7. Reproductive Toxicology:

The initial Pharmacology/Toxicology Reviewer, Dr. Will Coulter, reviewed the following studies. Additional comments by the current Reviewer are in italics

7.1 FERTILITY STUDIES - INCLUDING ASSESSMENT OF OVULATION AND CYCLICITY

7.1.1. Oral Fertility Study in Female Rats [Vol 1.25; C-860]

Study No.: TT#95-705-0
Compound: L-748,731 (-000R009)
Formulation: Suspensions prepared daily in 0.5% (w/v) aqueous methylcellulose
Route: Oral gavage at 5 mL/Kg
Dose Levels: Group- 1 2 3 4 5
mg/Kg/day- 0 10 30 100 300
Treatment: From 14 days prior to cohabitation, during cohabitation, and through GD7
Strain: Sprague-Dawley [Crl:CD®(SD) BR], approx. 9 weeks old, 206-295 g body weight
Number: 24F/group
Control Treatment: 0.5 % (w/v) aqueous methylcellulose
Study Site: Merck Research Laboratories, West Point, PA
Date: 12 Jan 95 to 6 Mar 95
GLP/QAU Statement: Both present and signed.

The purpose of this study was to determine the effects of L-748,731 on the fertility of F_0 female rats. Females were housed with one untreated male and limited to a maximum of 20 nights. Confirmed mating was designated GD 0. Body weights were determined at least 12 times during the study. Food consumption was measured over premating days 1-5, 8-12, and gestation days 1-5 and 8-12. Females were observed daily. All surviving females were killed GD 15 and 17 and viscera of the thoracic and abdominal region examined.

RESULTS AND DISCUSSION

- treatment related mortality: 2G3 sacrificed; 4G4 found dead, 2G4 sacrificed; 3G5 sacrificed,
- body weight gain: treatment related ↓ in G5 (23%) during premating - ↓ G2-5 (15%, 23%, 15%, 29% vs. control, respectively) during GD8-15-
- food consumption: no significant changes noted-
- treatment physical signs: reddish-brown ocular/nasal discharge, urine staining, soft feces, unkempt coat seen in the treatment related mortality animals - these signs also noted at times in other females-
- mating performance: nonpregnant: 2G1, 2G2, 2G3, 3G4, 6G5-
  pregnant F/mated F : 92%G1, 91%G2, 90%G3, 84%G4, 71%G5*-
  pregnant F/F cohabited: 92%G1, 88%G2, 90%G3, 80%G4, 68%G5*-
- corpora lutea: 390G1, 333G2, 266G3, 209G4, 206G5-
- corpora lutea/pregnant F: 17.7G1, 15.9G2*, 14.0G3*, 14.9G4*, 15.8G5*-
- treatment-related ↑ in peri/postimplantation loss, ↓ number of implants, and live fetuses/pregnant F in all drug treated groups - all but G2 were outside historical control range-
- gross findings in F_0 F: treatment related- ulcers in jejunum/ileum: 3G3, 5G4, 4G5-
  treatment related- peritonitis/adhesion: 4G3, 10G4, 6G5-
- histopath: ulcers in stomach, small intestine, and/or large intestine *with or* without peritonitis seen in all treated groups except G2, peritonitis was local and frequently widespread
- no treatment related ovarian histological changes reported in treated groups-
- the no effect level for GI lesions was 10 mg/Kg/day-

Maternal toxicity was seen at >10 mg/Kg/day as decreased body weight gain, physical signs (red-brown discharge from eyes and nose, urine staining, soft feces), peritonitis, ulcers, adhesions, and death.

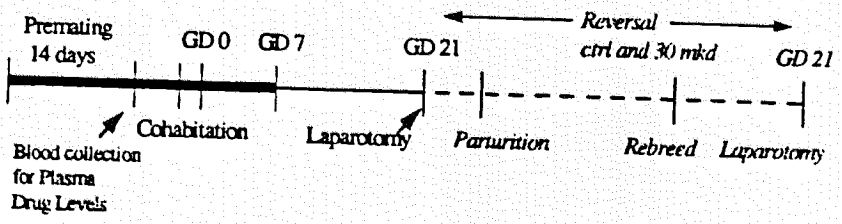
The decrease in the fertility and fecundity indices was significant in the 300 mg/Kg/day group. Embryo and fetal toxicity occurred in all drug groups and included reduced number of corpora lutea, implants, live and dead fetuses, as well as an increase in the number of resorptions. From the results, the sponsor stated the NOAEL could not be established - we would agree.

