REPRODUCTION TOXICITY STUDY REVIEW

STN number: 125297

DATS number/date/type of submission: DATS# 445153/7/11/2008/BLA

Relevant IND: --b(4)--

Sponsor : Novartis Vaccines, 1 Via Fiorentina, 53100 Siena, Italy

Manufacturer of vaccine product:
Novartis Vaccines, 1Via Fiorentina, 53100 Siena, Italy

Reviewer name: Marion F. Gruber, PhD

Office/Division name/HFM#: Office of Vaccines Research and Review/HFM 408

Review completion date: November 3, 2008

Vaccine:
Trade name: Agrippal
Nonproprietary name: Influenza Virus Trivalent Subunit (A/A/B haemagglutinin and neuraminidase; embryonated hen's egg) Vaccine, Inactivated (IVV)

Intended clinical population: Person 18 years of age and older

Clinical formulation: 0.5 ml solution

Route of administration: IM

Clinical protocol: refer to section 5 of STN 125297

Previous clinical experience: Since initial licensure of the vaccine in Italy in 1986, IVV has been licensed in over 50 countries worldwide. Since initial approval, over --b(4)-- doses have been distributed worldwide, with more than -b(4)-- distributed since the removal of thimerosal-preservative from the vaccine formulation in 2003. The sponsor has conducted 2 developmental toxicity studies as this product will be administered to a target population that includes females of childbearing potential.

Studies reviewed in this submission:
Intramuscular reproductive and developmental toxicity study of Agrippal vaccine in rabbits, including a postnatal evaluation (b(4)000-40)

Intramuscular reproductive and developmental toxicity study of Agrippal vaccine in rabbits, including a postnatal evaluation (b(4)000-43)

Executive Summary: The sponsor performed 2 studies to evaluate the reproductive and developmental toxicity potential of Agrippal vaccine in rabbits, including a postnatal evaluation.

b(4)000-40: Animals were dosed on study days (DS) -1, -15 and -29 prior to mating and on gestation days 7 and 20 either with Agrippal vaccine, 0.5 ml, I.M., 45 ug antigen or saline control. Animals were subdivided into subgroups of animals (27 rabbits/group), and either underwent Caesarean section on DG 29 or were allowed to rear their offspring. Data from animals assigned to the Caesarean sectioning group suggest no effects of the vaccine on mating, fertility, pregnancy and embryo-fetal development. However, of concern are the number of does with no surviving pups in animals assigned to the natural delivery subgroup, i.e. 5 does in the control group and 8 does in the vaccine group as well as mortality of does in control and vaccine treated groups during gestation and lactation phase. Furthermore, the low number of evaluable does and litters assigned to the natural delivery subgroups prohibits a meaningful assessment of developmental endpoints of the F1 generation as well as meaningful interpretation of maternal parameters. A total of 78 pups in control group III and 69 pups (80 pups according to CBER's calculations) in vaccine group IV were found dead, stillborn or were euthanized due to adverse clinical signs. Sponsor attributes the high pup mortality to handling of the pups between DL 1-5 and hence, has conducted a second study to repeat the natural delivery arm of the study, while using data from the Caesarean subgroups I (control) and II (vaccine) to demonstrate absence of vaccine related effects on Caesarean and fetal parameters. This reviewer agrees that the vaccine does not appear to affect embryo-fetal development and does not appear to exert teratogenic effects.

b(4)000-43: Animals were dosed on study days (DS) -1, -15 and -29 prior to mating and on gestation days 7 and 20 either with Agrippal vaccine, 0.5 ml, I.M., 45 ug antigen or saline control. Animals were allowed to rear their offspring. Three does died in the vaccine treated group, however data are within the range reported in historical data. Beginning on DG7 – DG 26, mean absolute and relative feed consumptions values in the vaccine treated group were significantly reduced compared to the control group values. There were slight differences in reproductive parameters, i.e., the number of rabbits achieving pregnancy in the vaccine treated group was slightly decreased compared to the control group, 21 and 25 does, respectively, there were differences in the fertility index (92.6% in control group and 80.0% in the vaccine group) and gestation index (100% in control group and 85.7% in the vaccine group) . The pregnancy rate was 92.6% in control group compared to 77.8% in vaccine treated group. In addition, the number of liveborn pups in the vaccine treated group (82.0%) was reduced compared to the number of liveborn pups in the control group (92.0 %). Importantly, when calculating the viability indices based on the numbers of pups dead/euthanized between DL 1-7, the results are 52.5% (control group) and 66.4% (vaccine group). These values are markedly lower than the viability index observed for the same interval in the historical control data base (87.3% (81.6-96.7), historical data from 3 studies). A total of 93 pups in control group I (100 pups according to CBER's calculations) and 46 pups in vaccine group II (55

pups according to CBER's calculations) were found dead, stillborn or were euthanized due to adverse clinical signs. Due to litters with no surviving pups, in particular in vaccine group II, the number of evaluable litters in group II was 15 and thus, slightly below the recommended litter number resulting in consistency of results.

In summary, data derived from the Caesarean subgroup evaluation from study b(4)-000-40 do not suggest adverse effects of Aggripal on embryo-fetal development and there was absence of vaccine-related teratogenic effects. However, in both studies, i.e., b(4)-000-40 and b(4)000-43, the observed high mortality of pups during the postpartum period, does not allow the conclusion of no effect of Agrippal on postnatal development.

The sponsor proposes language in section 8.1 of the product labeling according to the proposed rule entitled "Content and Format of Labeling for Human Prescription Drug and Biological products; Requirements for pregnancy and lactation labeling (May 29, 2008): The "Fetal risk Summary" section states "Based on reproductive toxicology data in rabbits, Agrippal is not predicted to increase the risk of developmental abnormalities." The "Clinical Considerations" section includes the statement "There were no teratogenic effects, and no findings of increased fetal loss, mortality or resorptions, reductions in body weight of fetuses, or other developmental abnormalities."

The proposed rule is not finalized and thus, the pregnancy labeling section 8.1 must include pregnancy categories and codified language. Furthermore, while it is agreed that, based on study b(4)000-40 Agrippal does not appear to increase developmental abnormalities, the data derived from the natural delivery subgroups from both studies, i.e., b(4) 000-40 and b(4)-000-43 are problematic due to the observed pup mortality across study arms, so that possible effects of Agrippal on postnatal development cannot be ascertained. The sponsor will be asked how data from postnatal evaluations will be described in product label.

Reproductive and developmental toxicology

Study title:
Intramuscular reproductive and developmental toxicity study of Agrippal vaccine in rabbits, including a postnatal evaluation (b(4)000-40)

Purpose of Study:
To test for toxic effects resulting from Agrippal vaccine treatment in the test species before cohabitation, through mating, gestation and lactation

Key study findings -b(4)-000-40: Animals were dosed on study days (DS) -1, -15 and -29 prior to mating and on gestation days 7 and 20 either with Agrippal vaccine, 0.5 ml, I.M., 45 ug antigen or saline control. Animals were subdivided into subgroups of animals (N =27), and either underwent Caesarean section on DG 29 or were allowed to rear their offspring. No adverse effects were observed on Casarean parameters evaluated, neither were there any vaccine related fetal malformations. However, of concern are the number of does with no surviving pups in animals assigned to the natural delivery subgroup, i.e. 5 does in the control group (III) and 8 does in the vaccine group (IV). Overall, in control groups I and III, two does were found dead, one doe aborted and was euthanized and one doe delivered early and was sacrificed. In the vaccine groups, four does were found dead, one doe aborted and was euthanized. Furthermore, adverse clinical observations, such as ungroomed coats and abnormal or scant feces were observed across groups. Also, body weight measurements and feed consumptions values in particular for animals assigned to the natural delivery subgroup are not interpretable, since only 16 and 9 litters were included in the analysis. This low number of evaluated litters also prohibits an assessment of developmental endpoints of the F1 generation. The sponsor has conducted serology on some animals to investigate possible infection of animals but findings were unremarkable. Sponsor did attribute the high pup mortality to handling of the pups between DL 1-5 and hence, has conducted a second study to repeat the natural delivery arm of the study.

Study no.: b(4)000-40

BLA section: 4.2.5.3.5.1 **page #**: 1 - 399

Conducting laboratory and location: -----b(4)----------
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Study Director:
------b(4)----------

Date of study initiation: June 1, 2007
Date of experimental study completion: October 5, 2007

Final report: 04 Feb 2008

GLP compliance:
US FDA Good Laboratory Practice Regulations (CFR Part 58)
Japanese Pharmaceutical Affairs Bureau, Ministry of Health, Labour and Welfare Good Laboratory Practice Standard for Safety Studies on Drugs, MHW Ordinance No. 21, March 1997
Revised OECD Principles of Good Laboratory Practice [C(97)186/Final]
Note that the immunology assays were performed by ------b(4)---------- not in compliance with GLP

QA reports: yes (X) no ( )

Test article, lot #, and purity:
Agrippal vaccine, clear, colorless liquid, refrigerated
Lot number -b(4)----
Information to document the identity, composition, strength, activity/purity and stability of the test article was provided by sponsor to testing facility, COA provided
Agrippal is comprised of hemagglutinin (HA) and neuraminidase antigens from each of three influenza strains (A/H1N1, A/H3N2 and B). Each 0.5 ml dose of Agrippal contained 15 ug HA of each strain (total 45 ug HA protein) in a non-adjuvanted, --b(4)------ solution. Prefilled syringes of the test article were used as received at the testing facility and stored refrigerated (2 ^{0}C to 8 ^{0}C).

