

REPRODUCTION TOXICITY STUDY REVIEW

STN number: 125285/0

DATS number: DATS# 439161

Date/type of submission: 4/18/2008/BLA

Sponsor: Protein Science Corporation,
1000 Research Parkway,
Meriden, CT06450-7159

Reviewer name: Marion F. Gruber, PhD

Office/Division name: Office of Vaccines Research and Review

HFM#: HFM 408

Review completion date: September 18, 2009

Vaccine: Flublok (trivalent recombinant influenza hemagglutinin protein vaccine)

Intended population/indication: Active immunization of adults 18 years of age and older against influenza disease caused by influenza subtypes A and type B represented in the vaccine

Route of administration: IM

Studies reviewed in this submission:

- STN 125285/0 Module 4.2.3.5 received April 18, 2008
Final Study Report; Influenza vaccine, FluBlok "Reproductive safety and immunogenicity evaluation of Flublok trivalent influenza vaccine in rats"
- Response to Complete Response Letter Items 25 - 28, received April 7, 2009

Executive Summary:

The sponsor has performed a developmental toxicity study to provide information on the potential of the test article to produce adverse maternal, reproductive, developmental or immunological effects when administered twice prior to mating and once during gestation to female rats. Fifty ---(b)(4)----- rats were dosed 5 and 2 weeks prior to gestation and again on gestation day 6 with 1X concentration of Flublok , administered as two 0.25 ml IM injections, one injection per hind leg, at each dosing interval of either test article or saline control. Animals were subdivided into subgroups of animals (25 rats/group), underwent Caesarean section on DG 20 or were allowed to rear their offspring. Treatment of animals with Flublok vaccine did not induce vaccine related

maternal death or abortion. Clinical signs during the pre-mating, gestation and lactation period, body weights and feed consumptions were comparable across treatment groups. Mating and fertility indices as well as Caesarean-sectioning, natural delivery and litter parameters were unaffected by treatment with Flublok vaccine. There appeared to be no treatment related effects on fetal viability, fetal body weight, sex, gross external or soft tissue or skeletal examinations. F1 pup viability, body weight, sex, reflex and physical development were not affected by treatment with the vaccine. Thus, under the conditions of the study, Flublok vaccine does not appear to affect embryo-fetal pre-and postnatal development and does not appear to exert teratogenic effects. Sponsor responded satisfactorily on April 7, 2009, to CBER's comments regarding the reproduction toxicity study," no. 2146-001.

Recommendation: Pregnancy category B

Supervisor concurrence: Yes ☒ No ☐

Background: Flublok (trivalent recombinant influenza hemagglutinin protein vaccine) is indicated for the active immunization of adults 18 years of age and older against influenza disease caused by influenza virus subtypes A and B represented in the vaccine. Since Flublok may be recommended for immunization of pregnant women and/or women of child bearing potential, the sponsor has conducted a developmental toxicity study.

Product description: Flublok, influenza vaccine is a purified recombinant influenza hemagglutinin vaccine derived from H1 (A/New Caledonia/20/1999), H3 (A/Wisconsin/67/2005), and B (B/Ohio/1/2005) influenza viral strains. The dosage form is sterile liquid in single dose vials (batch number 50-06020, Mfg date: September 2006), containing 45 ug of rHA of each strain per 0.5 ml dose (135 ug total rHA per 0.5 ml dose). The clinical route of administration is intramuscular. A certificate of analysis for the bulk test article (September 26, 2006) was contained in the report. The control was USP grade sterile saline for injection (0.9% sodium chloride; Lot # ---(b)(4)---), a copy of the certificate of analysis was included.

Sponsor: Protein Sciences Corporation
1000 Research Parkway
Meriden, CT 06450
USA

Testing Facility

----- (b)(4) -----
----- (b)(4) -----
----- (b)(4) -----
----- (b)(4) -----

The study was conducted in compliance with OECD Principles of GLP, OECD [C(97)186/Final] and in accordance with U.S. FDA GLP regulations as set forth in 21 CFR part 58. Status of GLP compliance for the serum antibody analyses is not known.

Study inspected and audited:

<u>Date of QA activity</u>	<u>Phase</u>	<u>Report to Study Director</u>
October 1&2, 2006	Protocol review	October 2, 2006
October 20, 2006	Dosing day 1/inspection	October 23, 2006
January 5, 2007	Inspect F0 and F1 necropsy, lactation, day 21	January 5, 2007
February/March 2008	Audit report/data, excluding Serum antibody report; Review protocol deviation	March 21, 2008
March 13, 2008	Review serum antibody report	March 13, 2008
March 21, 2008	Review protocol amendment no.1	March 21, 2008

Study title: “Reproductive safety and immunogenicity evaluation of Flublok trivalent influenza vaccine in rats,” no. 2146-001

Study Director: William D. Johnson, PhD, DABT

Quality assurance: Glenn B. Miller, M.S.

