

REVIEW MEMORANDUM

From: Marion F. Gruber, Ph.D., OVRR

To: Richard Daemer, DVRPA, OVRR

Date: July 24, 2008

Subject: Review memorandum

- STN 125280/0 Section 4.2.3.5.3.1
Final Study Report; Japanese Encephalitis vaccine, IC 51, “Pre and Post Natal Developmental Toxicity Study in Rats”
- STN 125280/0.2 (amendment) section 4.2.3.5.3.1.
Historical background data
- STN 125280/0.5 (amendment) section 4.2.3.5.3.1.
Individual animal data

Background: IC51 (Japanese encephalitis vaccine, JE-PIV) is developed by the sponsor as a vaccine against Japanese Encephalitis which is a mosquito-borne, flavivirus infection, and the most common viral encephalitis, with > 50,000 cases reported annually.

IC51 is purified and inactivated Japanese encephalitis virus (JEV) vaccine derived from the attenuated JEV strain SA14-14-2. The virus is grown in VERO cells, purified, inactivated with formalin, and then formulated with aluminium hydroxide. The final vaccine is in the form of a suspension in a pre-filled syringe. Each unit dose of IC51 contains 6 ug of the inactivated JE, strain SA14-14-2 per 0.5 ml. The vaccine does not contain preservative.

Two immunizations of the vaccine given 4 weeks apart are required to achieve optimal protection against JE with IC51. The vaccine elicits neutralizing antibodies thought to be protective. IC51 is injected intramuscularly in the deltoid region. IC51 is indicated for active immunization against JE for persons aged 18 years and older and should be considered for use in persons who plan to reside in or travel to areas where JEV is endemic. It is also indicated for people who work with JEV in laboratories and industry. In the US there is a licensed JE vaccine which however, will no longer be available in the future. Intercell has submitted a BLA for Japanese Encephalitis vaccine. The sponsor agreed to perform a developmental toxicity study in rats to evaluate the potential reproductive risk of this vaccine. The sponsor had submitted on July 8, 2005 a study proposal for the developmental toxicity study to IND -(b)(4)- and received CBER's comments on July 29, 2005.

Sponsor: Intercell AG
Campus Vienna Biocenter 2
A-1030 Vienna
Austria

Research Laboratory

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

Study was conducted in compliance with OECD Principles of GLP as set forth by the UK Department of Health. Blood serum was evaluated under non-GLP conditions by the sponsor as a separate study.

Study inspected and audited:

<u>Date of QA activity</u>	<u>Phase</u>	<u>Date of report to Study Director</u>
November 25, 2005	Protocol review	November 25, 2005
January 9, 2006	Dose dispensing	January 12, 2006
January 10, 2006	Dosing/protocol compliance	January 11, 2006
February 21, 2006	Necropsy/blood sampling	February 21, 2006
Exp completion date		December 28, 2006

June 26, 2007 QA assurance

Study title: “IC51 pre and post natal developmental toxicity study in rats”
STN 125280.0 , section 4.2.3.5.3.1

Study Director: --- (b)(6) -----

Quality assurance: --- (b)(6) -----

Objective: To detect the effects on pre- and postnatal development when administered one to three weeks before mating and at the start of the period of organogenesis (day 6 of gestation).

Test system: ----(b)(4)--- rat strain (a total of 148 females, -----(b)(4)-----, 29-31 days of age, no weight range specified) was used. According to the sponsor “the rat is a standard rodent species for the toxicological testing in animals required by regulatory authorities’ and “ has been proven a responsive species, which produces antibodies in response to vaccination with IC51.” Animals were acclimatized for 3 weeks and examined for signs of abnormality or disease; 74 males of the same strain from the same supplier were also received (ca 36 days old).

RoA: IM as it is the clinical route

Dosing regime: based on expected clinical regime(s)

Group assignment: Animals were allocated to 3 treatment groups by use of a repeated group order

Group number	Treatment regime	Animal numbers
1	Control	1-48
2	Vaccine I	49-96
3	Vaccine II	97-144

Treatment groups/dosages: The dosages used in this study (500 ul/occasion) was 4.9 ug of test article (planned dose was 6 ug in 0.5 ml), see below.

Study design: F0 females in group Vaccine I were dosed 3 and 1 weeks prior to mating and again on day 6 of gestation. Group Vaccine II received the test article one week prior to mating and a 2nd dose on day 6 of gestation. The schedule was based on data from preliminary studies demonstrating that 3 doses, administered 2 weeks apart, elicited a maximum antibody titer. Doses were administered by IM injection into the lateral compartment of the thigh muscle, the alternate thigh muscle used/occasion (dose volume 0.5 ml/rat), control animals received vehicle accordingly

Schedule	Control	Vaccine I	Vaccine II
Dose 3 wks prior to mating	all	all	-
Dose 1 wk prior to mating	all	all	all
Dose day 6 of gestation	all	all	all

Twenty-four females/group were allocated to the Caesarean subgroup and 24 females were allocated to the littering subgroup.