Control article, lot#, and purity:
b(4) sodium chloride injection, USP, clear, colorless, liquid, room temperature
Lot number --b(4)------

Animal diet:

Feed

Type/Name: Certified Rabbit Chow #5322 ------b(4)----------
Availability: 150 g per rabbit per day until GD 6, after that 180 g/rabbit/day, for rabbits assigned to groups III and IV beginning LD1, 230 g/rabbit/day
Analysis for contaminants: routinely performed by feed supplier, copies of results submitted to raw data, no contaminants detected
Comments: none

Water

Source: Local water passed through -----b(4)----------
Availability: *ad libitum* from individual water bottles
Analysis for contaminants: --b(4)---------- for chemical contamination and -b(4)--- for bacterial contamination, copies of results submitted to raw data
Comments: none

Husbandry

Environmental conditions:

Temperature: RT ---b(4)---
Humidity: --b(4)----
Air changes: positive airflow with minimum of b(4) changes per hour of 100% fresh air passed through 99.97% HEPA filters
Photoperiod: 12 hour light: 12 hours dark fluorescent light cycle
Housing: Females were individually housed in stainless steel, wire bottom cages, except during mating period, rabbits assigned to natural delivery were supplied with a nesting box and nesting materials no later than DG 27

Methods

Species/strain/supplier: --------b(4)---------- rabbits, -----b(4)----------
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Number/sex/group: 27/female rabbits/treatment group (male rabbits used for the purpose of breeding, not considered part of the test system)
Number of rabbits acclaimed: 112
Number of rabbits assigned to study: 108
Age: 7 months at arrival
Weight: 2.7 – 4.3 kg at study assignment
Doses/rationale: 3 doses prior to gestation to ensure induction of high antibody titers (days -1, -15, and -29) plus 2 doses administered during gestation to expose fetuses to vaccine antigen and to maintain antibody titers (GD 7 and 20). Dose (15 ug/strain antigen) was selected as the highest anticipated dosage that could be used in the clinic
Route, volume: IM, 0.5 ml for each injection, (consecutive injections were alternated between hind legs starting with the right leg) these represent proposed clinical routes and volume

Mating procedures

Description: After 35 days on study (first day of dosage administration considered DS1) virgin female rabbits were mated with male rabbits, one male rabbit per female rabbit, 20 USP units/kg of HCG was administered to female rabbits prior to mating, animals were observed continuously until mating confirmed to have occurred by observation, day of mating was designated DG 0. If females failed to mate they were placed with a second and then 3rd male, if mating was not confirmed following the third pairing, the female was recorded as no confirmed date of mating but considered to be at DG0. These rabbits continued on the assigned dosage regimen and were either Caesarean sectioned 29 days after the date of attempted mating or euthanized on day 34 when they did not naturally deliver a litter (group III and IV).

Culling procedures

Description: Litters were not culled during the lactation period to prevent selection bias in body weights and pup viability.

Study design:
Randomization: Rabbits were assigned to dosage groups based on computer-generated (weight ordered) randomization procedures

Table I: Study design: Allocation of animals and treatment schedule

TREATMENT	GROUP* (NUMBERS/ GROUP)	DOSE TO ANIMALS (total mcg HA protein)	DAYS TREATMENT ADMINISTERED	DOSAGE VOLUME (ML)	RABBIT NUMBER
		F_1 FEMALES			F_1 FEMALES
SALINE	I (27)	-	DS-1, -15, -29 GD 7 and 20	0.5	2201-2227
AGRIPPAL	II (27)	45 ug	DS-1, -15, -29 GD 7 and 20	0.5	2228-2254
SALINE	III (27)	-	DS-1, -15, -29 GD 7 and 20	0.5	2255-2281
AGRIPPAL	IV (27)	45 ug	DS-1, -15, -29 GD 7 and 20	0.5	2282-2300, 6491-6498

* Rabbits in groups I and II were Caesarean-sectioned, rabbits in groups III and IV were allowed to deliver naturally

Parameters and endpoints evaluated:
Clinical observation: Animals were inspected for viability 2x daily, injection sites were examined (onset, intensity and duration of signs recorded); rabbits were observed for abortions, premature deliveries and deaths before and approximately 60 minutes after dosage administration, once daily on non-dosage days and daily during postdosage period

Body weight: weekly during the acclimation period, weekly during the dosage period (including DG 1, 15, and 29 and DG 0, 7, 10, 13, 16 and 20), on DG 23, 26, 29 and 34 (when necessary). For rabbits in groups III and IV, body weights were recorded on days 1, 5, 8, 11, 15, 18, 22, 25 and 29 postpartum.

Food consumption: daily

Littering subgroup observations:
F0 generation: Day of parturition was considered day 0 of lactation; does were evaluated for adverse clinical signs during parturition, duration of gestation was evaluated, number of live and dead pups born in each litter was recorded after completion of parturition

F1 generation pups (natural delivery subgroups III and IV)
Litters evaluated for viability at least 2x daily, pups in each litter counted daily, pups observed for clinical observations and appearance once daily beginning DL 5, pup weights were recorded on DL 5, 8, 15, 22 and 29

Hair growth (from DL 5), eye opening (from DL 9), air righting reflex (from DL 10), acoustic startle (from DL 14) and pupil constriction was evaluated once (DL 22). The number of pups meeting the criterion was recorded on each day of testing, testing continued daily until the day the criterion was attained by all pups in the litter

Gross necropsy of F0 generation :

Gross necropsy of the thoracic and abdominal and pelvic viscera, blood samples collected, number and distribution of implantation sites recorded, rabbits that did not deliver a litter were euthanized on DG 34 and examined for gross lesions, uteri of apparent nonpregnant does were examined to confirm absence of implantation sites, uteri and ovaries of apparently nonpregnant does were retained in neutral buffered b(4) formalin.

Rabbits that died before scheduled termination were examined for cause of death, examined for gross lesions, hearts, lungs, liver, kidneys, stomach and spleen retained in b(4)formalin for possible histological evaluation, pregnancy status and uterine contents were recorded, aborted fetuses were examined to the extent possible.

Necropsy of F1 pups: Pups that died before DL1 were evaluated for vital status at birth by removing the lungs and immersion of the lungs in water, pups with lungs that sank were designated stillborns, pups with lungs that float were designated life born. Pups that died before DL 29 were examined for cause of death, hearts, lungs, liver, kidneys, stomach and spleen retained in b(4) formalin for possible histological evaluation, gross lesions retained in b(4) formalin.

Caesarean subgroup observations:
F0 generation: rabbits were euthanized by IV administration of sodium pentobarbital on DG 29, gross necropsy of thoracic, abdominal and pelvic viscera, blood samples collected, gravid uteri excised and weighed, uteri apparently non-pregnant does examined by being pressed between glass plates to confirm absence of implantation sites, these uteri and ovaries retained in b(4) formalin

Uterus of each rabbit examined for pregnancy status, for each animal the number of corpora lutea in each ovary and the number and location of implantation sites, the number and distribution of resorption sites and live and dead fetuses and early and late resorptions were recorded for each uterine horn

Rabbits that died before scheduled termination were examined for cause of death and examined for gross lesions, heart, lungs, liver, kidneys, stomach and spleen were retained in b(4) formalin, pregnancy status and uterine content were recorded, aborted fetuses were examined to the extent possible

Fetuses, F1 generation: removed from uterus and euthanized by IP injection of sodium pentobarbital, blood samples collected from each fetus via vena cava and samples from each litter pooled, externally examined for gross external alterations, weights of fetuses were recorded, sex of each fetus was recorded, cavitated organs were evaluated in all fetuses by dissection, all fetuses were eviscerated, cleared stained with ---b(4)---------- and examined for skeletal alterations.

Laboratory investigations
Blood samples for hematology and clinical chemistry were collected from adult females pre-dose administration and on DS 15 and 29 as well as DG 7, 20 and 29. Samples of 1 to 2 ml each were collected from the medial auricular artery (in-life) and inferior vena cava (terminal blood collection).

Statistical methods
Clinical observations and other proportion data were analyzed using the variance test for homogeneity of the binominal distribution. Data from F0 generation rabbits were evaluated with the individual rabbit as a unit measured. Litter values were used in evaluation of pup data.

Results F0 Generation

Mortality/Clinical signs (summarized in Table 1 and 29 of b(4)000-40):
Two does in the control group and four does in the vaccine groups were found dead, one doe in each of the control and vaccine treated groups aborted and were euthanized, and one doe in the control group delivered early and was sacrificed.

Control groups I and III:
Doe 2212 in group I delivered her litter and was sacrificed before DG 20. Adverse clinical signs consisted of ungroomed coat (DS 24 and 35), erythema grade 1 on DS 30 to 31, a laceration on the left hindlimb on DG 6-11, scab on left hindlimb on DG 12-15, ungroomed coat on DG 27-28, red substance in cage pan on DG 27 and 29, abnormal feces on DG 28. The animal lost weight on DG 23-26 (140 g). Feed consumption values were reduced after DG 25. All tissues appeared normal at necropsy. The litter consisted of 4 delivered late resorptions and 2 delivered live fetuses. At gross external, soft tissue and skeletal examination, the 2 fetuses appeared normal.

Doe 2227 in group I aborted and was euthanized on GD 19. Adverse clinical signs observed during in-life were ungroomed coat on DG0 to 4 and DG 7 to 9, erythema grade I on DG 9 and red substance in cage pan on DG 19. Body weight and feed consumption values were comparable to other rabbits in this group. All tissues appeared normal at necropsy. The litter consisted of one early resorption *in utero* and one aborted fetus.

In group III, on GD 32, doe 2264 was found dead. Adverse clinical signs were ungroomed coat (DS 6 to 24, DS 31 to 35, DG 0 to 5 and DG 8-31), abnormal feces (DG 25 and 26), weight loss (DS 15, and from DG 23 to 29 (490 g)). Feed consumption values were reduced during the premating period (DS 15-17) and after DG 22. Pale heart, kidneys and lobes of liver and lungs were observed, all other tissues appeared normal. The litter consisted of 8 fetuses *in utero* of which viability at time of maternal death could not be determined.

In group III, on DG 29, doe 2280 was found dead. Adverse clinical signs were ungroomed coat (DS 22, DS 29 to 35, DG 0 to 21 and DG 28), abnormal feces (DG 19 and 21, no feces on DG 24), weight loss occurred from DG 13 to 26 (470g). Feed

consumption values were reduced after DG 14. Necropsy revealed numerous ulcerations in the cardiac region of the stomach, all other tissues appeared normal. The litter consisted of 8 fetuses *in utero* of which viability at time of maternal death could not be determined, and one delivered pup.

Vaccine groups II and IV
In group II, doe 2249 was found dead on DG 27. Adverse clinical signs observed included ungroomed coat (DS to 22, DS 29 to 35 and DG 0-27), abnormal feces (DG 19-24, DG 21-24, no feces DG 26 and 27), decreased motor activity, lost righting reflex, vocalization to touch, ptosis, yellow perinasal substance, cold to touch, slight and moderate excess salivation and hyperpnea on DG 27. The doe lost weight on DS 15 and DG 13-26 (680 g). Feed consumption values were reduced on DG 13 to 15 and after DG 17. Necropsy revealed numerous ulcerations in the stomach; all other tissues appeared normal. The litter consisted of one late resorption and 8 fetuses *in utero*. One fetus had a domed head and medially rotated left hindlimb, all other fetuses in this litter appeared normal. At skeletal evaluation, seven fetuses had large fontanels and all had unossified pubic bones, these findings were not considered abnormal considering the stage of gestation.