Objective: To provide information on the potential of the test article to produce adverse maternal, reproductive, developmental or immunological effects when administered twice prior to mating and once during gestation to female rats.

Test system: 105 female -----(b)(4)----- rats, approximately seven weeks old (145-188 g), received from -----(b)(4)----- on October 11, 2006.

Randomization: Rats were held in quarantine for 9 days prior to randomization, and observed daily for mortality and moribundity. Animals were given physical exams, weighed and randomized using an in-house developed computerized randomization program based on body weight on October 19, 2006.

Mating: Upon receipt, rats were housed up to 2/cage and single-housed at time of randomization. Vaginal smears were collected daily starting 2 weeks before cohabitation on study day 32, to evaluate cyclicity and continued until a positive indication of mating was obtained or until the end of the approximately 2 week cohabitation period. During the approximately 2 week mating period, female/male, 1:1, were housed together, female rats determined to have mated (sperm-positive vaginal smear) were removed and single housed in the original cage. On GD 18, all females were transferred to polycarbonate

showbox-type cages with absorbent hardwood chip bedding equipped with automatic watering.

RoA: IM as it is the clinical route

Study design: Rats were assigned to 2 groups as outlined in table 1 below. All females were exposed by intramuscular (IM) injection to the control article (group 1) or Flublok (group 2) on study days 1 (10/20/06) and 20 (11/8/2006) and on gestation day 6 (ranging from 11/27/2006 to 12/10/2006). Both groups received two 0.25 ml injections, one injection per hind leg, at each dosing interval. Beginning day 32, females were individually cohabitated with 1 male (see above).

TABLE 1 – STUDY DESIGN

<i>Test material</i>	<i>Group</i>	<i>Total No. animals/sex</i>	<i>No. animals/subgroup</i>	<i>Immunization schedule</i>
Control	1	50 females	25 Caesarean/ 25 littering	-5 wk, -2 wk, GD 6,
Flublok	2	50 females	25 Caesarean/ 25 littering	Vaccine administration as per group 1

Clinical observation: Animals were inspected for moribundity and mortality 2x daily following treatment initiation, injection sites were examined on the day of each injection and daily thereafter for 5 days for signs of reactogenicity; during gestation and lactation, clinical observations were performed on the same day that body weights were measured; a complete physical examination was performed on all female rats prior to treatment initiation.

Body weight: recorded upon receipt, at randomization and weekly until confirmation of mating (days 1, 8, 15, 22 and 29), then on GD 0, 4, 6, 12, 15, 18 and 20 and on LD 0, 4, 7, 14, and 21.

Food consumption: at time of body weight measurement after initiation of treatment and prior to cohabitation (days 1, 8, 15, 22 and 29). Pregnant females had food consumption measured on the same day as body weights during gestation and lactation.

Parturition and lactation: Beginning on GD 18, females were examined 2x daily for delivery and possible dystopia.

Littering subgroup observations: Day of parturition was considered day 0 of lactation; duration of gestation was evaluated
Number of live and dead pups born in each litter was recorded after completion of parturition along with external abnormalities, pups were counted daily until weaning; pups were given a detailed examination on the day their body weight was recorded

Individual pup body weights were recorded on lactation days 1, 4, 7, 14 and 21

On lactation day 4, litters with more than 10 pups were culled randomly to an equal sex distribution, all pups were available for randomization

Pre-weaning developmental landmarks:

Pinna detachment (beginning on day 3 and then daily until positive response)

Eye opening (beginning on lactation day 13 and then daily until positive response)

Auditory startle response; beginning on lactation day 13 and then daily until positive response)

Weaning: On lactation day 21, all pups were removed from the litter and necropsied

Serum antibody determination:

For adult females serum samples (via orbital sinus) were taken on study days 1 and 20, on GD 20 for the Cesarean group and LD 21 for littering subgroup. For F1 pups, blood samples (via orbital sinus) were collected on LD 21 from 4 pups/litter (2/sex) randomly selected. Fetal blood samples (from 2 fetuses/sex/litter) on gestation day 20 via decapitation.

Postmortem analysis

F0 females: No F0 females were found dead during the study or sacrificed *in extremis*.

F1 pups; Pups found dead received gross external examinations and were externally sexed.

Caesarean group examinations:

F0 females were sacrificed on GD 20; tissue masses and suspect lesions fixed in 10% neutral buffered formalin. Apparent non-gravid uteri were examined by ammonium sulfide staining to confirm non-pregnant status. Uterus was weighed before removal of fetuses from the uterine horns, corpora lutea were counted for right and left ovaries, placentas were grossly examined; uterine horns were examined for implantations (early/late resorption), dead fetuses or live fetuses.