Mating: Females were paired on a 1 male:2 female basis with stock males from the same source, evidence of mating was confirmed, e.g., by ejected copulation plugs and presence of sperm in the vaginal smear.

For each female the time taken to show a positive mating sign, the fertility index and the number of failed opportunities to mate (oestruses without a sign of mating) were evaluated

Test substance:

Vaccine: IC-51 (JE-PIV) Lot number ICB05/501 (lot was also used for phase 3 clinical trial material); Certificate of Analysis were present in Appendix 1: The protein result of the drug substance (pre-formulation) was slightly below the range defined i.e., 10.3 ug/ml (specifications -----(b)(4)-----) equating to a dose of 4.9 ug in a 0.5 ml dose.

Vehicle: 0.1% alum/PBS placebo, Lot number ----(b)(4)----

Clinical observation: Animals were inspected for viability 2x daily, injection sites were examined on the day of each injection and daily thereafter until the first animal attained day 16 of gestation (onset, intensity and duration of signs recorded); once each week animals received a detailed clinical examination including appearance, movement and behavior pattern, skin and hair condition, eye and mucous membranes, respiration and excreta

Body weight: recorded once prior to commencement of dosing, then weekly until pairing for mating (weights also recorded 3 days after each dose in pre-mating period), then on days 0, 6-9, 13 and 20 after mating, and on days 1, 7, 14, and 21 of lactation.

Food consumption: weekly before pairing, days 0-6, 6-13, and 13-20 of gestation and days 0-7 and 7-14 of lactation.

Littering subgroup observations: Day of parturition was considered day 0 of lactation; duration of gestation was evaluated
Number of live and dead pups born in each litter was recorded after completion of parturition

Live pups were sexed, counted and examined for presence of milk in stomach, external abnormalities daily up to day 4 of lactation, sexed, counted and examined again on days 7, 14 and 21 of lactation

Live pups were weighed *en masse*, sexes separate on days 1, 4, 7 and 14 of lactation, on day 21 pups were weighed individually

Pups found dead were examined, when possible, prior to day 14 of lactation, any externally abnormal decedent pup was preserved, after day 14, no such pups were found

Deficiencies in lactation and maternal care were recorded

Reproductive indices

Reproductive indices such as fertility index (female), gestation index birth index, live birth index, viability index, lactation index and overall survival index were determined.

Littering phase observation:

Pinna detachment (from lactation day (LD) 1), upper incisor eruption (from LD 7), negative geotaxis (from LD 11), eye opening (from LD 11), auditory function (from LD 16), visual function (from LD 18); each litter was examined daily until all pups in that litter had reached the criterion (reproductive function was not assessed as detailed in protocol as study was not designed to allow such evaluation). For each sex in each litter the day at which criterion was reached in all pups and the median day to criterion were used as the bases of comparison between treatment groups for physical function.

Laboratory investigations

Blood samples for hematology and clinical chemistry were collected from adult females on GD 20 under terminal anesthesia, urinalysis samples were also obtained on GD 20. Blood samples for antibody titers were taken prior to administration of the 1st dose, one day prior to administration of the 3rd (2nd) dose, on GD 20 from adult females and pooled cord blood samples and on LD 21 from adults, on LD 21 samples of at least 0.5 ml were collected from one male and one female pup in each litter, when possible

Premature decent: was subjected to gross necropsy (cranial, thoracic and abdominal contents examined macroscopically) reproductive tracts were evaluated

Termination on GD 20

Necropsy

F0 generation: animals allocated to the embryo-fetal phases of the study were subject to a gross necropsy (thoracic and abdominal cavity examined macroscopically, abnormal tissues preserved; reproductive tract dissected and weighed intact then contents examined, 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 were recorded for each uterine horn

Fetuses: externally examined and any abnormalities were recorded, weights of live fetuses in litter recorded, sex of each fetus was recorded

Half of the fetuses in each litter were subjected to gross internal examination of the viscera of the neck, thorax and abdominal cavities, were then eviscerated and skeletons fixed in -----(b)(4)----- prior to staining with -----(b)(4)----; the remaining fetuses were fixed whole in -----(b)(4)----. Serial sections were prepared from the -(b)(4)-fixed fetuses and were examined under the microscope for visceral abnormalities, fetuses stained with -----(b)(4)---- were assessed for skeletal development and abnormalities

Termination on Lactation day 21

F0 generation: animals were necropsied consisting of external examination followed by macroscopic examination of the tissues and organs of the cranial, thoracic and abdominal cavities in situ, gross lesions preserved; organs were fixed in neutral buffered formalin (brain, GI tract, heart, kidney, liver, lumbar lymph node, lung, marrow smear, mesenteric lymph node, sciatic nerve, skin and mammary gland, spinal cord, spleen, submandibular lymph node, thymus, urinary bladder; reproductive tracts of all females were examined for signs of implantation and number of implantation sites).