The current Pharmacology/Toxicology Reviewer reviewed the following studies

7.1.2. L-748,731: Oral Fertility Study in Female Rats with a Recovery Period [Vol. 1.25; p. 926]

Study Identification: TT #95-721-0
Site: Merck Research Laboratories, West Point, PA
Study Dates: May 15 – Aug. 18, 1995
Formulation and Lot No.: L-748,731-000R009; [redacted]
Vehicle - 0.5% aqueous methylcellulose
Certificate of Analysis Submitted: No (X) Uniformity was assessed during Drug Week 1 and concentration during Drug Weeks 1 and 4; results were within acceptable limits, according to the Sponsor
Final Report (X) Mar 8, 1996
GLP and QA Statements Signed: Yes (X)
Objective: "The objective of this study was to evaluate the effects of L-748,731 administration on the fertility of F_0 female rats and to determine the reversibility of these effects."

The diagram of the study design, provided by the Sponsor, is included below.



All animals were treated 14 days prior to and during cohabitation, and through GD 7.

Test Material/ Group Designation	Dose and Regimen#				N	Sex	Species/ Strain
	mg/kg	ml/kg	Route	# of doses			
Group 1 -VH Control	-	5	oral, gavage	see above	48*	F	Sprague-Dawley - [Crl:CD®(SD)BR] [redacted]
Group 2 - L-748-731	1				24		
Group 3 - L-748-731	3				24		
Group 4 - L-748-731	10				24		
Group 5 - L-748-731	30				48*		

*24 animals were assessed for fertility and embryonic/fetal survival on GD 21; 24 underwent parturition and were rebred,
Natural breeding with cohabitation limited to ≤20 nights
#Animals were fed *ad libitum*

Parameter Evaluated	Time Point(s)
Toxicokinetic Parameters - 4 nonfasted anesthetized rats/ treated group/timepoint	Premating Day 10 - 1, 2, 4, 6, 10, and 24 hours
Cinical observations	Daily from Day 0 - GD 21 or LD0 During treatment - prior 1 and 1-5 hours after dosing Natural delivery group - GD 21- LD 0 - 4X/day 2nd Premating/gestation - mortality/daily, physical signs on weigh days
Body weight	Premating - Days 1, 4, 8, 11, and 14 No indication of mating - Days 22, 29, 36, 43, 50, and 55 During gestation - GD 0, 2, 4, 6, 8, 12, 15, 18, and 21 Lactation Day 0 2nd Premating - Days 1, 7, and 14 No indication of mating - Days 21, 28, 35, 42, 49, and 56
Food consumption	1st and 2nd Premating Days 1-5 and 8-12, GD 1-5, 8-12, and 15-19
Laparotomy - 1st and 2nd mating - Uterine/implantation data - corpora lutea, ovarian weights, gravid uterine weights, no. of implantations, early and late resorptions, viable and dead fetuses, placental morphology	Bred females on GD 21 Females with no indication of breeding Premating day 57 [Postcohabitation Day 23]
Fetal Examination (section subgroup) - C-sxn - fetal weight, sex distribution, gross external examination - Natural delivery - no., physical signs, gender	GD 21 PND 0
Necropsy after 1st and 2nd mating- thoracic and abdominal viscera	
Parturition Parameters - onset and completion, signs of difficulty	GD21-LD0

Results -

Mortality - One of twenty-four and 10/48 females at 10 and 30 mg/kg/day were either sacrificed or found dead secondary to treatment related effects from 1st Premating Day 11 - 1st GD 8.

Clinical Signs - The following signs, reddish-brown ocular/nasal discharge, urine staining, soft/no feces, dark-colored urine, and/or decreased activity, were observed in all females prematurely dying or undergoing unscheduled sacrifice. In addition, 5 females at 30 mg/kg/day that survived to terminal sacrifice exhibited signs. Signs were observed 1-5 days prior to death or for 1-7 days in the surviving rats.

Body Weight - Body weight gain was decreased compared to control values at 30 mg/kg/day by 40% during Premating Days 1-14 [most pronounced from Day 9-14] and by 23% for GD 0-8. The decrease in body weight gains at 10 and 30 mg/kg/day during GD 8-21 was attributed to an increase in embryonic/fetal loss [e.g. decrease in gravid uterine weights]. The absolute body weights were decreased by <10% through GD 15 and by ≤15% GD16-21. During the second phase of this study, body weight gain was comparable between the control and high dose groups.