In group II, doe 2253 was found dead on DG 28. Adverse clinical signs included ungroomed coat (DG 5, 13, 14, 21 and 27) abnormal feces (DG 21-24, 26-27), erythema grade 1 on DG 9. The doe lost weight on DG 0-28 (1130 g). Feed consumption values were reduced after DG 16. All tissues appeared normal at necropsy. The litter consisted of one aborted late resorption, two aborted fetuses and nine fetuses *in utero* (viability at time of maternal death could not be confirmed). One fetus had laterally rotated left hindlimb; all other fetuses in this litter appeared grossly normal. At skeletal evaluation, 4 fetuses had large fontanelles, one fetus had an asymmetric 2^{nd} sternal centra and the left 2^{nd} to 4^{th} sternal centra were fused, two fetuses had incompletely ossified first sternal centra, one fetus had misaligned right nasal of the skull, one fetus had asymmetric 1^{st} to 3^{rd} sternal centra, and 2 fetuses had a small hole in the frontal of the skull. Sponsor did not consider these findings abnormal considering the gestational age.

In group IV, doe 2291 was found dead on lactation day 2. Adverse clinical signs included ungroomed coat (DS 17-35, DG 0-8, DG 10, DG 13-28, and DL 1), erythema grade 1 on DG 29, discolored fur on DG 15-22, urine stained abdominal fur on DG 15-28 and DL 1, abnormal feces on DG 18, 19, 21-23, 25 and 26), sparse hair coat on DG 24-28 and DL 1 and red substance in cage pan on DG 28. This does lost weight on DG 16 to 26 (500 g). The feed consumption values were reduced after DG 18. All tissues appeared normal at necropsy. The litter consisted of 7 pups (3 stillborn pups, one pup found dead on day 1 postpartum and one male and 2 female pups were euthanized on day 2 postpatum), all appeared normal at necropsy.

In group IV, doe 2292 was found dead on DG 31. Adverse clinical signs observed included ungroomed coat (DG 8-16) erythema grade 1 on DG 9, abnormal feces on DG 23, 25-27, red perinasal substance and placentae in cage pen on DG 31. The doe lost weight on DS 15 and DG 16-29 (540 g). The feed consumption values were reduced after

DG 21. All tissues appeared normal at the time of necropsy. The litter consisted of 7 delivered pups and 2 dead fetuses *in utero*.

In group IV doe 6497 aborted and was euthanized on DG 24. Adverse clinical signs observed included ungroomed coat (DS 29-35, DG 0, DG 2-5, DG 7-19 and DG 22-24) abnormal feces on DS 22 and DG 19, red substance in cage pen on DG 24. The doe lost weight on DS 22 and DG 10-23 (420 g). The feed consumption values were reduced after DS 15-22 and after DG 12. All tissues appeared normal at the time of necropsy. The litter consisted of one late resorption, 5 early resorptions and 2 presumed cannibalized conceptuses.

Table II: Clinical observations and skin reactions -F0 Premating

PARAMETERS	F0 FEMEALS PRMAMTING (DS 1 -35)			
	C-sectioning		Natural delivery	
	I	II	III	IV
Mortality	0	2	2	2
Found dead	0	1	2	2
Aborted and found dead	0	1	0	0
Aborted and euthanized	1	0	0	1
Delivered and euthanized	1	0	0	0
Max. possible incidence	945/27	945/27	945/27	945/27
Appearance:				
Erythema grade I	10/6	5/3	4/3	4/2
Ungroomed coat	64/11	56/7	143/14	91/14
Abnormal Stool	4/1	3/1	2/1	4/1
Scant feces	0/0	0/0	8/1	2/2

Max. possible incidence: (days x rabbits)/no. rabbits exam/group

Table III: Clinical observations and skin reactions -F0 Presumed gestation

PARAMETERS	F0 FEMEALS PRESUMED GESTAION (ANIMALS WITH CONFIRMED MATING DATE)			
	C-sectioning		Natural delivery	
	I	II	III	IV
Mortality	0	2	2	2
Found dead	0	1	2	2
Aborted and found dead	0	1	0	0
Aborted and euthanized	1	0	0	1
Delivered and euthanized	1	0	0	0
Max. possible incidence	760/26	777/26	828/26	726/23
Appearance:				
Erythema grade I	11/6	3/2	7/6	10/9
Ungroomed coat	121/14	277/19	295/21	249/16
Abnormal Stool	3/3	35/14	10/5	17/9
Scant feces	13/4	40/12	26/8	20/10
No feces in cage pan	0/0	10/6	1/1	2/1
Red substance or placentae in cage	3/2	0/0	1/1	3/3
Urine-stained abdominal fur	6/2	0/0	3/1	15/2

Table IV: Clinical observations and skin reactions -F0 Natural delivery group

PARAMETERS	F0 FEMEALS ASSIGNED TO NATURAL DELIVERY	
	Natural delivery	
	III	IV
Mortality		
Found dead	2	2
Aborted and euthanized	0	1
Euthanized due to no surviving pups	5	9
Max. possible incidence	484/21	296/19
Appearance:		
Ungroomed coat	117/10	96/12
Sparse haircoat:	102/8	124/8
Abnormal Stool	3/1	0/0
Scant feces	0/80	2/1
No feces in cage pan	0/0	1/1
Red substance or placentae in cage	7/5	0/0
Urine-stained abdominal fur	0/0	1/1

Body weight: (summarized in Tables 2-7 and 30-32 of b(4)00040)
Observations during premating: Body weights and body weight gains during the premating period were not affected by administration of the test article (Table 2 and 3 of report b(4)00040).
Observations during gestation: There were reductions in body weight gain on DG 13-16 and DG 23-26 in group II (vaccine group), and on DG 20 to 23 in group IV (vaccine treated group). Sponsor states that these reductions in body weight gain were transient and not test article related as the overall body weight gains did not significantly differ between the control and vaccine treated groups.
Observations during lactation: There were reductions in body weight gain on DL 11- 15 in group IV (vaccine treated group).

Table V: Maternal Body weights[1] Gestation-Summary

EXAMINED F_0 PARENTS	GROUP	I	II	III	IV
RABBITS TESTES		27	27	27	27
PREGNANT		24	25	24	21
INCLUDED IN ANALYSIS		23*	25	24	20*
Body Weight GD 29		4.07±0.32	3.98 ±0.5	4.16±0.47	4.09 ±0.33
Body Weight Changes GD 29		0.25±0.18	0.08±0.34	0.22±0.24	0.15±0.24
Feed Intake absolute GD 0-29 (g/day)		117.5±21.6	116.1±34.8	125.0±25.8	114.8±20.0

[1]: Recorded in grams, rounded to 3 significant digits and reported in Kilograms
*: excludes animals without confirmed mating data

Food consumption: summarized in Tables 8-13 and 33-35 of b(4) 000-40)
Sponsor states that absolute (g/day) and relative (g/kg/day) feed consumption values during premating, gestation and lactation periods were comparable among treatment and control groups and did not significantly differ. There was a reduction in feed consumption during the latter stage of gestation between DG 26 and 29 of does in all

groups. Sponsor states that during early lactation, feed consumption of the does in groups III and IV returned to premating levels.

Comment: Food consumption and body weight measurements are difficult to interpret, in particular in the lactation phase of the study due to high pup mortality that resulted in euthanizing the does, number of does dead and number of animals not achieving pregnancy. These data are summarized in tables VI and VII below. For example, in animals assigned to the lactation phase, of the 27 animals assigned to control group III, only 16 animals contributed to the analysis of body weights and feed consumption. Of the total of 27 animals assigned to group IV, only 9 animals contributed to the analysis of body weights and feed consumption (Table VII).

Table VI: Gestation phase-F0 generation-mortality

Treatment group (no animals/group)	Animals not pregnant	Mating not confirmed	Animals found dead	Delivered and euthanized/or found dead	Animals aborted + euthanized/or found dead
Group I (27)	**3**	**1**		**1**(#2212)	**1**(#2227)
Group II (27)	**2**		**2**(#2249) GD27** (#2253) GD28		

**: Day event was discovered

Table VII: Lactation phase –F0 generation - mortality

Treatment group (no animals/group)	Animals not pregnant	Did not deliver a litter	Animals found dead	Delivered and euthanized/or found dead	Animals aborted + euthanized/or found dead	Animals euthanized during lactation period due to no surviving pups
Group III (27)	**3**	**1**	**2** (#2264 GD32 (#2280) GD29			**5**
Group IV(27)	**6**		**2** (#2291) LD2 (#2292) GD31		**1**(#6497)	**9**

Mating and fertility: (summarized in table 14 and 36 of b(4)00040)

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.): Mating and fertility indices appeared to be unaffected by treatment with the vaccine. Of 27 rabbits evaluated in each group, 27 (100%), 26 (96.3%), 26 (96.3%) and 24 (88.9%)

does mated, respectively. Pregnancy was reduced in group IV (21 does) compared to 24, 25, and 24 pregnancies in groups I, II, and III, respectively.

Table VIII: Reproductive parameters examined (in F_0 animals):

GENERATION		F_0			
DOSE (0.5 ml : DS 1, 15 , 29 ,GD 7 and 20)		**I CONTROL**	**II VACCINE**	**III CONTROL**	**IV VACCINE**
PREGNANCY					
Rabbits evaluated	N	27	27	27	27
Number of Females Paired	N %	27 100	26 96.3	26 96.3	24 88.9
Number of Females Achieving Pregnancy	N	24	25	24	21
Delivered a litter Included in analysis	N	N/A	N/A	21 21	20 19
Implantation sites Per delivered litter	N MEAN±SD	174 7.9± 2.8	160 7.0 ±2.8	194 9.2 ± 2.2	143 8.4 ± 3.0
Female Fertility Index	%	88.9	96.2	92.3	87.5
Gestation Index	%	100%	100%	91.3	85.0
Gestation Length	%	n/a	n/a	32.4±0.6	32.3±1.0
Live-born Index	%	100%	100%		
Number (Total and Per Liter) of Stillbirths at Day 0	N	0	0		

Necropsy (summarized in Tables 15 [16] and 37 of b(4)00040)
Necropsy observations included pale heart, lungs and kidneys (1 animal in group III), pale liver lobes (1 animal in group III and 2 group IV rabbits), ulcerations in stomach (1 animal in each of groups II and III) and mucoid white material or tissue present in stomach (1 rabbit in each of groups III and IV) and were observed across treatment groups.