F1 pups: From each litter, 2 males and 2 females were necropsied (external examination and macroscopic examination of the tissues and organs of the cranial, thoracic and abdominal cavities in situ), samples of abnormal tissue was preserved, remaining pups in each litter were checked for external visible signs of abnormalities and when found such abnormality was necropsied. Offspring found dead before day 14 of lactation was sexed, checked for presence of milk in stomach and for presence of externally visible abnormalities, no pups were found dead after 14 days of lactation.

STATISTICS

Statistical analysis was performed on body weight, fetal body weight, implant data, hematology, clinical chemistry, urinalysis, and organ weight data (analysis of variance and Kruskal-Wallis non-parametric analysis); organ weights also analyzed by ANCOVA, selected incomplete ossification parameters were analyzed as proportions in a Kruskal-Wallis analysis and by Fisher's exact probability test (2 sided, $p = 0.05$). For other data no formal statistical analysis was performed, data evaluated by individual and group values.

RESULTS

Necropsy and clinical observations (F0 generation); (Appendix 2): Findings from clinical and necropsy observations of the F0 generation were sporadic, distributed across groups and did not appear to be vaccine related. At the request of CBER the sponsor submitted on June 9, 2008, (125280/0.5) individual animal data from all animals evaluated and not only data from animals with findings as was submitted with the initial application. Changes in data presentation did not affect the overall interpretation.

Necropsy findings (F0 generation)

Necropsy findings	Control	Vaccine I	Vaccine II
Stomach: glandular mucosa reddened/thickened	4/11*	2/9	5/9
Skin : staining and encrusted	2/11	Not observed	Not observed
Hairloss: ventral abdomen	1/11	3/9	Not observed
Hairloss on hindlimbs/forelimbs	1/11	1/9	Not observed
Liver: all lobes pale	1/11	Not observed	Not observed
Liver: prominent lobulation on all lobes Lungs: many dark foci all lobes	1/11	1/9	3/9
Lungs: reddened dark patches	1/11	Not observed	Not observed
Lungs: all lobes reddened	2/11	Not observed	1/9
Uterus: dark, both horns dilated with fluid, greenish color Cervix; enlarged	Not observed	1/9	Not observed

*: total of animals presented with clinical observations per group, total number of animals examined: 48

Clinical observations (F0 generation)

Clinical observation	Control	Vaccine I	Vaccine II
Left/right eye, lid incrustated	2/11*	0/8	0/12
Left upper tooth damaged	0/11	0/8	0/12
Sparse hair/bald	6/11	6/8	8/12
Staining on fur/head	1/11	2/8	4/12
Found dead	1/11	0/8	0/8

*: total of animals presented with clinical observations per group, total number of animals examined: 48

Body weights (Table 2, 3, 13 & App. 3, 4, 16):*Body weight gains prior to mating:*

Body weight gains prior to mating were comparable among control and treatment groups (48 animals evaluated per group):

Control: 59 g \pm 10, Vaccine I: 56 g \pm 8, Vaccine II: 57 g \pm 9

Body weight gains during gestation (Caesarean subgroup):

Control: 150 g

Vaccine I: 139 g (93% of control)

Vaccine II: 143 g (95% of control)

Mean body weight of female F0 dams at GD 20:

Control: 404 g \pm 50

Vaccine I: 385 g \pm 40

Vaccine II : 385 g \pm 45

Body weight gains during gestation (Littering subgroup):

Control: 137 g , 24 animals evaluated

Vaccine I: 143 g (104% of control), 24 animals evaluated

Vaccine II: 144 g (105% of control), 24 animals evaluated

Body weight gains during Lactation (Littering subgroup):

Body weight gains during lactation were not provided, however group mean body weights (g) assessed on lactation days 1, 7, 14 and 21 suggest comparable weights of the animals among groups.

Sponsor notes that treated groups assigned to the Caesarean subgroup generally gained less weight than controls during gestation, whereas treated groups of animals assigned to the littering phase gained more weight than controls during gestation. Sponsor attributes this finding to the selection of animals, the first female from a pair of females mated to one male was assigned to the sub-group which was sacrificed on day 20 of gestation.

Food consumption: (Table 4, 5, 14 & App. 5, 6, 7, 17)

There were no treatment related effects on food consumption in the F0 generation.