Food Consumption - There was approximately a 10-15% decrease in food consumption when compared to controls in the high dose group on GD 5, 12, and 19.

Mating Performance and Fertility - Mating performance and fecundity and fertility indices in the treated groups were comparable to the respective controls for both the 1st and 2nd cohabitation.

Parturition - There was a slightly increased average length of gestation at 30 mg/kg/day [22.8 days] vs. controls [22.2 days]. The Sponsor states that this "was a reflection of the treatment-related decrease in litter size" and this conclusion is supported by their historical control data.

Embryonic/Fetal Survival - At 10 and 30 mg/kg/day, there were statistically significant decreases in the average numbers of implants and numbers of live fetuses per pregnant female following the 1st mating. This was associated with an increase in the number of resorptions and dead fetuses per pregnant female, an increase in the percentages of peri- and post-implantation loss, and a decrease in live pups per litter. Embryonic/fetal survival was comparable in the treated and control groups following the 2nd mating. The sex ratios difference at 30 mg/kg/day could be attributed to 2/5 litters having only 1 male. This finding was of questionable significance. The table below delineates these values.

Fetal Parameter	Treatment Group [mg/kg/day]		
	0	10	30
% peri-implantation loss	11.7	28.2*	66.1*
Implants/pregnant female	14.9	11.9*	5.7*
% resorptions/implants [litter mean]	3.8	19.0	48.9
% postimplantation losses	3.8	19.0*	56.1*
Live fetuses/pregnant female	14.4	10.0*	3.9*
Gravid uterine weight	94.5 ± 19.8	72.1 ± 30.6	30.4 ± 32.2

Fetal and Pup Weight - There were no treatment related effects. Pup weights in the 30 mg/kg/day group tended to be greater than the comparable controls which is considered secondary to the decrease in litter size and not to a direct treatment related effect on weight.

Fetal and Pup External Examinations - There were no treatment related effects.

Necropsy - In 1 and 10 females administered 10 and 30 mg/kg/day, respectively, which died or prematurely underwent unscheduled sacrifice, there was gross evidence of peritonitis secondary to gastrointestinal ulceration. Intestinal ulceration and peritonitis was also observed in 3 and 4 rats sacrificed after the 1st mating at 10 and 30 mg/kg/day, respectively, and in 6 females at 30 mg/kg/day rats sacrificed after the 2nd mating. Other treatment related findings included enlarged mesenteric lymph nodes and/or spleen [10 rats] and transmural nonglandular gastric ulcers [3 rats, 1 with peritonitis].

Ovarian Weight - There was a slight decrease [7.4%] in absolute ovarian weight in pregnant females sacrificed after the 1st mating. This change was generally observed in females with increased fetal resorptions.

Toxicokinetic Parameters - The table below outlines the AUC, t_{max}, and C_{max} for this study obtained from samples collected on premating day 10.

Dose [mg/kg/day]	Parameter		
	C_{max} [µg/ml]	T_{max} [hour]	AUC [µg•hr/ml]
1	0.19	1.0	1.72
3	0.88	1.0	8.16
10	3.03	2.0	32.83
30	6.19	4.0	81.47

Reviewer's Comments [Study Design and Data Presentation] – Based on the stated objective, the study design and data presentation were considered adequate.

Sponsor's Conclusions (numbered) and Reviewer's Comments -

1. The NOAEL for both maternal toxicity and embryofetal toxicity was 3 mg/kg/day. **Reviewer's Comment** – The incidence of toxicity/mortality was higher at comparable doses in this study than the preceding study. Embryo-fetal toxicity was characterized by a decrease in implants and live pups/litter and an increase in dead fetuses/pregnant female, resorptions, and peri/post-implantation loss.

2. The treatment-related effects on embryofetal survivability were reversed after a 14 day recovery period. **Reviewer's Comment** – The Reviewer concurs.