Fetal external examination: fetuses were removed, counted, and underwent external morphological examination, including body weight, all variations and malformations were recorded. Approximately half of the fetuses from each litter were preserved in Bouin's solution for visceral examination, transferred to 70% ethanol free hand sliced (Wilson). One-half of the fetuses were processed for skeletal examination (Alizarin Red-S staining)

Littering subgroup examinations:

F0 females were sacrificed on LD 21 and examined macroscopically, uteri were examined for implantation sites, non-gravid uteri were examined by ammonium sulfide staining, tissue masses and suspect lesions were fixed in 10% neutral buffered formalin. Culled F1 pups were sacrificed on LD 4, examined externally and discarded. F1 weaning pups were sacrificed on LD 21, examined macroscopically and discarded.

STATISTICS

Statistical analysis of Caesarean and fetal parameters were performed using the litter as the unit of analysis. The number of corpora lutea, implantations, viable/nonviable fetuses, early/late resorptions and gravid uterine weights were calculated as the total number for each group divided by the number of litters evaluated. Maternal food consumption, body weights, body weight gains, number of corpora lutea, implantation sites, viable fetuses and fetal body weights, by litter and sex, were analyzed by ANOVA. Percent pre/post-implantation loss, number of nonviable fetuses, early/late resorptions, percent male/female fetuses and gravid uterine weights were compared using the Kruskal-Wallis nonparametric test. Incidences of malformations and variations were compared using the Fisher's exact test with the litter as experimental unit. The total number of litters with external, visceral and skeletal malformations as well as the total number of litters with malformations and variations was statistically compared using the Fisher's exact test.

RESULTS

Premating/Cohabitation observations: The sponsor states that no adverse clinical observations were noted in the F0 females following pre-mating dosing on study days 1 and 20 and there was no death in F0 females during the study, nor did the F0 animals appear moribund (Table 1 and Table C-1; Appendix C of submission).

Body weights

Body weights prior to mating on study day 29 (Table 2, Table C-2 of submission):

Body weights prior to mating were comparable among control and treatment groups (50 animals evaluated per group):

Control: 255 g \pm 19.4 Vaccine: 251 g \pm 21.6

Body weight gains prior to mating (Table 3, Table C-3 of submission):

Total body weight gains prior to mating were comparable among control and treatment groups (50 animals evaluated per group):

Control: 66 g \pm 14.4 Vaccine: 62 g \pm 16

Mean body weight of F0 dams at GD 20 (Caesarean subgroup) (Table 9, Table C-8 of submission):

Body weights at GD20 were comparable among control and treatment groups

Control: 398 g \pm 34.2 (25 animals evaluated)

Vaccine : 398 g \pm 31.8 (25 animals evaluated)

Body weight gains during gestation (Caesarean subgroup) (Table 10, Table C-9 of submission):

Total body weight gains during gestation were comparable among control and treatment groups

Control: 137 g \pm 17.7 (25 animals evaluated)
Vaccine: 142 g \pm 20.0 (25 animals evaluated)

Mean body weight of F0 dams at GD 20 (Littering subgroup) (Table 18, Table C-17 of submission):

Body weights at GD20 were comparable among control and treatment groups.

Control: 406 g \pm 29.6 (24 animals evaluated)
Vaccine : 404 g \pm 35.5 (19 animals evaluated)

Body weight gains during gestation (Littering subgroup) (Table 19, Table C-18 of submission):

All animals gained weight during gestations. There was a statistically significant less weight gain on GD 18 in the vaccine treated group which was no longer observed on day 20 and there was no significant difference in total weight gain throughout pregnancy in animals of the littering subgroup.

Control: 134 g \pm 21.6 (24 animals evaluated)
Vaccine: 143 g \pm 16.2 (19 animals evaluated)

Body weight and weight gains during lactation (Littering subgroup) (Table 23 and 24, Table C-25 and C-26 of submission)

Mean body weights and mean body weight gains between control and Flublok treated females during lactation were comparable.

Mean body weight:

Control: Lactation day 0: 311g \pm 31.0 g	Lactation day 21: 338 g \pm 29.4g
Vaccine: Lactation day 0: 305g \pm 33.0 g	Lactation day 21: 344 g \pm 34.1g

Total body weight gain during lactation:

Control: 27g \pm 30.5 g
Vaccine: 39g \pm 23.6 g

Food consumption:

Premating phase (Table 4, Table C-4 of submission): Overall, there were no treatment related effects on food consumption in the F0 generation during the pre-mating phase. Mean food consumption in the vaccine treated group was statistically significantly decreased on day 29 but this decrease was transient and was not reflected in the total body weight gain of the vaccine treated group prior to mating.

Gestation phase (Caesarean subgroup): (Table 11, Table C-10 of submission). Overall, there were no treatment related effects on food consumption in the F0 generation allocated to the Caesarean subgroup during gestation. Mean food consumption in the vaccine treated group was statistically significantly increased on day 18 compared to controls, but this increase was transient and no longer observed on GD 20.

Gestation phase (Littering subgroup): (Table 20, Table C-19 of submission). Food consumption between control and vaccine-treated females allocated to the littering subgroup during gestation was comparable. Overall, food consumption during gestation of F0 animals allocated to the Caesarean and littering subgroup was comparable.