Caesarean data (Table 6 & reproduced below, Appendix 8)

Of the females selected for the embryo-fetal phase 23 of the 24 dams in the control group were pregnant, 22 of the 24 dams in the Vaccine I group and 21 of the 24 dams in the Vaccine II group. The mean number of corpora lutea, implantations, resorptions (early and late) live young and sex ratio (% males) were unaffected by treatment with the vaccines as data for these parameters derived from Vaccine groups I and II were comparable with concurrent control and historical background data (see Tables reproduced below entitled “Background data for pregnancy performance in developmental toxicity studies performed at -----(b)(4)----- laboratories in ---(b)(4)----- rats” and “Litter data - group mean values for females sacrificed on GD 20”). Total live male and female fetuses were similar in all groups and appeared unaffected by treatment. The number of live fetuses (live implantations) was slightly higher in the vaccine group II than in the control group (15.0 versus 13.8). The fetal weight in vaccine group II was slightly lower ($3.67 \text{ g} \pm 0.21$) compared to the control ($3.71 \text{ g} \pm 0.25$) and vaccine I group ($3.74 \text{ g} \pm 0.38$), probably attributable to the slightly higher number of live implantations in vaccine group II.

**Background data for pregnancy performance in developmental toxicity studies
performed at -----(b)(4)----- in -----(b)(4)----- rats**

Study	Start Date	Number of dams	Mean values								
			Corpora Lutea	Implants	Early Deaths	Late Deaths	Dead Foetuses	Total Deaths	Live Implants	Uterus Wt (g)	Foetal Wt (g)
1	Feb 2003	19	14.1 ± 2.5	13.5 ± 2.2	0.8 ± 1.0	0	0.1 ± 0.2	0.9 ± 1.0	12.8 ± 2.2	78 ± 13	3.73 ± 0.25
2	Mar 2003	18	13.0 ± 1.6	11.7 ± 2.9	0.3 ± 0.5	0	0	0.3 ± 0.5	11.4 ± 3.0	69 ± 18	3.79 ± 0.27
3	Jul 2003	22	13.7 ± 2.2	13.1 ± 2.1	0.6 ± 0.6	0	0	0.6 ± 0.6	12.5 ± 2.0	79 ± 11	3.87 ± 0.28
4	Apr 2004	18	14.4 ± 2.4	13.7 ± 1.9	0.2 ± 0.4	0.1 ± 0.3	0	0.3 ± 0.5	13.4 ± 1.9	81 ± 13	3.82 ± 0.41
5	Apr 2004	19	13.4 ± 2.7	12.2 ± 3.2	0.8 ± 1.0	0.1 ± 0.2	0	0.9 ± 1.0	11.3 ± 3.2	71 ± 19	3.91 ± 0.31
6	Jan 2005	19	13.3 ± 2.6	12.6 ± 2.6	0.7 ± 0.8	0.1 ± 0.2	0	0.7 ± 0.8	11.8 ± 2.4	70 ± 12	3.68 ± 0.15
7	Feb 2005	19	14.4 ± 1.7	13.5 ± 1.3	0.3 ± 0.6	0	0	0.3 ± 0.6	13.2 ± 1.2	81 ± 7	3.88 ± 0.16
8	May 2005	20	14.3 ± 1.9	14.0 ± 2.0	0.6 ± 0.7	0	0	0.6 ± 0.7	13.4 ± 1.8	85 ± 10	3.96 ± 0.22
9	Jun 2005	19	13.8 ± 1.4	13.5 ± 1.2	1.2 ± 1.5	0	0	1.2 ± 1.5	12.3 ± 1.9	77 ± 10	3.95 ± 0.20
10	Jul 2005	20	13.6 ± 2.0	12.9 ± 3.0	0.4 ± 0.9	0	0	0.4 ± 0.9	12.5 ± 2.9	77 ± 17	3.87 ± 0.26
11	Sept 2005	20	13.6 ± 1.8	12.9 ± 1.7	0.9 ± 1.2	0	0	0.9 ± 1.2	12.1 ± 2.5	77 ± 14	3.97 ± 0.23
12	Aug 2006	17	13.5 ± 1.4	13.4 ± 1.3	0.3 ± 0.6	0	0	0.3 ± 0.6	13.1 ± 1.5	79 ± 7	3.74 ± 0.24

Litter data - group mean values for females sacrificed on GD 20

	Group/Treatment		
	1 (Control)	2 (Vaccine I)	3 (Vaccine II)
Number of animals mated	24	24	24
Number of premature decedents	0	0	0
Number pregnant at Day 20 necropsy	23	22	21
Pregnancy frequency as %	96	92	88
Total corpora lutea graviditatis	352	335	333
Total number of implants	331	304	325
Pre-implantation loss as %	6	9	2
Total live implants (%)	317 (96)	294 (97)	316 (97)
Total dead implants (%)	14 (4)	10 (3)	9 (3)
Total early embryonic deaths (%)	14 (4)	9 (3)	9 (3)
Total late embryonic deaths (%)	0	0	0
Total dead fetuses (%)	0	1 (0.3)	0
Mean corpora lutea graviditatis	15.3 ± 1.9	15.2 ± 2.5	15.9 ± 1.8
Mean implants†	14.4 ± 1.9	13.8 ± 4.3	15.5 ± 1.8
Mean live implants	13.8 ± 1.9	13.4 ± 4.2	15.0 ± 1.8
Mean dead implants	0.6 ± 0.7	0.5 ± 0.8	0.4 ± 0.6
Mean early embryonic deaths	0.6 ± 0.7	0.4 ± 0.7	0.4 ± 0.6
Mean late embryonic deaths	0	0	0
Mean dead fetuses	0	0.05 ± 0.2	0
Total live male fetuses (%)	161 (51)	142 (48)	155 (49)
Total live female fetuses (%)	156 (49)	152 (52)	161 (51)
Live foetal sex ratio (M:F)	1:0.97	1:1.07	1:1.04
Mean total uterus weight (g)	82 ± 12	78 ± 22	87 ± 9
Mean litter mean foetal weight (g) †	3.71 ± 0.25	3.74 ± 0.38	3.67 ± 0.21

