REPRODUCTION TOXICITY STUDY REVIEW

STN number: 125297/0.4

DATS number/date/type of submission: 457801/Feb 2, 2009/BLA amendment

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: January 19, 2009

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

Novartis submitted its BLA (STN 125297) for Agrippal to CBER in electronic CTD format on July 10, 2008. CBER communicated its comments with regard to the two reproductive toxicity studies performed (b(4)00040 and b(4)00043) contained in BLA 125297 by secure email on December 8, 2008. On Feb 2, 2009, BLA amendment 125297/0.4 containing response to CBER comments, e.g., attachment 1 (health status reports), attachment 2 (health evaluation) and attachment 3 (letter from --b(4)---------- ---------- were uploaded into the EDR. Below are Novartis responses to Reproductive Toxicity Comments 1 through 7. For ease of review, CBER's original comments are restated, followed by the sponsor's response and the reviewer's response to the sponsor's response. The sponsor stated that the response to Reproductive Toxicity Comment 8 will be provided in a separate submission.

CBER original comment 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)A000-40 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**.**

Company Response to comment 1

For a complete discussion of the issues raised regarding mortality in the two reproductive and developmental toxicity studies (Study Nos. b(4)00040 and b(4)-00043), please see the response to Comment 2 (Question Identifier 003-2-N).

As noted by the reviewer, serology was evaluated in some animals during Study No. b(4)00040. We also conducted an assessment of environmental variables which included

1. location of cage within rack and within the room;
2. number and training of technicians;
3. water quality and chlorine concentration;
4. number of air changes;
5. Supplier recommendations.

There was no clear correlation with outcome for any of the parameters examined.

Reviewers' comment to sponsor' response: In addition to performing an assessment of the environmental variables that found no clear correlation with outcome, health status reports from the animal supplier (included as nonclinical attachment 1) and health evaluations for the ---------b(4)---------- rabbits that were also provided (included as nonclinical attachment 2) and also revealed no overt health problems during the conduct of these studies. Sponsor attributes the pup mortality observed that was higher than observed historically in control as well as test article treated groups to stress that the does undergo in a testing facility conducting GLP compliant studies as compared to a breeding facility. As a consequence of the results of studies b(4)00040 and b(4)00043, sponsor has implemented special procedures to eliminate stress in the room by limiting the number of technical staff entering the room,

having all animal care done by the technical staff and combining as many activities as possible to lower the number of interactions between the does and the technical staff.

Sponsor believes that these procedures resulted in better pup survival. Also, sponsor states that in our earlier studies, it had a dedicated staff of two people working on these studies. For the two b(4) studies, there was a much larger number of staff in and out of the room.

This reviewer agrees that pup mortality is not likely attributable to test article, because pup mortality occurred across treatment groups, in particular in study b(4)00043 pup mortality was increased in the control group (41.1 % during postpartum days 2-5) compared to the vaccine treated group (26.7%). Perhaps procedures implemented will prevent high pup mortality from occurring in follow-up studies, however, this reviewer maintains that 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.

CBER Comment 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**.**

Company Response to Comment 2:

Although the number of pups that died between days 2-5 of lactation in Study No. b(4)00043 is 26.7% and 41.1% for treated and control groups, respectively, these values are within the range of values reported in the historical control database (6.0 to 42.8%).

In Study No. b(4)00043, the viability index was calculated by comparing the number of live pups on day 5 with the number of live pups on day 1. While we agree that the viability index for the control group (57.9%) was slightly outside the historical control range (59.6 – 93.6%), there were sufficient numbers of control and treated animals to assess the effects of Agrippal on postnatal development.

Based on the data contained in Table 18 of the study report (page 62 of 314), the viability index for DL 1-8 can be calculated (52.5% and 66.4% for control and treated groups, respectively). The historical control database contains only a single study for this interval, the viability index is 75.9%.

Input was requested from the contract research organization that conducted the studies, ----b(4)---------- input was requested in answering the reviewer's questions because of his extensive experience in this field. ----b(4)---------- evaluation is provided, and the text below is extracted from the document provided.[1]

As noted, Agrippal does not affect embryo/fetal development. We do not agree that the postnatal development of kits exposed *in utero* or through milk to Agrippal cannot be adequately evaluated when data from both studies are evaluated. The number of litters evaluated in b(4)00040 was felt to be inadequate. However, when the total number of litters from both b(4)00040 and b(4)00043 are evaluated, (see table below) we believe that an adequate evaluation of postnatal development was conducted. Well over the ICH guidance of 16 to 20 litters were evaluated for the Control and Agrippal groups for litters alive on day 5 postpartum and day 29 postpartum.