Lactation phase (littering subgroup) (Table 25, Table C-27 of submission). Food consumption in the vaccine treated animals was statistically significantly increased compared to the control group in lactation days 4, 14, and 21.

Estrous stages, cyclicity data and cohabitation (TABLE 2 below)

Data in Table 5 and C-5 of the submission indicated that the average length of time of F0 females in diestrus, proestrus, estrus and metestrus was comparable among saline and vaccine treated groups.

A positive indication of mating was obtained for 49 of 50 control group animals and for 46 of 50 vaccine group animals. In the control group, all 50 animals were later found to be pregnant and the overall fertility index was 100%. In the vaccine treated group 4 of 50 animals did not have a positive indication of mating but were later found to be pregnant. However, 2 animals with positive indications of mating were later found not to be pregnant. Thus, the fertility index was 96% in the vaccine treated group (Tables 6 and C-6)

TABLE 2 -Cohabitation and Cyclicity Data

	Group name Group number Treatment	Control 1 saline	Treated 2 Flublok
Females paired with males	N	50	50
Total number mated	N	50	50
Female mating index	%	100%	100%
Pregnant	N	50	48
Female fertility index	%	100%	96%
Females with defined day 0 of gestation	N	49	46
No. of days until mating	Mean S.D	3.3 2.41	3.4 2.53

Normal cycles			
4-6 days	N	16	17
	%	32.0	34.0
Abnormal cycles			
Shortened cycle	N	23	22
	%	46.0	44.0
Prolonged cycle	N	4	2
	%	8.0	4.0
Extended estrus	N	5	2
	%	10.0	4.0
Not cycling	N	2	7
	%	4.0	14.0

Comment: Cyclicity data (see table below) indicate a difference in the number of animals not cycling (defined as at least ten days without estrus) in the vaccine group (7 of 50 animals (14%)) compared to the control group (2 of 50 animals (4%)). The sponsor states that this group difference is not statistically different. Regardless, this difference of 4 % versus 14 % seems remarkable especially when considering the test species, i.e. -- -----(b)(4)----- rats. The sponsor should provide historical control data for this parameter from studies conducted in the testing facility using this test species.

Caesarean data (refer also to Table 3 below)

F0 survival and pregnancy status (Caesarean subgroup)

All of the control and vaccine treated F0 animals allocated to the Caesarean subgroup were pregnant (25 of 25 animals in each group) all litters had viable fetuses and no litters had total implant loss. None of the dams died post-mating (Tables 7 and C-6 of submission).

F0 survival and pregnancy status (Littering subgroup)

All of the control and vaccine treated F0 animals allocated to the littering subgroup were pregnant (25 of 25 animals in each group). All litters had live born pups and none had stillborn pups. Of the vaccine treated dams allocated to the Littering subgroup, the fertility rate was 92%, with 23 of 25 dams delivering a litter. In the control group 25 of 25 dams delivered a litter (Tables 16 and C-6 of submission).

Clinical observations during gestation

Caesarean subgroup (Tables 8 and C-7 of submission): Sponsor did not note any clinical observation during gestation with the exception of one animal in each group that had alopecia on GD 20.

Littering subgroup (Tables 17 and C-16 of submission): Sponsor did not note any clinical observation during gestation with the exception of one animal in the control group that had alopecia with an onset on day 12 and continuing through GD 20.

Comment: There were 50 animals allocated to the littering subgroup, 25 animals/group. In the vaccine treated group 23 of 25 animals delivered a litter (Table 16), 2 were not

pregnant (Table 17). The total number of animals evaluated for clinical signs in the saline group was 24 and the total number of animals in the vaccine treated group was 21. Please provide information on the 4 animals in the vaccine treated group and the 1 animal in the saline group that were apparently not evaluated for clinical signs.

TABLE 3 – CAESAREAN DATA

		Control group 1	Flublok group 2
Pregnant		25	25
Dams with no viable fetusus		0	0
Dams with viable fetuses		25	25
Corpora lutea	Total	406	414
	Mean	16.2	16.6
	SD	2.54	3.04
Implantation sites	Total	378	356
	Mean	15.1	14.2
	SD	1.62	1.88
Preimplantation loss	Total	32	58
No per animal	Mean	1.3	2.3
	S.D.	1.7	2.82
% per animal	Mean	6.9	12.3
	S.D.	8.74	13.27
Postimplantation loss	Total	14	8
No per animal	Mean	0.6	0.3
	SD	1.5	0.75
% implants per animal	Mean %	3.7	2.3
	SD	9.95	5.68
Early resorptions	Total	13	8
No. per animal	Mean	0.5	0.3
	SD	1.48	0.75
Late resorptions	Total	1	0
No. per animal	Mean	0.0	0.0
	SD	0.2	0.0
Dead Fetuses	Total	0	0
Live fetuses			
No. per animal	Total	364	348
	Mean	14.6	13.9
	SD	2.14	2.00
Live males	Total	162	175
	Mean%	44.0	50.3
	SD	14.76	9.43
Live females	Total	202	173
	Mean%	56	49.7
	SD	14.76	9.43
Fetal body weight	Mean	3.6	3.59
	SD	0.241	0.244
Gravid uterine weight mean		81.9	79.0
SD		13.08	13.05

The mean number of corpora lutea, resorptions (early and late) live young and sex ratio (% males) were unaffected by treatment with the vaccines.