7.1.3. L-748,731: Oral Ovulation/Fertilization Study in Female Rats [Vol. 1, 26; p. C-1214]

Study Identification: TT #95-715-0
Site: Merck Research Laboratories, West Point, PA
Study Dates [In-life]: April 3 - May 6, 1995
Formulation and Lot No.: L-748,731-000R009; [redacted]
Vehicle - 0.5% methylcellulose
Certificate of Analysis Submitted: No (X) Assayed for uniformity during Drug Week 1; assayed for concentration Drug Weeks 1 and 3; assays were within acceptable limits, according to the Sponsor
Final Report: Oct. 11, 1995
GLP and QA Statements Signed: Yes (X)
Objective: 'The objective of this study was to evaluate the effects of L-748,731 on ovulation and fertilization following administration of the compound to F_0 female rats.

Test Material/ Group Designation	Dose and Regimen#				N	Sex	Species/ Strain
	mg/kg	ml/kg	Route	# of doses			
Group 1 -VH Control	-	5	oral gavage	14 days prior to cohabitation through cohabitation* until confirmed breeding	24	F	Sprague-Dawley - [Crl:CD®(SD)BR] [redacted] App. 9 wks at study start 202-259 g
Group 2 - L-748-731	10						
Group 3 - L-748-731	30						

#fed *ad libitum*
*cohabitation limited to 20 nights

Parameter Evaluated	Time Point(s)
Clinical observations	Daily During treatment - prior to and 1-5 hours post dosing
Body weight	Premating interval - Days 1, 4, 8, 11, and 14 If no mating - Days 22 and 29 GD0
Necropsy-thoracic and abdominal viscera *in situ*	GD1
Ovulation/Fertilization Assessment - no. corpora lutea, no. embryos/unfertilized oocytes	GD1
Ovary weights and histopathology	GD1

Results-

Mortality - There were no deaths in any group

Clinical Observations - Treatment related signs in 4/24 females at 30 mg/kg/day included brown nasal discharge, brown staining of the forepaws, urine staining, and soft feces. At necropsy, 3/24 females were found to have peritonitis.

Body Weight Changes - There were no treatment-related effects.

Mating Performance and Fertility Parameters - The increase in time to mating, which was observed in the 30 mg/kg/day group [1.46 vs. 1.04 in controls], was attributed to 2 pseudopregnant females. Based on [1] a lack of effect on time to mating up to a dose of 300 mg/kg/day in Study TT #95-705-0 and [2] the occasional observance of pseudopregnancy in their lab, the Sponsor concluded that the increase in time to mating was an incidental finding.

There was a statistically nonsignificant decrease in fecundity and fertility at 30 mg/kg/day compared to controls.

Ovulation/Fertilization Parameters - There were statistically significant decreases in the average numbers [1] of corpora lutea per female; [2] preimplantation embryos and/or unfertilized oocytes per females at ≥10 mg/kg/day.

There was a nonsignificant dose dependent increase in ovulation failure [no. of corpora lutea - no. of preimplantation embryos and/or unfertilized oocytes per female]. There were 14, 16, and 17 rats at 0, 10, and 30 mg/kg/day that exhibited some degree of ovulation failure. In the control animals, 2 had ovulation failure values >3. In the 10 and 30 mg/kg/day groups, there were 6 and 7, respectively, which had ovulation failure values >3.

The table below provides the values for the parameters that demonstrated changes.

Parameter	Dose [mg/kg/day]		
	0	10	30
Time to mating [4-day periods] - days	1.04 ± 0.20	1.12 ± 0.34	1.46 ± 1.18
Fertility index -%	96	91	83
Fecundity index - %	96	91	83
# of corpora lutea/F	17.1 ± 2.4	14.8 ± 2.5*	14.0 ± 4.8*
# of preimplantation embryos and/or unfertilized oocytes/F	15.5 ± 2.7	12.8 ± 3.8*	11.1 ±4.7*
Ovulation failure	1.6 ± 2.1	2.0 ± 2.2	3.0 ± 3.3[a]

*denotes statistical significance
[a]p=0.055

Necropsy - At 30 mg/kg/day, there were 4 females which exhibited treatment related peritonitis. All other findings were sporadic and were considered incidental by the Sponsor.

Ovarian Weights and Histopathology - There were no treatment related effects.

Reviewer's Comments [Study Design and Data Presentation]: For the stated purpose, the study design and data presentation were adequate.

Sponsor's Conclusions (numbered) and Reviewer's Comments:

1. "[T]here was a treatment-related, statistically significant decrease in both the average numbers of corpora lutea and numbers of preimplantation embryos and/or unfertilized oocytes per female compared to control." **Reviewer's Comment** – The Reviewer concurs.