Caesarean data (summarized in Tables 17-18, and 38-40 in b(4)00040)

Table IX: Caesarean-sectioning observations F0 generation

GENERATION		F_0	
DAILY DOSE ((0.5 ml : DS 1, 15 , 29 ,GD 7 and 20)		GROUP I CONTROL	GROUP II VACCINE
PREGNANCY			
Rabbits tested	N	27	27
Number of Females Paired	N	27	27
MATERNAL MORTALITY			
Number of Females Pregnant	N	24	25
Found dead	N	0	1
Aborted + found dead	N	0	1
Aborted + euthanized	N	1	0
Delivered + euthanized	N	1	0
Rabbits pregnant + C-sectioned	N	22	23
GESTATION			
Gestation Length	MEAN S.D.	29*	29
Gestation Index	%	-	-
CORPORA LUTEA			
Total # Corpora Lutea	N	196	180
Corpora Lutea/Dam	MEAN S.D.	8.9 2.7	7.8 2.9
IMPLANTATIONS			
Total # Implantations	N	174	160
Implantations/Dam	MEAN S.D.	7.9 2.8	7.0 2.8
RESORPTIONS			
Total # Resorptions	N	10	8
Resorptions/Dam	MEAN S.D.	0.4 0.7	0.3 0.6
Total # Early Resorptions		5	4
Early Resorptions/Dam	MEAN S.D.	0.2 0.5	0.2 0.4
Total # Late Resorptions	N	5	4
Late Resorptions/Dam	MEAN S.D.	0.2 0.5	0.2 0.4
% Resorbed Conceptuses/litter	MEAN S.D.	6.1 11.4	4.1 7.9
BIRTHS – DAY 0			
Number Stillborns – Total Per Liter	N MEAN S.D.	0	0
Number Live-born – Total Per Liter	N MEAN S.D.	164 7.4 2.7	152 6.6 2.7
% Live Male Fetuses Litter	MEAN S.D.	55.0 25.3	50.6 23.1
LIVE FETAL BODY WEIGTS/LITTER (G)	MEAN S.D.	41.65 6.97	40.00 6.98

* Caesarean group, thus all animals sacrificed on DG 29

Comment: Litter averages for corpora lutea, implantations, litter sizes, live fetuses, resorptions, percent live male fetuses, fetal body weights and % resorbed conceptuses were comparable between the saline (group I) and vaccine treated (Group III) groups. There were no stillborn fetuses.

Results F1 generation

Caesarean-sectioning group-fetal necropsy (summarized in Table 19- 23 and Tables 41 of b(4)000-40)

Fetal alterations: defined as a) malformations (irreversible changes occurring at low incidences in the species and strain, not compatible with survival or normal life) and b) variations (common findings in the species and strain and reversible delays or accelerations in development). Evaluations were based on 164 and 152 live DG 29 Caesarean-delivered fetuses in 22 and 23 litters in groups I and II, respectively.

Table X: Fetal Alterations- F1 generation-Summary

GENERATION		**Rabbits assigned to C-sectioning**	
DOSAGE Group (ug/0.5 ml)		**I 0**	**II** 45
LITTER EVALUATED	N	22	23
FETUSES EVALUATED	N	164	152
LIVE	N	164	152
Litters with fetuses with any alteration observed	N (%)	14 (63.3)	11 (47.8)
Fetuses with any alteration observed	N (%)	27 (16.5)	18 (11.8)
% fetuses with any alteration/litter	MEAN S.D.	15.6 15.0	15.2 26.0
GROSS EXTERNAL ALTERATIONS			
Body: umbilical hernia			
Litter incidence	N(%)	0	1 (4.3)
Fetal incidence	N(%)	0	1 (0.6)*
SOFT TISSUE ALTERATIONS			
Malformation:			
Protuding small intestine	N(%) N(%)	0 0	1 (4.3) 1 (0.6)
FETAL SKELETAL ALTERATIONS			
Malformation:			
Vertebrae/ribs			
Fused ribs (right 8th and 9th)	FETAL INCIDENCE N(%)	0	1 (0.6)**
Fused arches (7th and 8th thoracic vertebrae (right) and fused thoracic vertebral centra (7th to 8th)		0	

* also occurring in historical control, albeit with lower incidence (litter incidence 0.7%, fetal incidence 0.08%), (91 studies, 1151 live litters examined, 9721 live fetuses examined (Jan 2005-2007)

** also occurring in historical control, i.e., 2 or more fused ribs: litter incidence 1.02, fetal incidence 0.12, (91 studies, 1151 live litters examined, 9721 live fetuses examined (Jan 2005-2007)

Comment: There were no tissue or skeletal malformation that were attributable to vaccine treatments. Skeletal alterations, such as irregularities in skull ossification, irregularities in cervical, thoracic and caudal vertebra, incompletely ossified sternal centra, fused sternal centra occurred across treatment groups and not attributable to vaccine treatment. There were no statistically significant differences across groups in the

average numbers of ossifications sites per fetus for the hoid, vertebrae, ribs, sternum, forelimbs or hindlimbs.

Natural delivery observations (summarized in Tables 24- 26, Tables 42-47 of -b(4)000-40)

Group III and IV delivered 21 and 20 litters, respectively. Sponsor states that values for the number of does delivering litters, duration of gestation, averages implantations sites per delivered litter and live litter size, gestation index, number of does with stillborn pups and does with no liveborn pups, pup sex ratios, litter size and pup body weights were comparable among the 2 groups.

Table XI: Natural delivery observations (F0 Generation)

GENERATION		**F0 GENERATION**	
DOSE (0.5 ml : DS 1, 15 , 29 ,GD 7 and 20)		**III** **SALINE**	**IV** **VACCINE**
Rabbits assigned to natural delivery		**27**	**27**
PREGNANT	N (%)	24 (88.9)	21 (77.8)
Delivered a litter included in analysis	N (%) N (%)	21 (87.5) 21	20 (95.2) 19*
Duration of gestation	N	32.4 ± 0.6	32.3. ± 1.0
Implantation sites Per delivered litter	N MEAN ± SD	194 9.2 ± 2.2	143 8.4 ± 3.0
Does with stillborn pups	N (%)	5 (23.8)	8 (42.1)
Does with no liveborn pups	%	0	2 (10.5)
Gestation Index	%	91.3	85.0**
Pups delivered total	N MEAN ± SD	183 8.7 ± 2.1	136 8.0 ± 2.9
Liveborn	N MEAN ± SD	176 8.4 ± 2.1	124 0.7± 1.1
Stillborn	N MEAN ± SD	7 0.3 ± 0.7	12 7.3 ± 2.9
Does with all pups dying days 1-4 postpartum	%	4 (19.0)	5 (29.4)
Does with all pups dying days 5- 29 postpartum	N	1 (4.8)	3 (17.6)
Viability index***	%	59.6 106/176	60.5 75/124

*excludes does 2292, delivered and found dead on GD 31
** number of does with live offspring/number of pregnant does
*** number of live pups on day 5 postpartum/number of liveborn pups on day 1 postpartum

F_1 physical development:

Table XII: F1 – Generation-Physical development

GENERATION		F_1 LITTER	
		GROUP III CONTROL	GROUP IV VACCINE
LITTER SIZE			
Number Born – Total	N	183	136
Per Litter	MEAN	8.7	8.0
	S.D.	2.1	2.9
Total number found dead, stillborn or euthanized due to clinical sign	N	78	69
SURVIVING PUPS			
Day 0 – Total	N	176	124
Per Litter	MEAN	8.4	7.3
	S.D.	2.1	2.9
Day 5 – Total			
Per Litter	MEAN	4.7	4.1
	S.D.	3.2	3.8
Day 8 – Total	N		
Per Litter	MEAN	4.2	3.4
	S.D.	3.0	3.8
Day 15 – Total	N		
Per Litter	MEAN	4.0	3.2
	S.D.	2.7	3.5
Day 22 – Total	N		
Per Litter	MEAN	4.0	3.2
	S.D.	2.7	3.5
Day 29 – Total			
Per Litter	MEAN	4.0	3.2
	S.D.	2.7	3.5
LITTER WEIGHT (G)			
Day 0	MEAN	Not recorded	Not recorded
	S.D.		
Day 5	MEAN	82.8 ± 15.7	84.0 ± 11.5
	S.D.		
Day 8	MEAN	121.8 ± 25.6	122.3 ± 19.1
	S.D.		
Day 15	MEAN	228.2 ± 51.8	228.6± 56.1
	S.D.		
Day 21	MEAN	334.2 ± 77.5	329.5 ± 103.5
	S.D.		
Day 29	MEAN	550.8 ± 93.9	542.5 ± 107.0
	S.D.		

Comment: The sponsor states that in groups III and IV of study b(4)000-40, 78 and 69 pups were found dead, were still born or were euthanized, respectively. The numbers of dead pups in group IV need to be clarified, i.e., Table 25 shows that 136 total pups were delivered, 12 were stillborn and an additional 68 pups died between LD 1 and 15. Thus,

the total number of dead pups should be 80, rather than 69. Moreover, Table 28 states that the number of pups found dead in the vaccine group IV was 57.

F_1 evaluation/Developmental endpoints: (summarized in Table 27 and Table 47-51 of b(4)000-40)
There were no significant differences between groups III and IV in the average postpartum day that at least 50% of the pups in a litter met the criteria for hair growth, eye opening, air righting, auditory startle and pupil constriction in the surviving F1 generation pups. However, only 16 and 9 litters from group III and IV were included in the analysis, respectively. This number of litters does not allow a meaningful analysis of the data.

F_1- generation Necropsy observations: (summarized in Table 28 and Table 52 of b(4)000-40).
A total of 78 pups in group III and 69 pups in group IV were found dead, stillborn, or were euthanized due to adverse clinical signs. Of these, 2 control and 4 vaccine group pups had no milk present in the stomach. In the control group, 1 pup had slight ventricular dilation in the brain and 1 pup had red areas in the bladder. One control group pup and 2 vaccine group pups had tan areas on the lateral or median liver lobe. Pups that survived to scheduled sacrifice appeared normal at necropsy observation.