Comment: There was a difference in regard to total preimplantation loss in the vaccine treated group (58) compared to the saline control (32). In addition, data in Tables C11 and C 12 of the study report (not shown here) showed that in the saline treated group 8 of 25 animals experienced a preimplantation loss above 10% ranging from 11.1 – 25.0%. In the vaccine treated group 11 of 25 animals experienced a preimplantation loss above 10% ranging from 11.1 – 38.1%. The sponsor should provide the historical control data from studies conducted using this test species from the testing facility with regard to this parameter.

Fetal pathology (Table 4 below and Tables 13, 14 & Table C-13 of submission)

Malformations: In the control group (364 fetuses in 25 litters examined) there was one animal with malformations, i.e., exencephaly, open eye lids and severely bent ribs dam (# 427, male fetus # 12). Of 348 fetuses in 25 litters examined in the vaccine treated group, one animal presented with a cleft lip (dam# 465, female fetus# 16).

Variations: Minor skeletal variations such as unossified sternebrae and unossified hoid were observed in both groups. Visceral variations included dilated kidneys in 5 animals in 3 litters in the control group and in 3 fetuses in 2 litters in the vaccine treated group. There was 1 fetus in the vaccine treated group with a ventricular septal defect (dam# 475, female# 8), and 1 fetus with a small testicle (dam# 480, male fetus# 5). In the control group, 1 fetus had a supernumary spleen (dam 3 413, female fetus #6) and 1 fetus had undescended testes (dam # 425, male fetus# 4). There were no variations upon external examination.

TABLE 4 - Summary of fetal observations - malformations

	Control group 1	Vaccine group 2
Litter exam. externally	25	25
Fetuses examined	364	348
Exencephaly		
Fetal incidence	1	0
Litter incidence	1	0
Open eyelid		
Fetal incidence	1	0
Litter incidence	1	0
Litters exam. Viscerally	25	25
Fetuses examined	135	126
Cleft lip		
Fetal incidence	0	1
Litter incidence	0	1
Litters exam. skeletally	25	25
Fetuses examined	123	115
Ribs bent-severe		
Fetal incidence	1	0
Litter incidence	1	0

TABLE - Summary of fetal observations -variations

	Control group 1	Vaccine group 2
Litter exam. externally	25	25
Fetuses examined	364	348
Litters exam. Viscerally	25	25
Fetuses examined	135	126
Ventricular septal defect		
Fetal incidence	0	
Litter incidence	0	1
		1
Dilated kidneys		
Fetal incidence	5	
Litter incidence	3	3
		2
Spleen supernumery		
Fetal incidence	1	
Litter incidence	1	0
		0
Testes undescended		
Fetal incidence	1	
Litter incidence	1	0
		0
Testicle very small		
Fetal incidence	0	
Litter incidence	0	1
		1

Litters exam. skeletally	25	25
Fetuses examined	123	115
Hyoid bone unossified		
Fetal incidence	22	24
Litter incidence	12	11
Entire skeleton-red. ossification		
Fetal incidence	2	2
Litter incidence	2	2
Skull bones-red. Ossification		
Fetal incidence	2	2
Litter incidence	2	2
Vertebra-red. Ossification		
Fetal incidence	0	1
Litter incidence	0	1
Sternebra 5 and/or 6 - unossified	51	44
Fetal incidence	19	18
Litter incidence		
Sternebra misaligned		
Fetal incidence	0	1
Litter incidence	0	1
Ribs 14 th rudimentary		
Fetal incidence	5	3
Litter incidence	3	3
Carpal/metacarpal-unossified		
Fetal incidence	9	11
Litter incidence	7	6
Tarsal/metatarsal-unossified		
Fetal incidence	7	6
Litter incidence	5	5

The fetal incidence with visceral variations was 7 (litter incidence 5) in the control compared to 5 (litter incidence 3) in the vaccine treated group. The fetal incidence with skeletal variations was 68 (litter incidence 24) in the control compared to 59 (litter incidence 20) in the vaccine treated group.

In summary, findings with regard to malformations occurred sporadic, were observed on both, the control as well as the vaccine treated group and were isolated in nature.

Overall skeletal and visceral examinations do not suggest that the vaccine is teratogenic.

Necropsy of F0 animals (Table 15, Tables C-14 and C-15 of submission)

There were no remarkable findings upon necropsy of the F0 generation allocated to the Caesarean subgroup.