Means are given ± Standard Deviation

Note: Premature decedents excluded below double line

† = No statistical significance attained

Females assigned to Day 20 of gestation necropsy

Fetal pathology (Tables 7-9 & App. 9-11)

At the request of CBER the sponsor submitted on June 9, 2008, (125280/0.5) individual animal data from all animals evaluated and not only data from animals with findings as was submitted with the initial application. Changes in data presentation did not affect the overall interpretation.

Major fetal abnormalities: There were no major abnormalities due to the administration of vaccine antigen. Results in the table below entitled “Group incidence of major fetal

abnormalities” shows that these findings occurred across study groups and sometimes with higher incidence in the control group.

“Group incidence of major fetal abnormalities”

Parameter	Group/treatment		
	1	2	3
	Control	Vaccine I	Vaccine II
	Incidence of fetuses (litters)		
Omphalocele	1(1)	0	0
Duplicated/partially duplicated inferior vena cava	3(3)	1(1)	0
Partially split sternum	1(1)	0	0
Ribs (kinked)	1(1)	0(0)	0(0)
No. with major abnormality	5(5)	1(1)	2(2)
Total no. examined	317(23)	294(22)	316(21)

Minor visceral abnormalities: The type and number of visceral minor abnormalities and variants were similar across study groups. Of 159 fetuses (23 litters) evaluated by -(b)(4)-sectioning in the control group there were 62 fetuses (20 litters) with minor visceral abnormalities/variants. Of 145 fetuses (22 litters) evaluated in the vaccine I group there were 41 fetuses (18 litters) with minor visceral abnormalities/variants. Of 158 fetuses (21 litters) evaluated in the vaccine II group there were 39 fetuses (17 litters) with minor visceral abnormalities/variants.

Minor skeletal abnormalities: The type and number of skeletal minor abnormalities and variants were similar across study groups. Of 158 fetuses (23 litters) evaluated skeletally in the control group there were 8 fetuses (6 litters) with minor skeletal abnormalities/variants. Of 149 fetuses (22 litters) evaluated in the vaccine I group there were 4 fetuses (2 litters) with minor skeletal abnormalities/variants. Of 158 fetuses (21 litters) evaluated in the vaccine II group there were 4 fetuses (3 litters) with minor visceral abnormalities/variants.

However, there was an increase in the incidence of incomplete ossification in some parameters in the fetuses of dams in the vaccine II group (see table below entitled “Group incidence of skeletal ossification parameters”). These included incomplete ossification of ≥ 4 skullbones (25 fetuses, 10 litters), pubes (21 fetuses, 8 litters), ischia (7 fetuses, 4 litters), sacral vertebral arches (37 fetuses, 13 litters) and 2nd and/or 4th metacarpals (11 fetuses, 6 litters). For comparison incidence in the control group were: incomplete ossification of ≥ 4 skullbones (4 fetuses, 3 litters), pubes (9 fetuses, 6 litters), ischia (0 fetuses, 0 litters), sacral vertebral arches (24 fetuses, 13 litters) and 2nd and/or 4th metacarpals (1 fetuses, 1 litters). Statistical significance was attained for the parameters “ ≥ 4 skull bones” and “ischia” when the control group and Vaccine group II (2 injections) were compared. The control group and the Vaccine I group (3 injections) were well

within the historical control data value for these parameters. The incidence in the vaccine II group (2 injections) was higher both based on the litter and fetal incidence for the skull but only based on the fetal incidence for the ischium.