2. The decrease in preimplantation embryos and/or unfertilized oocytes at 10 mg/kg/day compared to controls is secondary to treatment related decrease in corpora lutea. At 30 mg/kg/day, however, this decrease in preimplantation embryos and/or unfertilized oocytes is a function of both a treatment-related decrease in corpora lutea as well as a "partial inhibition of ovulation". **Reviewer's Comment** – Although the number of rats demonstrating ovulation failure was comparable across all groups, the magnitude of the failure was mildly increased at both 10 and 30 mg/kg/day.

3. Fertilization was not affected by administration of L-748,731 up to doses of 30 mg/kg/day administered for 14 days prior to cohabitation. **Reviewer's Comment** - There was a mild, nonsignificant decrease in both fertility and fecundity at 30 mg/kg/day.

Additional Reviewer Comments – The NOAEL for maternal toxicity [GI ulceration/peritonitis] is 10 mg/kg/day. The NOAEL for embryo-fetal toxicity is <10 mg/kg/day.

7.1.4. L-748,731: Oral Estrous Cyclicity/Ovulation/Fertilization Study in Female Rats [Vol. 1.26: C-1316]

Study Identification: TT #95-724-0
Site: Merck Research Laboratories, West Point, PA
Study Dates [In-life]: May 20 - July 24, 1995
Formulation and Lot No.: L-748,731-000R014;
Vehicle - 0.5% methylcellulose
Certificate of Analysis Submitted: No (X) Assayed for uniformity during Drug Week 1; assayed for concentration Drug Weeks 1 and 3; assays were within acceptable limits, according to the Sponsor
Final Report: Dec. 22, 1995
GLP and QA Statements Signed: Yes (X)
Objective: "The objective of this study was to evaluate the effects of L-748,731 on estrous cyclicity, ovulation, and fertilization following administration of the compound to F_0 female rats".

Test Material/ Group Designation	Dose and Regimen#				N	Sex	Species/ Strain
	mg/kg	ml/kg	Route	# of doses			
Group 1 -VH Control	-	5	oral gavage	app. 20 days prior to cohabitation -ovarian follicular development through cohabitation until confirmed breeding -daily	24	F	Sprague-Dawley - [Crl:CD®(SD)BR] App. 9 wks at study start 168-259 g
Group 2 - L-748-731	10						
Group 3 - L-748-731	30						

#fed *ad libitum*, dosing begun in proestrus

Parameter Evaluated	Time Point(s)
Clinical observations	daily during treatment - prior to and 1-5 hours post-dosing
Body weight	Pretreatment Days 1, 7, 14, 21 Premating interval Days 1, 4, 8, 11, and 14 If no indication of mating during cohabitation - Days 22, 29, 36, and 43 GD0
Necropsy-thoracic and abdominal viscera *in situ*	GD 1
Ovulation/Fertility - no. corpora lutea, no. of preimplantation embryos and/or unfertilized eggs	GD 1
Ovary weights and histopathology	Gd 1

Results -

Mortality - Four of 24 animals dosed at 30 mg/kg/day underwent unscheduled sacrifice or died prematurely after receiving 9-23 doses.

Clinical Observations - Sign[s] included brown oculonasal discharge and urine staining and were observed in 5/24 females at 30 mg/kg/day. Signs occurred between Premating Days 9-17. Peritonitis was observed at necropsy in all animals exhibiting treatment-related signs.

Body Weight- The decrease in weight gain at 30 mg/kg/day compared to controls [-14%] was not statistically significant.

Estrous Cyclicity - There were no treatment related effects.

Mating Performance and Fertility - There were no statistically significant treatment related effects.

Ovulation/Fertilization - A statistically significant decrease in the average numbers of corpora lutea and preimplantation embryos and/or unfertilized oocytes per female and a subsequent increase in ovulation failure indices was observed in rats at ≥10 mg/kg/day when compared to controls.

The table below provides the values for the parameters that demonstrated changes.

Parameter	Dose [mg/kg/day]		
	0	10	30
No. of corpora lutea per female	16.9 ± 1.82	15.0 ± 2.65*	14.1 ± 2.79*
No. of preimplantation embryos and/or unfertilized oocytes per female	15.3 ± 1.84	11.7 ± 3.03*	9.5 ± 4.16*
Ovulation failure	1.6	3.4*	4.7*

Necropsy - Five high dose and 2 low dose females exhibited peritonitis/intestinal ulceration.

Ovary Weight - There were no treatment related effects.