Delivery and litter data (Littering subgroup Table 6 below)

Sponsor reports a statistically significant decrease in the number of dams delivering at least one still born pup in the Flublok group compared to the control group. In the

Flublok group, one dam (4.3%) delivered 2 stillborn pups, and in the control group, seven dams (28%) delivered at least one stillborn pup. Furthermore, on lactation day 0, 44.6% of the pups of Flublok treated dams in the littering subgroup of the study were males, compared to 52.0% of the pups born to dams in the control group representing a statistically significant decrease in the ratio of male pups to total pups in the vaccine group. This gender distribution difference was not observed in the Caesarean subgroup in which 50.3% of the fetuses in the vaccine treated group were male compared to 44.0% of the fetuses in the control. Thus, this sex difference observed in the littering subgroup is likely not test article related. Overall, Flublok treatment did not appear to effect delivery and litter parameter.

TABLE 6 - Duration of gestation & overall litter performance (Littering subgroup)

Group	1	2
	Control	Flublok
Females on study	25	25
Females mated (Mating index%)	25 (100%)	25 (100%)
Females pregnant (fertility index%)	25 (100%)	23 (92%)
Females with liveborn (gestation index%)	25 (100%)	23 (100%)
Females completing delivery	25	23
With still born pups	7 (28%)	1 (4.3)
With all stillborn	0	0
Duration of Gestation (days) Mean	21.8	22.1
SD	0.41	0.71
N	24*	19*
Mean no. of implant sites N	387	359
per litter \pm SD	15.5 \pm 1.85	15.6 \pm 1.9
Mean total no. of live pups day 1/litter	14.6 \pm 2.06	14.0 \pm 2.4
Sex ratio – male pups:total pups	193	146
	52.6%	44.6%
Pup weight/litter (grams) day 1		
Mean \pm SD	6.6 \pm 0.87	6.8 \pm 0.66
Pup weight/litter (grams) day 21		
Mean \pm SD	46.1 \pm 4.39	46.1 \pm 6.27
N	25	23

For the parameter "duration of gestation" the number of dams is 24 in the control group and 19 in the Flublok treated group, because for 1 animal in the control group and 4 animals in the vaccine treated group, there was no positive indicator of mating observed (sperm plug or sperm in vaginal smear), thus duration of gestation could not be determined for these animals. However, the animals were later found to be pregnant.

F0 clinical observations during lactation:

Two animals in the control group and one animal in the Flublok treated group presented with alopecia during lactation with an onset on LD 7-14 and continuing through lactation day 21. There were no other adverse clinical observations noted during lactation. (Table 22, Table C-24 of submission).

Necropsy findings (F0 generation, littering subgroup)

Overall, there were no remarkable findings with the exception of one dam treated with vaccine that was observed to exhibit a thymus with multiple dark red foci. (Table 26, Tables C-14 and C-15 of submission).

F1 Clinical observations

No adverse clinical signs were observed in the F1 pups of vaccine treated dams at any lactation time interval (323 pups evaluated in 23 litters up to day 4). In the control group, one F1 pup was pale on lactation days 1 and 4. On lactation day 4 in the control group, 2 pups were pale, cold to the touch and exhibited rapid breathing. All control pups were found to be normal on lactation days 7, 14, and 21 (365 pups evaluated in 25 litters up to day 4) (Table 27, Table C-28 of submission).

F1 body weights and body weight gains

There were no differences in body weight or body weight gains in pups of dams treated with vaccines compared to dams treated with saline control (Tables 28, 29, 30, C-29 of submission).

F1 Morphological development and reflexes

There were no group differences in the age at which pinna detachment, acoustic startle reflex and eye opening were noted in the F1 generation (Tables 31 and C-30, C-31, C-32, C-33, C-34 and C-35 of submission and Table 7 below). Sixty four (64%) of F1 pups had pinna detachment on day 3 and 93% achieved this developmental landmark on day 4, compared to 75% and 100% in the FluBlok treated group, respectively (Table C-31 of submission.)

TABLE 7 Pup reflex and morphological development

Group Group number Treatment		Control 1 Saline (day)	Treated 2 Flublok (day)
Eye opening	Mean	15.5	15.2
	SD	0.64	1.13
	N	25	23
	%	100	100
Pinna detachment	Mean	3.4	3.3
	SD	0.61	0.36
	N	25	23
	%	99	100
Auditory startle response	Mean	13.2	13.2
	SD	0.40	0.31
	N	25	23
	%	100	100

F1 necropsy observations

Overall necropsy findings of F1 pups on lactation day 21 were non remarkable. There were no significant differences in pup necropsy observations between the pups of Flublok treated (225 pups evaluated) and control animals (247 pups evaluated) (Table 32 and Table C-36, C-37 of submission). In 4 pups of the control group (2 litters) and in 1 pup of the vaccine treated group, red pigmentation of the lungs were observed. In the Flublok group, dilated kidneys were observed in 6 pups from 3 litters.