Number of Litters Evaluated	**Day 5**	**Day 29**
b(4)**00040**		
Control	16	16
Agrippal	11	9
b(4)**00043**		
Control	21	21
Agrippal	16	15
Total		
Control	37	37
Agrippal	27	24

It is also important to note that the average litter size delivered in both b(4)00040 and b(4)00043 is very close to the average for this Testing Facility and close to the average litter size for the does that were assigned to Caesarean-section in b(4)00040 and the average for this Testing Facility. See table below.

[1] For clarity, the treated group is referred to as 'Agrippal' instead of '15mcg/0.5mL' Both 'kits' and 'pups' are used to refer to the F1 offspring in this document.

	b(4)00040 Caesarean-Section [a]	**b(4)00040 Delivered** [a]	**b(4)00043 Delivered** [a]	**Historical Control Caesarean-Section** [b]	**Historical Control Deliveries** [b]
Implantations					
Control	7.9 ± 2.8 (22)	9.2 ± 2.2 (21)	8.6 ± 2.2 (25)	8.8 (93) 7.5 – 11.2	8.1 (13) 6.2 – 10.3
Agrippal	7.0 ± 2.8 (23)	8.4 ± 3.0 (19)	8.0 ± 1.9 (18)	NA	NA
Litter Size					
Control	7.4 ± 2.7 (22)	8.7 ± 2.1 (21)	8.0 ± 2.2 (25)	8.4 (93) 6.5 – 11.2	7.6 (13) 6.2 – 9.2
Agrippal	6.6 ± 2.7 (23)	8.0 ± 2.9 (17)	7.4 ± 2.4 (18)	NA	NA

a. Mean ± Standard Deviation (number of litters)
b. Mean (number of studies), mean range.

Based on the above data it is clear that the delivered litter size was comparable for the two studies and to our extensive historical control data both for Caesarean-sectioned does (data for the last two years) and our studies with delivered litters. The delivered values are also comparable to those of our supplier (---b(4)--- Products) for all of these studies. Our Supplier states that litter sizes of 8 to 12 are typical.

The reviewer's concerns relate to the high number of kit deaths that occurred in both of these studies. The number of deaths was higher than we have observed historically but the deaths were not related to any health problem with either the does or kits or any effect of the test article. From our investigations, it appears that the higher incidence of kit deaths that resulted in lower viability indices was the result of stress that the does undergo in a Testing Facility conducting GLP compliant studies as compared to a breeding facility.

We have found that while commonly used out-bred mice and rats have good survival rates postpartum, transgenic and in bred strains have lower fertility rates and poor survival in research environments relative to breeding environments.

A similar phenomenon appears to be occurring with rabbits when they are stressed or subjected to more changes in their environment than occurs at the breeders. There are major differences in the handling of kits delivered at our Supplier and those delivered in our research facility. The differences include the number of staff that enters a room to make observations at various times during the day and the amount of cleaning and animal care that takes place. At our supplier, the pregnant does are allowed to deliver and rear their litters essentially undisturbed until weaning of their litters at five weeks of age. Caging is not changed, only bedding and feed and water is *ad libitum*. Room temperature is usually kept low in the --b(4)----- range.

At our facility, does are housed in cages with delivery boxes, but the cages have cage papers that are changed three times a week, feed is restricted and provided daily and the room temperature is maintained at --b(4)---. The survival of delivered kits in this

environment has always been lower than those observed at the breeders. We do agree that the survival in these two studies was below what has been occurring at out Testing Facility. Below is a listing of the delivered litters and viability indices for each study. The studies are presented in time order of conduct. Since completion of b(4)00043, we conducted an additional delivery study. In this study we took special procedures to eliminate stress in the room by limiting the number of technical staff that entered the room, having all animal care done by the technical staff and combining as many activities as possible to lower the number of interactions between the does and the technical staff.

We believe that these procedures resulted in better pup survival and that in our previously conducted studies we had less disruption of does. In our earlier studies, we had a dedicated staff of two people working on these studies. For the two b(4)-- studies, we had a much larger number of staff in and out of the room. Also attached are two files with health status reports [see response to Comment 1, Question Identifier 003-1-N]. One file contains the health status reports from the Supplier and the other has the health evaluations of our in-house sentinel rabbits. No health problems were occurring during the conduct of these studies.