Serology results (Study No. 769-N106856, -b(4)-, page 342-354 of b(4)000-40)
Serum samples were obtained from study groups I, II, III and IV prior to test article administration, on premating study days 15 and 29, gestation days 7, 20 and 29 and lactation day 29 (8 does each). On gestation day 29, blood from fetuses from 8 does assigned to Caesarean sectioning was pooled for HAI antibody analysis. A total of 52 pups were used to evaluate pup serology on LD 29. For the influenza strain analyzed (H1N1 strain IVR-116 (A/New Caledonia/20/99-like strain) HAI antibody titers were not detected in control groups I and III on DG 29 or DL 29. Similar levels of antibodies were observed in Group II rabbits on DS 29 and DG7, and in group II and IV rabbits on DG 20. A decrease titer was measured in samples from Group II does collected on DG 29 and from group IV does on DL 29. On GD 29, fetal sera titers were similar to maternal titers. Pup sera sampled on DL 29 showed low HAI titers approaching baseline. In summary, these results suggest that Agrippal induced HAI antibody titers that were transferred to offspring, at least against influenza H1N1.

Serology report (Appendix 5, page 355 - 372 of b(4)000-40)
In order to further investigate potential causes of doe and pup mortality observed in b(4)00040 serum sample from a limited number of does and pups were evaluated to detect antibodies for infectious agents. Results were negative for parainfluenza type 1, and 2, reovirus type 3, lymphocytic choriomeningitis, *Encephalitozoon cuniculi*, Cilia-associated respiratory bacillis and *Clostridium piliforme.* Positive results for epizootic diarrhea of infant mice (EDIM) were obtained using the --b(4)---------- test with test samples from 2 of 3 sampled does (6493 and 6498) and from both of the fetuses from each of these 2 does. The test was repeated using diluted samples. The repeat test yielded positive results for the 2 dams and negative results for the 4 pups. Sponsor states

that Rotavirus is present in the Supplier's colony of rabbits and considered endemic in most b(4) commercial rabbitries. Sponsor states that rapid spread of rotavirus and high mortality would only be expected if it were introduced into a colony not previously exposed. In summary, the sponsor cannot provide an explanation for the observed high pup mortality in does assigned to the delivery subgroup and/or does found dead during the course of the study.

Reproductive and developmental toxicology

Study title:
Intramuscular reproductive and developmental toxicity study of Agrippal vaccine in rabbits, including a postnatal evaluation (b(4)000-43)

Purpose of Study:
To further test for toxic effects resulting from Agrippal vaccine treatment in the test species before cohabitation, through mating, gestation and lactation. This study is a repeat study of the natural delivery group previously evaluated in b(4)000-40 in which the limited number of litters available for evaluation was deemed insufficient to allow a meaningful interpretation of data. Sponsor states after discussion of study findings from b(4)000-40 with animal supplier, it was determined that the most likely cause of the low number of surviving litters in b(4)000-40 (16 control and 9 Agrippal treated) was handling of the does and litters for the purpose of weighing and counting in the immediate postpartum period (DL1 – DL4). Thus, a number of changes were made to current study design a) body weight measurements for does beginning DL5, b) no in-cage examination of does or pups on DL 1 – 3, c) no viability checks within the nesting box until DL 4, d) no counting of pups in a litter until DL 5.

Key study findings:
Animals were dosed on study days (DS) -1, -15 and -29 prior to mating and on gestation days 7 and 20 either with Agrippal vaccine, 0.5 ml, I.M., 45 ug antigen or saline control. Animals were allowed to rear their offspring. Three does died in the vaccine treated group. These data are within the range reported in the historical data. Beginning on DG7 – DG 26, mean absolute and relative feed consumptions values in the vaccine treated group II were significantly reduced compared to the control group values. Sponsor attributes this to 3 does (# 3831, 3837, 3843) in vaccine group II with severely reduced feed consumption. Body weight and food consumption data are concerning because losses in body weight and feed consumption occurred primarily in animals assigned to the vaccine treated group. There appear to be differences in reproductive parameters, the number of rabbits achieving pregnancy in the vaccine treated group was slightly decreased compared to the control group, 21 and 25 does, respectively, there were differences in the fertility index (92.6% in the control group and 80.0% in the vaccine group) and gestation index (100% in the control group and 85.7% in the vaccine group). Note that the gestation index in the vaccine group (85.7%) is below the gestation index observed in historical controls (97.8% (88.9-100). The pregnancy rate (number of rats pregnant/number of rats mated) was 92.6% and 77.8% in the control groups and vaccine group, respectively.
In addition, the number of liveborn pups in the vaccine treated group (82.0%) was reduced compared to the number of liveborn pups in the control group (92.0). The number of pups that died in the control group (41.1% during postpartum days 2-5) was increased compared to the vaccine group (26.7%) resulting in a viability index (defined as number of live pups on day 5 postpartum/number of liveborn pups on day 1 postpartum) of 70.0% in the vaccine group versus 58.0% in the control group . Historical

control data from 4 studies show that the % pups found dead or presumed cannibalized between days lactation 2-5 is 23% (6.0-42.8). Thus, the % of pups found dead in the current study between lactation days 2-5 (26.7% and 41%) is above the mean observed in the historical control data base. Furthermore, when calculating the viability indices based on the numbers of pups dead/euthanized between DL 1-7, the results are 52.5% (control) and 66.4% (vaccine). These values are markedly lower than the viability index observed for the same interval in the control data base (87.3% (81.6-96.7), data from 3 studies). The total number of pups stillborn, found dead or euthanized due to adverse clinical observations was 93 in the control group (100 according to CBER's calculations) and 46 in the vaccine group (55 according to CBER's calculations). While 16 litters in the vaccine group were evaluated for eye opening, parameters such as air righting, auditory startle, pupil construction was evaluated in only 15 litters in the vaccine group due to litters with no surviving pups. This number of litters is slightly below the recommended litter number to be evaluated resulting in consistency of results (i.e., the ICH S5A document recommends that 16 - 20 litters for rabbits provide a degree of consistency between studies).

Study no.: b(4)000-43

BLA section: 4.2.3.5.2 **page #**: 1 - 314

Conducting laboratory and location: ----b(4)----------
----b(4)----------
----b(4)----------
---b(4)----------
-b(4)-

Study Director:
----b(4)----------

Date of study initiation: November 8, 2007
Date of experimental study completion: April 9, 2008

GLP compliance:
US FDA Good Laboratory Practice Regulations (CFR Part 58)
Japanese Ministry of Health and Welfare, Good Laboratory Practice Standard for Safety Studies on Drugs, MHW Ordinance No. 21, March 26, 1997
Revised OECD Principles of Good Laboratory Practice [C(97)186/Final]

QA reports: yes (X) no ( )

Test article, lot #:
Agrippal vaccine, clear, colorless liquid, refrigerated
Lot number --b(4)---
Information to document the identity, composition, strength, activity/purity and stability of the test article was provided by sponsor to testing facility, COA provided

Agrippal is comprised of hemagglutinin (HA) and neuraminidase antigens from each of three influenza strains (A/H1N1, A/H3N2 and B). Each 0.5 ml dose of Agrippal contained 15 ug HA of each strain (total 45 ug HA protein) in a non-adjuvanted, --b(4)----------. Prefilled syringes of the test article were used as received at the testing facility and stored refrigerated (2^0C to 8 ^{0}C).

Control article, lot#, and purity:
b(4) sodium chloride injection, USP, clear, colorless, liquid, room temperature
Lot number --b(4)---

Animal diet

Feed

Type/name: Certified rabbit Chow #5322 ----b(4)----------
Availability: 150 g per rabbit per day until GD 6, after that 180 g/rabbit/day, beginning DL 1, 230g/rabbit/day, beginning LD21, 500 g/rabbit/day
Analysis for contaminants: routinely performed by feed supplier, copies of results submitted to raw data, no contaminants detected
Comments: none

Water

Source: Local water passed through ----b(4)----------
Availability: *ad libitum* from individual water bottles
Analysis for contaminants: ---b(4)------ for chemical contamination and --b(4)--- for bacterial contamination, copies of results submitted to raw data
Comments: none

Husbandry

Environmental conditions:

Temperature: RT --b(4)----
Humidity: --b(4)----
Air changes: positive airflow with minimum of b(4) changes per hour of 100% fresh air passed through 99.97% HEPA filters
Photoperiod: 12 hour light: 12 hours dark fluorescent light cycle
Housing: Females were individually housed in stainless steel, wire bottom cages, except during mating period, rabbits assigned to natural delivery were supplied with a nesting box and nesting materials no later than DG 27

METHODS
Species/strain/supplier: ----b(4)---------- rabbits, -b(4)----------
Rationale for test system: rabbits widely used for developmental toxicity studies, historical data available at testing facility, test article pharmacologically active in species and strain

Number/sex/group: 27/female rabbits/treatment group (male rabbits used for the purpose of breeding, not considered part of the test system)
Number of rabbits acclaimed: 56
Number of rabbits assigned to study: 54
Age: 6.5 months at arrival
Weight: 2.9 – 3.9 kg at study assignment
Doses/rationale: 3 doses prior to gestation to ensure induction of high antibody titers (days -1, -15, and -29) plus 2 doses administered during gestation to expose fetuses to vaccine antigen and to maintain antibody titers (GD 7 and 20). Dose (15 ug/strain antigen) was selected as the highest anticipated dosage that could be used in the clinic
Route, volume: IM, 0.5 ml for each injection, (consecutive injections were alternated between hind legs starting with the right leg) proposed clinical routes and volume

Mating procedures
Description: After 35 days on study (first day of dosage administration considered DS1) virgin female rabbits were mated with male rabbits, one male rabbit per female rabbit, 20 USP units/kg of HCG was administered to female rabbits prior to mating, animals were observed continuously until mating confirmed to have occurred by observation, day of mating was designated DG 0. If females failed to mate they were placed with a second and then 3rd male, if mating was not confirmed following the third pairing, the female was recorded as no confirmed date of mating but considered to be at DG0. These rabbits continued on the assigned dosage regimen and were euthanized on day 34 when they did not naturally deliver a litter.

Culling procedures
Description: Litters were not culled during the lactation period to prevent selection bias in body weights and pup viability

Study design:
Randomization: Rabbits were assigned to dosage groups based on computer-generated (weight ordered) randomization procedure.

Table I: Study design: Allocation of animals and treatment schedule

TREATMENT	GROUP* (NUMBERS/ GROUP)	DOSE TO ANIMALS (total mcg HA protein)	DAYS TREATMENT ADMINISTERED	DOSAGE VOLUME (ML)	RABBIT NUMBER
		F_1 FEMALES			F_1 FEMALES
SALINE	I (27)	-	-1, -15, -29 GD 7 and 20	0.5	3801-3827
AGRIPPAL	II (27)	45 ug	-1, -15, -29 GD 7 and 20	0.5	3828-3854

Parameters and endpoints evaluated:
Clinical observation: Animals were inspected for viability 2x daily, injection sites were examined (onset, intensity and duration of signs recorded); rabbits were observed for clinical observations, abortions, premature deliveries and deaths before and approximately 60 minutes after dosage administration, once daily on non-dosage days and daily during postdosage period, except during parturition and on days 1, 2 and 3 postpartum. Does were observed for maternal behavior on DL 4, 5, 8, 15, 22 and 29.