Group incidence of skeletal ossification parameters

Abnormality	Group/Treatment			Fisher's Exact Test/ Group	
	1	2	3	2	3
	Control	Vaccine I	Vaccine II	Control vs Vaccine I	Control vs Vaccine 2
	Incidence of Foetuses (Litters)			Control vs Vaccine I	Control vs Vaccine 2
<u>Incomplete ossification affecting</u>				<u>p-value</u>	<u>p-value</u>
≥4 skull bones ^a	4(3)	3(3)	25(10)*	1.0000	0.0199
≤3 skull bones	38(14)	37(14)	42(16)	-	-
Cervical vertebral arch(es) ^a	1(1)	0	4(3)	1.0000	0.3347
Thoracic centrum(a)	6(6)	10(6)	6(5)	-	-
Pubis(es) ^a	9(6)	9(7)	21(8)	0.7494	0.5206
Ischium(a) ^a	0	2(2)	7(4)*	0.2333	0.0441
Lumbar vertebral arch(es)	0	1(1)	1(1)	-	-
Sacral vertebral arch(es) ^a	24(13)	25(13)	37(13)	1.0000	0.7667
2 nd and/or 4 th metacarpal(s) ^a	1(1)	1(1)	11(6)	1.0000	0.1027
5 th metatarsal(s)	0	0	1(1)	-	-
<u>Unossified</u>					
2 nd and/or 5 th metacarpal(s)	72(19)	61(19)	84(18)	-	-
5 th metatarsal(s)	0	1(1)	0	-	-
<u>Ossified</u>					
Anterior arch of atlas ossified	44(16)	37(15)	43(15)	-	-
>2 cervical vertebral centra ossified	5(4)	10(3)	3(2)	-	-
One or more sacrocaudal vertebra with connection between centrum and arch(es)	45(15)	29(9)	52(16)	-	-
Phalangeal elements ossified	3(2)	13(4)	5(3)	-	-
Mean number of caudal vertebral centra	3.9	4.0	3.9	-	-
<u>Number of sternebrae retarded:</u>					
0	48(19)	58(18)	57(16)	-	-
1	67(20)	50(19)	61(21)	-	-
2	41(18)	38(15)	32(14)	-	-
>2	2(1)	3(2)	8(4)	-	-
Total number examined skeletal	158(23)	149(22)	158(21)	-	-

* - achieved statistical significance $p \leq 0.05$ using Fisher's Exact Test

a = analysed by Fisher's Exact Test and Kruskal- Wallis

- = not analysed

Group incidence of skeletal ossification parameters in vaccine group II compared to concurrent control and historical range

Abnormality	Group/Treatment		
	1	3	-
	Control	Vaccine II	Control Historical Range
	Incidence of Foetuses (Litters)		
<u>Background Data</u>			
Range taken from 12 studies over 2003-2006			
<u>Incomplete ossification affecting</u>			
<u>≥4 skull bones</u>	4(3)	25(10)	1(1)-7(5)
Pubis(es)	9(6)	21(8)	1(1)-12(6)
Ischium(a)	0	7(4)	0-4(4)
Sacral vertebral arch(es)	24(13)	37(13)	9(7)-29(10)
2 nd and/or 4 th metacarpal(s)	1(1)	11(6)	0-8(4)

Control historical range taken from 12 studies over 2003-2006

Control group data for the incidence of incomplete ossification affecting ≥ 4 skullbones, pubes, ischia, sacral vertebral arches and 2nd and/or 4th metacarpals appeared to be in the range of what is observed in the historical control data (submitted at the request of CBER on April 7, 2008 (STN 125280.02). Except for the incomplete *in utero* ossification in some skeletal regions there were no other parameters that would have suggested *in utero* growth delay. Fetal weight on gestation day 20 in vaccine group II was slightly lower ($3.67 \text{ g} \pm 0.21$) than in the control ($3.71 \text{ g} \pm 0.25$) and the vaccine I group ($3.74 \text{ g} \pm 0.38$), however, it is unclear whether this accounts for the differences observed in ossification. Pup weights at birth was comparable between groups. There appeared to be no vaccine related effect on *in utero* survival of fetuses and there was no incidence of teratogenicity. Also, postnatal growth and development did not appear to be affected (see below) by vaccine administration. Notably, this observation was made in vaccine II, the vaccine treated group that received only 2 and not 3 vaccine administration.

No maternal toxicity was noted in the study. The mean body weight of female F0 dams at GD 20 was $404 \text{ g} \pm 50 \text{ g}$ in the control group, $385 \text{ g} \pm 40 \text{ g}$ in Vaccine I and $385 \text{ g} \pm 45 \text{ g}$ in Vaccine II. Even though the mean body weight of dams in the treated groups was slightly less than the control dams, there was no difference in weights in Vaccine I compared to Vaccine II. In addition, a review of raw data of vaccine group II showed that dams # 100, 114, 115, 116, and 138 with litters in which fetuses exhibited the described incomplete ossifications parameters did not show differences in gestational body weight (Appendix 4) and gestational food consumption (Appendix 7). Furthermore, the mean fetal weight of their offspring was in the range observed of other offspring in this group (Appendix 8). Mistimed pregnancies may result in different ossification status, however, based on body weight all litters were considered by the sponsor to be the gestation age indicated. Gestation length was within the expected range for females that were assigned to the delivery subgroup. In summary, the observed higher incidences of

incomplete ossification of fetuses in vaccine II does not appear to be a vaccine related event.