Study Number	Pregnancy (%)	Delivered Litters (%)	Viability Index [a] (%)
b(4)134	15 (75.0)	14 (93.3)	78.0
b(4) 163	17 (85.0)	17 (85.0)	84.6
b(4) 214	20 (95.2)	18 (100)	88.9
b(4) 281	4 (80.0)	4 (100)	100
b(4)350	23 (95.8)	23 (100)	81.1
b(4)486	16 (80.0)	16 (100)	70.3
b(4) 521	19 (95.0)	19 (100)	97.0
b(4) 522	20 (100)	20 (100)	96.5
b(4)551	17 (85.0)	17 (100)	75.9
b(4) 567	19 (79.2)	16 (88.9)	93.6
b(4)00040	24 (88.9)	21 (87.5)	59.6
b(4)00043	25 (92.6)	25 (100)	57.9
b(4)600	26 (96.3)	26 (96.3)	87.0

a. Includes viability indices calculated from day 1 to either 4, 5, 7 or 8 postpartum

Reviewers' comment to sponsor' response:

As noted by the sponsor, the reviewer's concerns relate to the high number of kit deaths that occurred in both studies, b(4) 000-40 and b(4)000-43. In addition, and as acknowledge by the Director of Research at b(4), the number of deaths was higher than was observed historically. It is acknowledged that the values of pup death observed between LD 2-5, i.e., 26.7% and 41.1% for treated and control groups are within the range of values reported in the historical control database (6.0 to 42.8%), however, they are 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). Sponsor does not provide any response to this observation but acknowledges that the viability index (calculated by comparing the number of live pups on day 5 with the number of live pups on day 1) for the control group (57.9%) was outside the historical control range (59.6 – 93.6%). Sponsor states that there were sufficient numbers of control and treated animals to assess the effects of Agrippal on postnatal development when the total number of litters from both studies were evaluated. Although it is reassuring that the delivered litter sizes were comparable for the 2 studies and to the historical control data it is problematic to combine evaluable litters across studies to arrive at a sufficient number of litters for an evaluation of postnatal parameters. In summary, from the sponsor's investigations, it appears that the higher incidence of kit deaths that resulted in lower viability indices was the result of stress that the does undergo in a Testing Facility conducting GLP compliant studies. This reviewer recommends that when the results from the studies conducted are described in the labeling, a statement should be included that postnatal evaluations could not be performed because of high (non-test article related) pup mortality.

CBER Comment 3:

The proposed language in section 8.1 of the product labeling 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). It is acceptable to include the subheadings as described in the proposed rule in product labeling. However, as the proposed rule is not finalized, the pregnancy labeling section 8.1 must include a pregnancy category and language as prescribed in current 21 CFR 201.57(9)(i)(A). This will need to be addressed when negotiating the product labeling.

Company Response to 3:

We acknowledge the need for a pregnancy category and language. We propose revising the label to include the following statement: "Pregnancy Category B**:** Two reproductive and developmental toxicity studies have been performed in rabbits with a dose level that was approximately 15 times the human dose based on body weight. The studies revealed no evidence of impaired female fertility or harm to the fetus due to Agrippal. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed."

Furthermore, in response on how the sponsor would describe data from postnatal observations in labeling the sponsor stated "No developmental differences were detected in the F1 offspring from the control group or the Agrippal-treated group in either Study No. b(4)00040 or b(4)00043. Parameters evaluated included eye opening, hair growth, air righting, acoustic startle and pupil constriction."

Reviewers' comment to sponsor' response:

The proposed language will need to be revised to include a statement about the limitations of the study to evaluate post-weaning development.

CBER Comment 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**.**

Company response to comment 4:

The two reproductive and developmental toxicity studies conducted to assess Agrippal were performed at -------b(4)---------- input was requested in answering the reviewer's questions because of his extensive experience in this field. --b(4)---------- evaluation was provided as Nonclinical Attachment 3. The text below is extracted from this document and from discussions with --b(4)---------.

"The losses in body weight and reductions in feed consumption which occurred in the vaccine group were due to inclusion of data from the two does that died early and the one doe that aborted. As these early deaths were not considered related to the vaccine, the data for body weight and feed consumption has been recalculated excluding these does.

The recalculation of the body weight and feed data for gestating does excluding the two does that were found dead and the one doe that aborted resulted in the following:

a) no statistical difference in body weights;

b) no statistically significant differences in body weight gain from day 23 to 26;

c) lowered significance from $p \leq 0.01$ to $p \leq 0.05$ for body weight gain for day 7 to 10;

d) no statistical significance for absolute feed consumption for days 13 to 16 and 16 to 20;

e) lowered significance from $p \leq 0.01$ to $p \leq 0.05$ for absolute feed consumption for days 20 to 23;

f) no statistical significance for relative feed consumption for days 7 to 10, 13 to 16, 16 to 20 and 20 to 23;

g) Lowered significance from $p \leq 0.01$ to $p \leq 0.05$ relative feed consumption for days 20 to 23.