Serum antibody determination

Sponsor states that immunization of the F0 generation with Flublok produced a time dependent immune response against each of the influenza antigens with antibody titers peaking at gestation day 20, 2 weeks following the last immunization (GD 6). Antibody was transferred to fetuses as determined by fetal blood analysis on GD 20 and at study completion (LD 21 samples).

Comment: Data in Table 1 of Appendix D show that on study day 1 (day of 1st injection) the maternal antibody titer to A/New Caledonia/20/1999 (H1N1) was about 9 x above that observed in the control group. Please explain. Furthermore, data in Table 1 Appendix D show that antibody titers to A/Wisconsin/67/2005 (H3N2) in fetal blood on GD 20 were approximately 5 x higher (GMT:338.2) compared to the titer in maternal blood on GD 20 (GMT: 62.3) and approximately 2 fold higher in pups on lactation day 2 (GMT:114.9). Please explain this finding as pups were not immunized with the test article.

SUMMARY

Under the conditions of the study, there were no overt signs of treatment related maternal toxicities. Treatment did not affect body weights and body weight gains of the F0 generation neither did it affect body weight gain of the F1 generation born to treated dams, F1 reflexes or development. There was no test article related effects on food consumption of F0 and F1 animals. The influenza vaccine, Flublok, did not affect embryo-fetal and postnatal development.

Cyclicity data indicated a difference in the number of animals not cycling (defined as at least ten days without estrus) in the vaccine group (14%) compared to the control group (4%). In addition, total preimplantation loss was higher (58) in the vaccine treated group compared to the saline control (32). In addition, in the saline treated group 8 of 25 animals experienced a preimplantation loss above 10% ranging from 11.1 – 25.0%. In the vaccine treated group 11 of 25 animals experienced a preimplantation loss above 10% ranging from 11.1 – 38.1%.

There were no observed treatment related effect on the incidence of major and minor abnormalities and skeletal variants in the offspring of dams treated with the test article. Also, postnatal growth and development of the F1 generation did not appear to be affected by vaccine administration.

The sponsor requests a pregnancy category B. Sponsor should comment on the observed differences in cyclicity and pre-implantation loss and should provide the historical control data for these parameters before a decision with regard to the pregnancy category can be made.

Comments to sponsor with regard to the reproduction toxicity study no. 2146-001

1. Fifty (50) animals were allocated to the littering subgroup, 25 animals/group. In the vaccine treated group 23 of 25 animals delivered a litter (Table 16), 2 were not pregnant (Table 17). The total number of animals evaluated for clinical signs in

the saline group was 24 and the total number of animals in the vaccine treated group was 21. Please explain and provide information on the 4 animals in the vaccine treated group and the 1 animal in the saline group that were apparently not evaluated for clinical signs.

2. Data in Table 5 and C-5 indicated that the average length of time of F0 females in diestrus, proestrus, estrus and metestrus was comparable among saline and vaccine treated groups. However cyclicity data indicate a difference in the number of animals “not cycling” (defined as at least ten days without estrus) in the vaccine group (7 of 50 animals (14%)) compared to the control group (2 of 50 animals (4%)). You state that this group difference is not statistically different. We find this difference of 4 % versus 14 % remarkable especially when considering the test species, i.e., -----(b)(4)----- rats. Please comment and provide historical control data for this parameter from studies conducted in the testing facility using this test species.
3. Total preimplantation loss was higher (58) in the vaccine treated group compared to the saline control (32) (Table 12). In the saline treated group 8 of 25 animals experienced a preimplantation loss above 10% ranging from 11.1 – 25.0%. In the vaccine treated group 11 of 25 animals experienced a preimplantation loss above 10% ranging from 11.1 – 38.1%. In this group there were an additional 2 animals with a % preimplantation loss above 7.7% (Tables C-11 and C012). Please provide the historical control data from the testing facility with regard to this parameter.
4. Data in Table 1 of Appendix D show that on study day 1 (day of 1st injection) the maternal antibody titer to A/New Caledonia/20/1999 (H1N1) was about 9 x above that observed in the control group. Please explain. Furthermore, data in Table 1 Appendix D show that antibody titers to A/Wisconsin/67/2005 (H3N2) in fetal blood on GD 20 were approximately 5 x higher (GMT:338.2) compared to the titer in maternal blood on GD 20 (GMT: 62.3) and approximately 2 fold higher in pups on lactation day 2 (GMT:114.9). Please explain this finding as pups were not immunized with the test article.

**Review of applicant's response to Complete Response Letter Items 25 – 28,
received April 7, 2009**

The following restates CBER's comments communicated to sponsor regarding the reproduction toxicity study no. 2146-001 followed by the sponsor's response and reviewer's assessment.

CBER's CR question 25:

Fifty (50) animals were allocated to the littering subgroup, 25 animals/group. In the vaccine treated group 23 of 25 animals delivered a litter (Table 16), 2 were not pregnant (Table 17). The total number of animals evaluated for clinical signs in the saline group was 24 and the total number of animals in the vaccine treated group was 21. Please explain and provide information on the 4 animals in the vaccine treated group and the 1 animal in the saline group that were apparently not evaluated for clinical signs.