Body weight: weekly during the acclimation period, weekly during the dosage period (including DG 1, 15, and 29 and DG 0, 7, 10, 13, 16 and 20), on DG 23, 26, 29 and 34 (when necessary). Body weights were recorded on days 5, 8, 11, 15, 18, 22, 25 and 29 postpartum.

Food consumption: daily

Littering group observations:

F0 generation: Day of parturition was considered day 0 of lactation; does were evaluated for adverse clinical signs during parturition, duration of gestation was evaluated, litter size (all pups delivered) and pup viability at birth.

F1 generation pups
On DL 1-3 postpartum, litters were not disturbed. Dead pups found outside of the nesting box on DL 1-3 were processed as described below. Litters evaluated for viability at least 1x daily beginning on DL 4 and continuing through DL 14. Beginning DL 5, pups in each litter were counted, pups observed for clinical observations and appearance once daily, pup weights were recorded on DL 5, 8, 15, 22 and 29.

Hair growth (from DL 5), eye opening (from DL 9), air righting reflex (from DL 10), acoustic startle (from DL 14) and pupil constriction was evaluated once (DL 22). The number of pups meeting the criterion was recorded on each day of testing, testing continued daily until the day the criterion was attained by all pups in the litter

Gross necropsy of F0 and F1 generation:
Gross lesions were retained in neutral buffered b(4) formalin, skin and muscle from the injection sites were retained in b(4) formalin, representative photographs of maternal gross lesions and fetal gross alterations are on file. After completion of the 29 day lactation period, all surviving female rabbits were sacrificed and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. The number and distribution of implantation site was recorded. Rabbits that did not deliver were sacrificed on DG 24 and examined for gross lesions. Uteri were evaluated to confirm absence of implantation sites.

F1 generation pups were sacrificed by IV administration of sodium pentobarbital on DL 29, blood samples were collected for HAI analysis and a gross necropsy was performed. Pups were examined internally to determine sex.

F0 generation: Rabbits that died before scheduled termination were examined for cause of death, examined for gross lesions, hearts, lungs, liver, kidneys, stomach and spleen retained in b(4) formalin for possible histological evaluation, pregnancy status and uterine contents were recorded, aborted fetuses were examined to the extent possible. The body weight of each fetus was recorded, fetuses were examined for gross external alterations and internally to examine sex, all fetuses were examined for skeletal

alterations. Does with no surviving pups were euthanized after the last pup was found dead, a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Postpartum data from these animals were excluded from summary tables.

F1 pups: Pups that died before examination of the litter for pup viability on DL4 were evaluated for vital status at birth by removing the lungs and immersion of the lungs in water; pups with lungs that sank were designated stillborns, pups with lungs that float were designated life born. Pups that died between DL 5 and DL 29 were examined for cause of death, hearts, lungs, liver, kidneys, stomach and spleen retained in b(4) formalin for possible histological evaluation, gross lesions retained in b(4) formalin.

Laboratory investigations

Blood samples for hematology and clinical chemistry were collected from adult females pre-dose administration and on DS 15 and 29 as well as DG 7, 20 and 29. Samples of 1 to 2 ml each were collected from the medial auricular artery (in-life) and inferior vena cava (terminal blood collection).

Statistical methods

Clinical observations and other proportion data were analyzed using the variance test for homogeneity of the binominal distribution. Data from F0 generation rabbits were evaluated with the individual rabbit as a unit measured. Litter values were used in evaluation of pup data.

Results F0 Generation

Mortality/Abortion (summarized in Table 1 and 16 of b(4)000-43):

Two does in the vaccine group were found dead, one doe aborted and was euthanized. Sponsors states that these events were not considered vaccine related since they were within historical control ranges.

Doe 3831 was found dead on GD 29. Adverse clinical signs consisted of ungroomed coat (DG 16, 20, 23 - 28), abnormal feces on DG 14, 17, 20, 22, 24 and 26 and red substance on cage wall on DG 22. The does lost weight between DG 10-26 (780 g). Feed consumption values were reduced after DG 13. All tissues appeared normal at necropsy. The litter consisted of 9 pups and 2 late resorptions. At gross external and soft tissue examination, the pups appeared normal. At skeletal examination, the pubes of 2 pups were not ossified. No skeletal alterations were observed in the remaining pups.

Doe 3837 was found dead on DG 30 while in the process of delivering a litter. Adverse clinical signs were ungroomed coat (DG 29), abnormal feces (DG 23 to 24 and 28, no feces on DG 29), weight loss occurred from DG 23 to 29 (480g). Feed consumption values were reduced after DG 23. Necropsy revealed red and dark lungs, all other tissues appeared normal. The litter consisted of 11 pups and one late resorption. All pups in the litter appeared normal upon gross external, soft tissue and skeletal examinations.

Doe 3843 aborted and was euthanized on DG 28. Adverse clinical signs were ungroomed coat (DS 30 – 35, and DG 0-6), abnormal feces (DG 23 and 26), weight loss occurred from DG 10 to 26 (420g). Feed consumption values were reduced on DG 19 - 27. Necropsy revealed red and dark lungs, all other tissues appeared normal. The litter consisted of 8 aborted fetuses. All pups in the litter appeared normal upon gross external, soft tissue and skeletal examinations, except partial cannibalized pups, one of which only had 9 caudal vertebrae at examination.

Table II: Clinical observations and skin reactions -F0 Premating

PARAMETERS	F0 FEMEALS PRMAMTING (DS 1 -35)	
	I	II
Mortality Found dead Aborted and euthanized	0 0 0	3 2 1
Erythema grade I	0/0	1/1
Max. possible incidence	945/27	945/27
Clinical observations Ungroomed coat	9/2	28/3
Abnormal Stool Scant feces	1/1	4/2

Max. possible incidence: (days x rabbits)/no. rabbits exam/group

Table III: Clinical observations and skin reactions -F0 gestation

PARAMETERS	F0 FEMEALS PRESUMED GESTATION (ANIMALS WITH CONFIRMED MATING DATE)F0	
	I	II
Mortality Found dead Aborted and euthanized	0 0 0	3 2 1
Max. possible incidence	878/27	846/26
Clinical observations Ungroomed coat	22/3	67/10
Abnormal Stool (soft/liquid) Scant feces	6/4 16/10	10/7 29/14

Table IV: Clinical observations and skin reactions -F0 Lactation

PARAMETERS	F0 FEMEALS	
	I	II
Mortality Found dead Aborted and euthanized	0 0 0	3 2 1
Max. possible incidence	626/25	453/18
Clinical observations Ungroomed coat	88/10	53/7
Abnormal Stool (soft/liquid) Scant feces	1/1 4/3	3/2 12/3

Body weight: (summarized in Tables 2-7 and 23-25 of b(4)000-43)

Premating body weight gains appeared to be comparable between group I and II (Figure 1 and Table 2 of b(4)-000-43). Transient, but significant reductions in body weight gain and body weight loss were observed on DG 7 - 10 and DG 23 - 26 and mean body weights were reduced in the vaccine treated group II compared to the control group I. Sponsor states that this is dues to severely reduced food consumption of 3 does (# 3831, 3837, 3843) in group 2.

Table V: Maternal Body weights[1-] Gestation-Summary

EXAMINED			
F_0 PARENTS	GROUP	I	II
RABBITS TESTED		27	27
PREGNANT		25	21
Body Weight	GD 0	3.87±0.26	3.86 ±0.20
Body Weight	GD 13	4.08±0.30	4.02 ±0.22
Body Weight	GD 23	4.22±0.35	4.08 ±0.32
Body Weight	GD 26	4.24±0.34	4.03 ±0.36*
Body Weight	GD 29	4.20±0.31	4.10 ±0.37
Body Weight Changes GD 29		0.33±0.16	0.26±0.28
Feed Intake absolute GD 0-29 (g/day)		138.7±19.9	124.0±23.2*

[1]: Recorded in grams, rounded to 3 significant digits and reported in Kilograms
*: significantly different from group I value

Food consumption: summarized in Tables 8-13 and 26 - 28 of b(4)000-43)
Sponsor states that absolute (g/day) and relative (g/kg/day) feed consumption values during premating, gestation and lactation periods were comparable among treatment and control group. Beginning on DG7 – DG 26, mean, and absolute and relative feed consumptions values in group II were reduced or significantly reduced compared to the control group values. Sponsor states that 3 does (# 3831, 3837, 3843) in group 2 had severely reduced feed consumption. There was a reduction in feed consumption during the latter stage of gestation between DG 26 and 29 of does in the control group. Sponsor states that during lactation, feed consumption of the does were comparable between groups.

Treatment group (no animals/group)	Animals not pregnant	Animals found dead	Animals aborted + euthanized	Animals euthanized during lactation period due to no surviving pups
Group I (27)	2	-	-	4
Group II (27)	6	2*	1**	3

*2 does # 3831 and 3837
** doe # 3843

Mating and fertility: (summarized in Table 14 and 29 of b(4)000-43)

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):
The number of group II does which mated during the first pairing with a male rabbit was significantly reduced compared with the number in group I, however, all females mated in subsequent pairings (27/27 in group I versus 26/27 matings in group II)
Pregnancy occurred in 25 and 21 group I and II does, respectively. The fertility index was 92.6% in group I and 80.8% in group II, the pregnancy rate (number pregnant/number mated) was 92.6% versus 77.8% respectively.

Table VI: Reproductive parameters examined (in F_0 animals):

DOSE (0.5 ml : DS 1, 15 , 29 ,GD 7 and 20)		**I CONTROL**	**II VACCINE**
PREGNANCY			
Rabbits evaluated	N	27	27
Number of Females Paired	N %	27 100	26 96.3
First pairing Second paring Third pairing	N (%) N(%)	22 (100) 0 (0) 0	22(84.6) 3.911.5) 1 (3.8)
Number of Females Achieving Pregnancy	N	25	21
Female Fertility Index	N/N %	25/27 92.6	21/27 80.8
Gestation Index (#rabbits with liveborn/#pregnant rabbits)	%	100.0	85.7
Pregnancy rate (number pregnant/number mated)	%	92.6	77.8

Comment: There appear to be slight, but not statistically significant differences in reproductive parameters, i.e. the fertility index, gestation index and pregnancy rate in group II is reduced compared to Group I. Note that the gestation index in group II (85.7%) is below the gestation index observed in historical controls (97.8% (88.9-100).