Hematology (Table 10 & App. 12, 14)

Hematology parameters of pregnant animals evaluated on GD 20 were comparable between the control, vaccine I and vaccine II groups.

Clinical Chemistry (Table 11 & App 12, 14)

Clinical chemistry parameters of pregnant animals evaluated on GD 20 were comparable between the control, vaccine I and vaccine II groups.

Urinalysis (Table 12 & Appendix 12, 15)

There were no treatment related effects on urinary parameters. Sponsor states a slight decrease in urine volume on day 19/20 of gestation in vaccine I adult animals which attained statistical significance. However, an evaluation of water consumption was not performed. There was no treatment related effect on organ weights.

Mating performance, duration of gestation, fertility indices, litter size, pup survival and sex ratio (Littering subgroup)

Overall, there appeared to be no treatment related effect on the mating performance, fertility, duration of gestation, litter size, pup survival or sex ratio in the F1 generation. However, the fertility index (%) in groups Vaccine I and Vaccine II was lower than in the control group, 85%, 85% and 96%, respectively. There was no apparent difference on other interrelated endpoints such as estrous cycle, number of corpora lutea, and number of implantation sites. At the request of CBER, the sponsor submitted on April 7, 2008 (STN 125280.02) historical control data for reproductive indices, however, these did not contain data on fertility indices.

Mating performance and fertility indices

	Group/Treatment		
	1 (Control)	2 (Vaccine 1)	3 (Vaccine 2)
Median no. nights to positive mating sign	3	3	3
No. passing one oestrus	4	3	5
No. passing 2 oestruses	0	0	0
No. females paired	48	48	48
No. females pregnant	46	41	41
Female fertility Index (%)	96	85	85

In the control arm of the littering subgroup, 1 animal showed no positive sign of mating, 1 experienced total litter loss, 1 animal died during parturition, a total of 24 animals were evaluated.

In the vaccine I arm of the littering subgroup, 4 animals were not pregnant, 1 animal had no positive sign of mating, 1 animal had total litter loss, a total of 24 animals were evaluated.

In the vaccine II arm of the littering subgroup, 4 animals were not pregnant, a total of 24 animals were evaluated.

Duration of gestation and overall litter performance

	Group/Treatment		
	Control	Vaccine I	Vaccine II
Number pregnant	23	19	20
Duration of Gestation (days)			
21	8	7	11
22	13	11	9
23	2	1	0
Mean duration	21.7	21.7	21.5
Number of females with live litter	23	19	20
Gestation index (%)	100	100	100
Mean no. of implant sites* per pregnancy \pm SD	14.3 \pm 2.8	14.5 \pm 2.1	15.0 \pm 2.5
Mean total no. of pups* born/litter	13.6 \pm 2.9	13.4 \pm 3.3	14.3 \pm 2.1
No. of males on day 1 of lactation	141 (51)	124 (53)	140 (50)
No. of males on day 1 of lactation	137 (49)	108 (47)	141 (50)

*excludes litters where all pups died

Females assigned to day 21 of lactation necropsy

Survival indices

		Group/treatment		
		Control	Vaccine I	Vaccine II
Birth index	Mean litter (%)	95	92	96
	No. loosing > 2 pups	1	2	2
	No. litters	22	19	20
Live birth index	Mean litter Index (%)	99	98	100
	No. loosing > 1 pups	0	1	0
	No. litters	22	19	20
Viability index Days 0-4	Mean litter Index (%)	91	94	97
	No. loosing > 3 pups	3	1	0
	No. litters	22	19	20
Lactation index Days 4-21	Mean litter Index (%)	99	94	100
	No. loosing > 1 pups	0	1	1

	No. litters	21	19	20
Overall survival index	Mean litter Index (%)	89	90	97
	No. loosing > 4 pups No. litters	3	1	0
		22	19	20

F1 litter and pup weights (Table 18 & App 20-21)

Group mean litter and pup weight (g) evaluated on lactation days 1, 4, 7, 14 and 21 were not affected by treatment and comparable across study groups.

F1 preweaning physical and functional development (Tables 19/20 & App 20-24)

Postnatal physical development was assessed by pinna detachment, upper incisor eruption, open eyes and was assessed for males and females separately. There were no treatment related effects on the physical development of the F1 generation. Similarly, negative geotaxis, auditory function or visual function assessed as developmental parameters were also not affected as a result of treatment with vaccine.

Organ weights (Tables 21-22 and App 25)

Group mean values for body weight, brain, heart, kidneys, liver, lung, spleen and thymus were shown in Table 21 of the submission for the control vaccine I and vaccine II groups of the F0 generation and there were no effects of treatment on any of the organ weights assessed.