As the reviewer has noted, there were no statistically significant differences in pregnancy rate, fertility index, gestation index, or the number of live born pups. In addition, with the exception of gestation index, these parameters were all within the historical control ranges at the Testing Facility. In this type of study, large group sizes are used in order to power statistical analysis because sporadic differences in parameters do occur. For example, in Study No. b(4)00040 where four groups were evaluated the fertility indices in the Caesarean sectioned groups were 88.9% and 96.2% for control and treated groups, respectively, while in the natural delivery groups the fertility index was 92.3% (control) and 87.5% (treated).

Evaluating all the available data across the two studies, we do not see any cause for concern because Agrippal did not cause overt systemic toxicity or adverse effects on mating, fertility, or litter or natural delivery parameters."

Reviewers response to sponsors response to comment: Addressed satisfactorily

CBER 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**.**

Company Response to comment 5:

In the report for Study No. b(4)00040, the text on page 43 of 399 refers to values from Table 28 (Necropsy Observations – Summary – F1 Generation Pups) and includes only those pups for which a complete necropsy could be performed. Pups that were autolyzed or partially cannibalized did not receive a complete necropsy. If a gross lesion was observed in this autolyzed or partially cannibalized pup then it was noted. The difference in numbers in Table 25 compared with Table 28 is due to the fact that not all pups were necropsied.

The total number of deaths for all reasons including stillborn, found dead, cannibalized, missing, and euthanized can only be found in Study No. b(4)00040 Table 25 (Litter Observations – (Naturally Delivered Pups) Summary F1 Generation Litters).

Reviewer comment to sponsor's response: addressed satisfactorily

CBER Comment 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.

Company Response to comment 6:

In the report for Study No. b(4)00043, the text on page 35 of 314 refers to values from Table 21 (Necropsy Observations – Summary – F1 Generation Pups) and includes only those pups for which a complete necropsy could be performed. Pups that were autolyzed or partially cannibalized did not receive a complete necropsy. If a gross lesion was observed in this autolyzed or partially cannibalized pup then it was noted. The difference in numbers in Table 18 compared with Table 21 is due to the fact that not all pups were necropsied.

The total number of deaths for all reasons including stillborn, found dead, cannibalized, missing, and euthanized can only be found in Study No. b(4)00043 Table 18 (Litter Observations – (Naturally Delivered Pups) Summary F1 Generation Litters).

Reviewer comment to sponsor's response: addressed satisfactorily

CBER Comment 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.

Company Response to comment 7:

The variability in the number of studies included in the historical control database for individual parameters is due to slight differences in the study designs and the days observations were made.

For example, the viability index was calculated based on the number of live pups on days 4, 5, 7, or 8 compared to the number of live pups on day 1 depending on the study design, and this difference is reflected in the historical control database.

Reviewer comment to sponsor's response: It is acknowledged that the historical data base is limited.

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

Company response to comment 7 : Company will submit a response with separate submission

Reviewer's comment to sponsor's response: acknowledged

Action Item: The pregnancy section (8.1) of the labeling should state the following:

8.1 Pregnancy

Pregnancy Category B.

Reproduction studies have been performed in female rabbits at a dose approximately 15 times the human dose (on a mg/kg basis) and have revealed no evidence of impaired fertility or harm to the fetus due to AGRIPPAL. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, AGRIPPAL should be given to a pregnant woman only if clearly needed.

In two reproduction toxicity studies, the effect of AGRIPPAL on embryo-fetal or post-natal development was evaluated in pregnant rabbits. Animals were administered AGRIPPAL 3 times prior to gestation, during the period of organogenesis (gestation day 7) and later in pregnancy (gestation day 20), 0.5 ml/rabbit/occasion (appr. 15-fold excess relative to the projected human dose on a body weight basis) by intramuscular injection. No adverse effects on mating, female fertility, pregnancy, and embryo-fetal development attributable to the vaccine were observed. There were no vaccine related fetal malformations or other evidence of teratogenesis noted in this study. Potential effects on post-natal development were not assessed due to pup mortality occurring between lactation days 2 and 5 in the vaccine treated group and in the control group at an incidence higher than that observed historically. However, pup deaths were not considered related to any effect of the vaccine or associated health problem with either the does or kits. The higher incidence of pup deaths was likely a result of coincidental stress that the does underwent while on study.

Supervisory concurrence : Yes _____ No_____