Necropsy (summarized in Tables 15 [16] and 30 of b(4)000-43)
Necropsy observations included red and dark lungs (2 group II rabbits), friable abdominal adipose and pale spleen (one group I rabbit) and tan area on median liver lobe (one group II rabbit). Overall, gross lesions occurred with low frequency in both groups.

Results F1 generation

Natural delivery observations (summarized in Tables 17- 18, and 32-35 of b(4)000-43)

Pregnancy occurred in 25/27 and 21/26 mated female rabbits in each of groups I and II, respectively. Twenty-five (25) does in group I and 18 does in group II delivered litters. Sponsor states that these rates are within the historical control data range of the facility.

Sponsor states that values for the number of does delivering litters, duration of gestation, averages implantations sites per delivered litter, gestation index, number of does with stillborn pups and does with no liveborn pups, pup sex ratios, the lactation index and pup body weights were comparable among the 2 groups. Sponsor states that the *total* number of liveborn pups delivered was significantly reduced in group II as compared to group I, however, sponsor states that there was no significant difference in the number of liveborn pups/litter.

Table VII: Natural delivery observations (F0 Generation)

GENERATION		F0 GENERATION	
DOSE (0.5 ml : DS 1, 15 , 29 ,GD 7 and 20)		**I** **SALINE**	**II** **VACCINE**
Rabbits assigned to natural delivery		**27**	**27**
PREGNANT	N (%)	25 (92.6)	21 (77.8)
Delivered a litter included in analysis	N (%) N (%)	25 (100.0) 25	18 (100.0)) 18*
Duration of gestation	N	32.3 ± 0.6	32.3. ± 0.9
Implantation sites Per delivered litter	N MEAN ± SD	197 8.6 ± 2.2	136 8.0 ± 1.9
Does with stillborn pups	N (%)	8 (32.0)	9 (50.0)
Does with no liveborn pups	%	0	0
Gestation Index**	%	100.0	85.7
Pups delivered (total)	N MEAN ± SD	199 8.0 ± 2.2	134 7.04± 2.4
Does with all pups dying days 1-4 postpartum	N %	3 (12.0)	2 (11.1)
Does with all pups dying days 5- 29 postpartum	N %	1 (4.0)	1 (5.6)
Viability index***	% N/N	57.9 106/183	70.0 77/110

*excludes does found dead
** number of does with live offspring/number of pregnant does
*** number of live pups on day 5 postpartum/number of liveborn pups on day 1 postpartum

Comment: Of note are the numbers of does with all pups dying during the postpartum period, i.e., 4 in the control group and 3 in the vaccine group. Does with all pups dying days 1-4 postpartum was 12.0% and 11.1% in groups I and II, respectively compared to 7.4 % (0-19%) in the historical control group. Does with all pups dying days 5 - 29 postpartum was 4.0% and 5.6 % in groups I and II, respectively compared to 0.6 % (0-5.6%) in the historical control group. Even though the values observed in the control and vaccine treated groups were within the range of what is observed in the historical control data, the mean % values are increased in this study compared to historical control data.

F_1 physical development:

Table VIII: Natural delivery observations (cont.) (F1 Generation)

GENERATION		F_1 LITTER	
		GROUP I CONTROL	GROUP II VACCINE
LITTER SIZE			
Number Born – Total	N	199	134
Per Litter	MEAN	8.2	7.4
	S.D.	2.2	2.4
Liveborn	N	183 **(92%)**	110 **(82%)**
	MEAN	7.3	6.1
	S.D.	2.1	2.3
Stillborn	N	13 (6.5 %)	15 **(11.2%)**
	MEAN	0.5	0.8
	S.D.	1.0	1.1
Unknown vital status	N	3	9
Pups dead/euthanized			
Days 0-1	N (%)	3/183 (1.6)	5/110) (4.5)
Days 2-5	N(%)	74/180 (41.1)	28/105 (26.7)**
Days 6-8	N(%)	10/106 (9.4)	4/77 (5.2)
Days 9-15	N(%)	0/96	3/73(4.1)
Days 16-22	N(%)	0/96	0/70
Days 23-29	N(%)	0/96	0/70
SURVIVING PUPS			
Day 5 – Total			
Per Litter	MEAN	4.2	4.3
	S.D.	2.5	2.6
Day 8 – Total	N		
Per Litter	MEAN	3.9	4.1
	S.D.	2.3	2.6
Day 15 – Total	N		
Per Litter	MEAN	3.8	3.9
	S.D.	2.2	2.6
Day 22 – Total	N		
Per Litter	MEAN	3.8	3.9
	S.D.	2.2	2.6
Day 29 – Total			
Per Litte	MEAN	3.8	3.9
	S.D.	2.2	2.6
LITTER WEIGHT (G)			
Day 0	MEAN	Not recorded	Not recorded
	S.D.		
Day 5	MEAN	99.1 ± 24.6	102.3 ± 38.9
	S.D.		
Day 8	MEAN	144.0 ± 34.8	147.4 ± 55.7
	S.D.		
Day 15	MEAN	261.4.2 ± 56.8	269.9± 77.4
	S.D.		
Day 21	MEAN	366.2 ± 69.3	373.0 ± 108.9
	S.D.		
Day 29	MEAN	608.5 ± 84.8	602.1 ± 130.4
	S.D.		

** statistically lower than group 1

Comment: There appeared to be differences in the natural delivery parameters, a) the number of rabbits achieving pregnancy in the vaccine treated group was slightly decreased compared to the control group (21 (vaccine group) and 25 (control group), b) the gestation index in vaccine group II was decreased compared to control group I and so was the pregnancy rate (92.6% in control group I versus 77.8 % in vaccine group II). In addition, the number of liveborn pups in the vaccine treated group IV (82.0%) is reduced compared to the number of liveborn pups in group I (92.0). The number of stillborn pups was slightly enhanced in vaccine group II (11.2%) compared to group I (6.5%), however, it appears to be within the range observed in the historical control database for this facility (mean: 13.7% (0-28.8). The number of pups that died in the control group I (41.1% during postpartum days 2-5 was increased compared to the vaccine group II (26.7%) resulting in a viability index (defined as number of live pups on day 5 postpartum/number of liveborn pups on day 1 postpartum) of 70.0% in vaccine group II versus 58.0% in control group I. Historical control data from 4 studies show that the % pups found dead or presumed cannibalized between days lactation 2-5 is 23% (6.0- 42.8). Thus, the % of pups found dead in the current study between lactation days 2-5 (26.7%) is still above the mean found in the historical control data base. Furthermore, when calculating the viability indices based on the numbers of pups dead/euthanized between DL 1-7, the results are 52.5% (group I) and 66.4% (group II). These values are markedly lower than the viability index observed for the same interval in the control data base (87.3% (81.1.6-96.7), data from 3 studies).

Pups dead/euthanized LD 0-29

		GROUP I CONTROL	GROUP II VACCINE
Pups dead/euthanized			
Days 0-1	N (%)	3/183 (1.6)	5/110) (4.5)
Days 2-5	N(%)	74/180 (41.1)	28/105 (26.7)
Days 6-8	N(%)	10/106 (9.4)	4/77 (5.2)
Days 9-15	N(%)	0/96	3/73(4.1)
Days 16-22	N(%)	0/96	0/70
Days 23-29	N(%)	0/96	0/70

Comment: The sponsor states that the total number of pups stillborn, found dead or euthanized due to adverse clinical sign was 93 and 46 in groups I and II in b(4)000-43, respectively. These numbers need to be clarified. In Table 18 it is stated that in group I, there were 13 stillborn pups and 87 pups dying between LD 0-15, thus, the total number of dead pups should be 100. Furthermore, Table 18 shows that in group II, there were 15 stillborn pups (in contrast, Table 21 states that there were 11 stillborn pups) and that 40 pups died between LD 0-15. Thus, the total number of dead pups in group II should be 55, not 46, not accounting for the 9 pups for which the viability status could not be confirmed (Note that the sponsor did clarify these numbers satisfactorily in its response to CBER's comment that were received Feb 2, 2009 to STN 125297/0.4.)

<u>Clinical observations – F1 generation pups (summarized in Table 19 and Table 36 of b(4)000-43)</u>

Clinical signs were transient and included events such as dehydration, limited use of left hindlimb, ungroomed coat, not nursing, soft/liquid feces, gasping, hyperprnea, labored breathing, decreased motor activity and occurred across study groups, albeit with low frequency. Observations associated with pup death were no milk in stomach, not nesting/nursing, cold to touch, prostrate, gasping, hyperpnea, labored breathing, decreased motor activity, apparent dehydration.

F_1 evaluation/Developmental endpoints: (summarized in Table 20 and Table 37- 41 of b(4)000-43)
Reflex and physical development in F_1 pups was assessed in 21 and 16 (15) litters of group I and II, respectively (because litters with no surviving pups were excluded from the analysis). There were no significant differences between groups I and II in the average postpartum day that at least 50% of the pups in a litter met the criteria for hair growth, eye opening, air righting, auditory startle and pupil constriction in the surviving F1 generation pups.

Comment: While 16 litters in group II were evaluated for eye opening, parameters such as air righting, auditory startle, pupil construction was evaluated in only 15 litters in group II due to litters with no surviving pups. This number of litters is slightly below the recommended litter number to be evaluated resulting in consistency of results (i.e., the ICH S5A document recommends that 16-to 20 litters for rabbits provide a degree of consistency between studies).

F_1- generation Necropsy observations: (summarized in Table 21 and Table 42 of b(4)000-43).
Twenty-five litters in group I and 18 litters in group II were evaluated. The total numbers of pups stillborn, found dead or euthanized due to adverse clinical observations was 93 in group I and 46 in group II. There was finding of significantly fewer pups in the vaccine group II (N = 23) with no milk in the stomach compared to the saline control group (N = 72). In the control group, 1 pup had lateral bilateral moderate ventricular dilation in the brain, four pups had red or dark red lung lobes and 1 pup had a black liver. Nine control pups and 4 vaccine group pups had tan areas on various liver lobes; of these pups, 1 control pup also had a yellow accessory liver lobe and 1 vaccine group pup had a pale heart. Pups that survived to scheduled euthanasia appeared normal at necropsy examination. Note that necropsy was restricted to pups in which complete necropsy were performed. In some pups, autolysis or cannibalization precluded full necropsy which explains the disparity in numbers between pups found dead or euthanized in Tables 21 and 18 of study report b(4)000-43.