Serological analysis

(From: Updated final report on serological analysis (PRNT) of sera from IC51 pre- and post natal developmental toxicity study in rats (----- (b)(4) -----))

Serum samples were measured using a Plaque Reduction Neutralization Test (PRNT) conforming to GLP principles at Intercell, AG, Vienna, Austria. The assay allows detection and quantification of neutralizing anti-Japanese encephalitis virus (JEV) antibodies. Serial dilutions of sera are incubated with a fixed amount of plaque forming units of JEV. The latter are quantified by plaque formation on a ----- (b)(4) ----- . The reduction in plaques gives a measure of the antibody titer which is calculated from the serial serum dilutions. A PRNT₅₀ titer, the reciprocal of the test serum, dilution at which a 50% plaque reduction is observed compared to a sample lacking antibody, is calculated by ----- (b)(4) ----- analysis.

As shown in Tables 3 and 4 reproduced below entitled “ JEV neutralizing antibody levels (GMT) in adults and in fetal and cord blood,” groups 1-3 tested negative prior to the 1st vaccination 3 weeks prior to mating. The control group remained negative at all subsequent time points (i.e., GD 5, GD 20 and LD 21). In vaccine groups I and II on GD 5 (prior to the last test article administration on GD 6) 100 % sera conversion occurred and remained at that level at all subsequent time points. Vaccine group II displayed lower GMTs at day 5 of gestation compared to group Vaccine I because animals in group Vaccine II received only 1 vaccine administration (- 1 week) prior to mating. Fetal and cord blood on GD 20 as well as pup blood on LD 21 showed 100 % seroconversion in vaccine groups I and II.

Table 3: JEV neutralizing antibody levels (GMTs) in adults

adults	GMT values			
vaccination	prior first dose	day 5 of gestation	day 20 of gestation	day 21 of lactation
control	5	5	5	5
scheme 1	5	>609	>578	>611
scheme 2	5	>254	>621	>584

Table 4: JEV neutralizing antibody levels (GMTs) in foetal cord blood and off-spring

foetuses/pups	GMT values		
vaccination	day 20 of gestation	day 21 of lactation, male	day 21 of lactation, female
control	8	5	5
scheme 1	>520	>640	>635
scheme 2	>503	>614	>478

Summary

Under the conditions and design of the study, the Japanese encephalitis vaccine, IC-51, did not affect F0 female fertility, mating performance, embryo-fetal development and postnatal development. There were no overt signs of treatment related maternal toxicity.

Treatment did not affect body weights and body weight gains of the F0 generation neither did it affect body weight gain of the F1 generation born to treated dams. There was no effect on food consumption.

There were no observed treatment related effect on the incidence of major and minor abnormalities and skeletal variants in the offspring of dams treated with the test article. However, in the group of dams that received 2 injections of IC-51 1 week prior to mating and then again on GD6 (Vaccine II), there were delays in *in utero* ossification in some regions (in particular in pelvis and skull) in some of the fetuses. This was not observed in the group treated with 3 injections of vaccines (Vaccine I). Control group data for the incidence of incomplete ossification affecting ≥ 4 skullbones, pubes, ischia, sacral vertebral arches and 2nd and/or 4th metacarpals appeared to be in the range of what is observed in the historical control data. There were no other parameters that would have suggested *in utero* growth delay. Fetal weight on gestation day 20 in vaccine group II was slightly lower ($3.67 \text{ g} \pm 0.21$) than in the control ($3.71 \text{ g} \pm 0.25$) and the vaccine I group ($3.74 \text{ g} \pm 0.38$); however, it is unclear whether this accounts for the differences observed in ossification. Pup weights at birth were comparable between groups. There appeared to be no vaccine related effect on *in utero* survival of fetuses and there was no incidence of teratogenicity. Also, postnatal growth and development did not appear to be affected by vaccine administration. There were no signs of maternal toxicity. The mean body weight of female F0 dams at GD 20 was $404 \text{ g} \pm 50 \text{ g}$ in the control; $385 \text{ g} \pm 40 \text{ g}$ in Vaccine group I and $385 \text{ g} \pm 45 \text{ g}$ in vaccine group II. Thus, even though the mean body weight of dams in the treated groups was slightly less than the control group, there was no difference in weights in Vaccine I compared to Vaccine II. Review of individual animal data of vaccine group II showed that dams # 100, 114, 115, 116, and 138 with litters in which fetuses exhibited the described incomplete ossifications parameters did

not show differences in gestational body weight (Appendix 4) and gestational food consumption (Appendix 7). Furthermore, the mean fetal weight of their offspring was in the range observed of other offspring in this group (Appendix 8). Mistimed pregnancies may result in different ossification status, however, based in body weight all litters were considered to be the gestation age indicated. In addition, gestation lengths were within the expected range for females that were assigned to the delivery subgroup. Together, the observed higher incidences of incomplete ossification of fetuses in vaccine II does not appear to be a vaccine related event.