Sponsor's response:

One rat in the saline group and 4 rats in the vaccine group did not have a positive copulation indicator (Table C-20 of study report). Thus, clinical observations, in addition to body weight and food consumption measurements, were not performed on these 5 animals during gestation since the day of gestation was unknown. Therefore, in Table 17, the total number of animals observed in saline group is 24 and, in the vaccine treated group, is 21.

Reviewer comment:

The question is satisfactorily addressed. The number of animals evaluated for clinical signs is sufficient to allow a meaningful interpretation of the data (International Conference on Harmonization (ICH) Harmonized Tripartite Guideline for Industry (ICH-S5A) Detection of Toxicity to Reproduction for Medicinal Products, (59 FR 48746, September 22, 1994).

CBER's CR question 26:

Data in Table 5 and C-5 indicated that the average length of time of F0 females in diestrus, proestrus, estrus and metestrus was comparable among saline and vaccine treated groups. However cyclicity data indicate a difference in the number of animals "not cycling" (defined as at least ten days without estrus) in the vaccine group (7 of 50 animals (14%)) compared to the control group (2 of 50 animals (4%)). You state that this group difference is not statistically different. We find this difference of 4 % versus 14 % remarkable especially when considering the test species, i.e., ----(b)(4)---- rats. Please comment and provide historical control data for this parameter from studies conducted in the testing facility using this test species.

Sponsor's response:

The 2 rats in the saline group that were not cycling were assigned to the repro-phase and delivered. For the seven rats in the vaccine treated group which were not cycling, five were assigned to the repro-phase and delivered. Sponsor states that the "estrus" stage of

the cycle was missed during the vaginal smear evaluation. The remaining 2 rats in the vaccine treated group were not pregnant.

Reviewer comment: no further comment

CBER's CR question 27:

Total preimplantation loss was higher (58) in the vaccine treated group compared to the saline control (32) (Table 12). In the saline treated group 8 of 25 animals experienced a preimplantation loss above 10% ranging from 11.1 – 25.0%. In the vaccine treated group 11 of 25 animals experienced a preimplantation loss above 10% ranging from 11.1 – 38.1%. In this group there were an additional 2 animals with a % preimplantation loss above 7.7% (Tables C-11 and C012). Please provide the historical control data from the testing facility with regard to this parameter.

Sponsor's response:

The historical control data for preimplantation loss from the four most recent teratology studies using -----(b)(4)----- rats (n = 103 rats with approximately 25 rats/study) conducted at IITRI is as follows:

Preimplantation Loss	
Total	Range: 31-54 (31, 48, 50, 54)
No. per animal	Range: 1.1 – 2.1 (1.1, 1.9, 2.0, 2.1)
% Preimplantation Loss	
Range per animal: 6 - 86%	
> 10% (11-86%) 43/103 = 41.7%	
≤ 10 % (6-10%) 28/103 = 27.2%	
Mean per study: 13.9% (range: 8.7 – 16.3)	

Reviewer's comment:

The total number of preimplantation loss appeared to be in the range of what is observed in the (limited) historical control database, with the total number of preimplantation loss in the vaccine treated group (n= 58) slightly higher. The relevance of this is unknown. The % preimplantation loss in both groups appears to be in the range of what is observed in the historical control data base. Thus, the observed differences are likely not vaccine related.

CBER's CR question 28:

a) In Table 1 of Appendix D show that on study day 1 (day of 1st injection) the maternal antibody titer to A/New Caledonia/20/1999 (H1N1) was about 9 x above that observed in the control group. Please explain.

b) Furthermore, data in Table 1 Appendix D show that antibody titers to A/Wisconsin/67/2005 (H3N2) in fetal blood on GD 20 were approximately 5 x higher (GMT:338.2) compared to the titer in maternal blood on GD 20 (GMT: 62.3) and approximately 2 fold higher in pups on lactation day 2 (GMT:114.9). Please explain this finding as pups were not immunized with the test article.

Sponsor's response:

- a) The maternal antibody titer to the A/New Caledonia/20/1999 H1N1 antigen on day 20 (97.1) and gestation day 20 (89.4) in the control was equivalent to that of the vaccine treated group on day 1 (81.1), the titer values in the vaccine treated group was considered in the range of background with the control group on maternal day 1 showing a low background titer (9.4).
- b) The reason for the high titers of H3N2 in the pups as compared to the maternal animals is unknown and possibly due to a preferential transport of the antibodies of A/Wisconsin/67/2005 (H3N2) from mother to fetus.

Reviewer's comment:

No further comment. Data derived from this assay can be taken to support “proof of concept” that an active immune response was induced in the dams and that varying antibody transfer took place to the fetuses.

RECOMMENDATION:

No further action indicated, pregnancy category B