Serology results (Study No. 827-N106859, --b(4)--, page 291- 303 of b(4)000-43)
Serum samples were obtained from study groups I and II prior to test article administration, on premating study days 15 and 29, gestation days 7, 20 and 29 and lactation day 29 (8 does each sampling day). A total of 41 pups were used to analyze pup serology on LD 29 and were offspring from animals assigned to group II. For the influenza strain analyzed (Influenza A (H1N1) strain A/Solomon Island/3/2006 (BMISOLOMON-E01) antibody titers were observed in group II but not in group I, they

peaked on GD 20 and where still detected on DL 29, albeit markedly decreased when compared to GD 20. Titers in pups on DL 29 confirmed maternal antibody transfer, antibody titers were markedly lower than maternal titers on LD 29.

Discussion and Conclusions

The sponsor performed 2 studies to evaluate the reproductive and developmental toxicity potential of Agrippal vaccine in rabbits, including a postnatal evaluation. In study b(4)-000-40 animals were dosed on study days (DS) -1, -15 and -29 prior to mating and on gestation days 7 and 20 either with Agrippal vaccine, 0.5 ml, I.M., 45 ug antigen or saline control. Animals were subdivided into subgroups of animals (27 rabbits/group), underwent Caesarean section on DG 29 or were allowed to rear their offspring. No adverse effects were observed on Casarean parameters evaluated and neither were there any vaccine related fetal malformations. These data allow the conclusion that Agrippal does not adversely effect embryo-fetal development. However, of concern are the number of does with no surviving pups in animals assigned to the natural delivery subgroup, i.e., 5 does in control group III and 8 does in vaccine group IV. The low number of evaluable does and litters assigned to the natural delivery subgroups prohibits a meaningful assessment of developmental endpoints of the F1 generation as well as meaningful interpretation of maternal parameters. The sponsor has evaluated the serology on some animals to investigate possible infection of animals but findings were unremarkable. Sponsor did attribute the high pup mortality to handling of the pups between DL 1-5 and hence, has conducted a second study to repeat the natural delivery arm of the study.

In the second study (b(4)000-43) animals were dosed on study days (DS) -1, -15 and -29 prior to mating and on gestation days 7 and 20 either with Agrippal vaccine, 0.5 ml, I.M., 45 ug antigen or saline control. Animals were allowed to rear their offspring. Three does died in the vaccine treated group, however data are within the range reported in historical data (0-3). Beginning on DG7 – DG 26, mean absolute and relative feed consumptions values in the vaccine treated group II were significantly reduced compared to the control group values. There appear to be slight, but not statistically significant differences in reproductive parameters, e.g., the number of rabbits achieving pregnancy in the vaccine treated group was slightly decreased compared to the control group, 21 and 25 does, respectively, additionally differences occurred in the fertility index (92.6% in control group I and 80.0% in vaccine group II) and gestation index (100% in control group I and 85.7% in vaccine group II). Note that the gestation index in group vaccine II (85.7%) is below the gestation index observed in historical controls (97.8% (88.9-100)). The pregnancy rate was 92.6% in control group I versus 77.8 % in vaccine group II. In addition, the number of liveborn pups in the vaccine treated group IV (82.0%) is reduced compared to the number of liveborn pups in group I (92.0). The total numbers of pups stillborn, found dead or euthanized due to adverse clinical signs was 93 (100 according to CBER's calculations) in group I and 46 in group II (55 according to CBER's calculations).

The number of pups that died in the control group I (41.1% during postpartum days 2-5) was increased compared to the vaccine group II (26.7%) resulting in a viability index

(defined as number of live pups on day 5 postpartum/number of liveborn pups on day 1 postpartum) of 70.0% in group II versus 58.0% in group I. Historical control data from 4 studies show that the % pups found dead or presumed cannibalized between days lactation 2-5 is 23% (6.0-42.8). Thus, the % of pups found dead in the current study between lactation days 2-5 (26.7% and 41%) is above the mean observed in the historical control data base. Furthermore, when calculating the viability indices based on the numbers of pups dead/euthanized between DL 1-7, the results are 52.5% (group I) and 66.4% (group II). These values are markedly lower than the viability index observed for the same interval in the control data base (87.3% (81.1.6-96.7), data from 3 studies). While 16 litters in group II were evaluated for eye opening, parameters such as air righting, auditory startle, pupil construction was evaluated in only 15 litters in group II due to litters with no surviving pups. This number of litters is slightly below the recommended litter number to be evaluated resulting in consistency of results (i.e., the ICH S5A document recommends that 16-to 20 litters for rabbits provide a degree of consistency between studies). Sponsor was asked to provide further explanation for the finding of high pup mortality during LD 2-5 as the data from b(4)000-43 appear to confirm findings from study b(4)000-40. Attributing the pup death in study b(4)000-40 to handling of the litter during LD 1-5 does not seem to fully account for the observed events of pup mortality in studies b(4)000-40 and b(4)000-43. The sponsor was asked how findings from postnatal evaluations will be described in the product labeling. Furthermore, since this the vaccine is licensed in Europe since 1986, the sponsor was asked if human pregnancy outcome data are available.

Comments to sponsor:

1. We concur that data derived from the Caesarean subgroup of study b(4)000-40 suggest that Agrippal does not adversely affect embryo-fetal development. We further concur that the limited number of litters in control group III and vaccine group IV in study b(4)000-40 is insufficient to allow a meaningful interpretation of possible effects of the Agrippal vaccine on postnatal development. We note that you have evaluated the serology in study b(4)000-40 on some animals to investigate possible infection of animals and findings were unremarkable. You further state that you attribute the high pup mortality to handling of the pups between DL 1-5. However, data from the follow-up study b(4)000-43 suggest that handling of pups during lactation days 2-5 only partially account for pup mortality (see item 2). Please provide any additional information on investigations you have performed to explain the finding of high pup mortality during LD 2-5 in studies b(4) 000-40 and b(4) 000-43. In addition, please provide information with regard to the data the animal supplier has on file to demonstrate health of the animals.
2. In the repeat study b(4)000-43 you state that the total numbers of pups stillborn, found dead or euthanized due to adverse clinical signs was 93 in control group I and 46 in vaccine group II. You state that number of pups that died in the control group I (41.1% during postpartum days 2-5) was increased compared to the vaccine group II (26.7%) resulting in a viability index (defined as number of live pups on day 5 postpartum/number of liveborn pups

on day 1 postpartum) of 70.0% in group II versus 58.0% in group I. Historical control data from 4 studies show that the % pups found dead or presumed cannibalized between days lactation 2-5 is 23 % (6.0-42.8). Thus, the % of pups found dead in the current study between lactation days 2-5 (26.7% and 41 %) is still above the mean observed in the historical control data base. Furthermore, when calculating the viability indices based on the numbers of pups dead/euthanized between DL 1-7, the results are 52.5% (control group I) and 66.4% (vaccine group II). These values are markedly lower than the viability index observed for the same interval in the control data base (87.3% (81.1.6 - 96.7, data from 3 studies). Thus, as in study b(4)000-40, pup mortality across study groups does not allow a meaningful interpretation of the data with regard to potential effects of the Agrippal vaccine on postnatal development.

3. You are proposing language in section 8.1 of the product labeling that follows the format of the proposed rule entitled "Content and Format of Labeling for Human Prescription Drug and Biological products; Requirements for pregnancy and lactation labeling" (May 29, 2008):

   a. The proposed rule is not finalized and thus, the pregnancy labeling section 8.1 must include a pregnancy category and language as prescribed in current 21 CFR 201.57(9)(i)(A).

   b. While we agree that, based on study b(4)000-40, Agrippal does not affect embryo-fetal development, the data derived from the natural delivery subgroups from both studies, i.e., b(4)000-40 and b(4)000-43, are problematic due to the observed pup mortality across study arms, so that potential effects of Agrippal on postnatal development cannot be ascertained.

   c. Please comment on how data from postnatal observations will be described in product labeling.

4. In study b(4)000-43 body weight and feed consumption values of the F0 generation are concerning because losses in body weight and feed consumption occurred primarily in animals assigned to the vaccine treated group. Moreover, there appear to be some differences in reproductive parameters, e.g., the number of rabbits achieving pregnancy in the vaccine treated group (21) was slightly decreased compared to the control group (25), there were differences in the fertility index (92.6% in the control group and 80.0% in the vaccine group), gestation index (100% in the control group and 85.7% in the vaccine group) and pregnancy rate (92.6 % in the control group and 77.8% in the vaccine group). We note that the gestation index in the vaccine group (85.7%) is below the gestation index observed in historical controls (97.8% (88.9-100). In addition, the number of liveborn pups in the vaccine treated group IV (82.0%) was reduced compared to the number of

liveborn pups in group I (92.0). Even though these differences did not reach statistical significance, a possible vaccine related effect cannot be ruled out. Please comment.

5. You state that in groups III and IV of study b(4)000 - 40, 78 and 69 pups were found dead, were stillborn or were euthanized, respectively (page 43 of 399). Please clarify the numbers of dead pups in group IV, i.e., Table 25 shows that 136 total pups were delivered, 12 were stillborn and an additional 68 pups died between LD 1 and 15. Thus, the total number of dead pups in group IV should be 80 rather than 69. Moreover, Table 28 states that the number of pups found dead in vaccine group IV was 57.

6. You state that the total number of pups stillborn, found dead or euthanized due to adverse clinical sign was 93 and 46 in groups I and II in b(4)000-43, respectively (page 35 of 314). Please clarify these numbers. In Table 18 it is stated that in group I, there were 13 stillborn pups and 87 pups dying between LD 0-15, thus, the total number of dead pups should be 100. Furthermore, Table 18 shows that in group II, there were 15 stillborn pups (in contrast, Table 21 states that there were 11 stillborn pups) and that 40 pups died between LD 0-15. Thus the total number of dead pups should be 55, not 46, and not accounting for the 9 pups for which the viability status could not be confirmed.

7. Please explain the variability in the number of studies included in the historical control database (period 1997-2007) for study b(4)000-43. For example, the number of studies included regarding viability indices ranges from 1 - 6 and the number of studies included regarding pups found dead on lactation days 1 - 29 ranges from 2 - 10.

8. Your proposed labeling indicates that limited data are available from vaccinating pregnant women with Agrippal. Please provide these data.

Note that the sponsor submitted a response to CBER's comments on Feb 2, 2009 to STN 125297/0.4. Thus, reference is made to review memorandum of STN 125297/0.4.

Supervisor Concurrence: Yes ___ No ___