Reviewer's Comments [Study Design and Data Presentation] – For the stated objective, study design and data presentation were adequate.

Sponsor's Conclusions [numbered] and Reviewer's Comments

1. L-748,731 at ≥10 mg/kg/day, when administered prior to cohabitation, did not induce changes in estrous cyclicity or fertilization.

2. L-748,731, when administered at ≥10 mg/kg/day prior to cohabitation, resulted in a decrease in corpora lutea and the number of preimplantation embryos and/or unfertilized oocytes per female and a subsequent partial inhibition of ovulation.

Reviewer's Comment - The Reviewer concurs with the Sponsor.

7.1.5. L-748,731: Oral Fertility Study in Female Rats with Gestation Days 15 and 21 Laparotomies [Vol 1.26; p. C-1379]

Study Identification: TT #95-739-0
Site: Merck Research Laboratories, West Point, PA
Study Dates [In-life]: Nov. 6 - Dec. 30, 1995
Formulation and Lot No.: L-748,731-000R009; [redacted]
Vehicle - 0.5% methylcellulose
Certificate of Analysis Submitted: No (X) Assayed for uniformity during Drug Week 1; assayed for concentration Drug Weeks 1 and 4; assays were within acceptable limits, according to the Sponsor
Final Report: May 20, 1996
GLP and QA Statements Signed: Yes (X)
Objective: "The objective of this study was to evaluate the effects of L-748,731 oral administration on the fertility of F_0 female rats".

Test Material/ Group Designation	Dose and Regimen#				N	Sex	Species/ Strain
	mg/kg	ml/kg	Route	# of doses			
Group 1 -VH Control	-	5	oral gavage	app. 14 days prior to and during cohabitation*, until GD7	48**	F	Sprague-Dawley - [Crl:CD®(SD)BR] App. 9 wks at study start 203-283 g
Group 2 - L-748-731	10						
Group 3 - L-748-731	30						

#fed *ad libitum*
*limited to 20 nights
**24 sacrificed at each scheduled time point [GD 15 and 21]

Parameter Evaluated	Time Point(s)
Clinical observations	daily during treatment - prior to and 1-5 hours post-dosing
Body weight	Premating interval Days 1, 4, 8, 11, and 14 If no indication of mating - during cohabitation on Days 22 and 29 and after cohabitation on Days 36, 43, and 49 GD0, 2, 4, 6, 8, 12, 15, 18, and 21
Necropsy-thoracic and abdominal viscera *in situ*	GD 15 and 21
Fertility - no. corpora lutea, no. of implants, live and dead fetuses, resorptions	GD 15 and 21
Ovary weights	GD 15 and 21

Results -

Mortality – Treatment-related premature deaths/unscheduled sacrifices were seen in 1/48 and 5/48 females at 10 and 30mg/kg/day, respectively, after 22 - 27 doses of L-748,731.

Clinical Observations - Clinical signs in premature decedents included brown oculonasal discharge and/or urine staining.

Body Weight- There was a statistically significant decrease in body weight gain Premating Days 1-14 and GD 0-8 in females at 30 mg/kg/day when compared controls. Body weight gain [absolute body weight] was 24 ± 8 g [259 ± 20 g] and 19 ± 8 g [257 ± 18 g] for 0 and 30 mg/kg/day groups. Decreases in body weight gain in rats administered 10 and 30 mg/kg/day during GD 8-21 were considered to be secondary to a treatment related increase in embryonic/fetal loss.

Mating Performance and Fertility - There were no statistically significant treatment related effects on time to mating. There was a dose-dependent decrease in fecundity and fertility that reached statistical significance at 30 mg/kg/day.

Embryonic/Fetal Survival - There were no treatment related effects on the number of corpora lutea at either the GD 15 or 21. At both GD 15 and 21, there was a decrease in the average number of implants and live fetuses with a concomitant increase in the number of resorptions, dead fetuses, and peri- and post-implantation losses in the treated groups compared to controls. Four litters from females dosed with 30 mg/kg/day underwent total resorption and/or fetal death.

Gross Abnormalities/Malformations The craniorachischisis in 1/177 live fetuses from rats administered 10 mg/kg/day was considered incidental.

Ovarian Weights - There were no treatment-related effects.

The table below provides the values for the parameters that demonstrated changes.

Parameter	Dose [mg/kg/day]		
	0	10	30
Fecundity index#	94	80	73*
Fertility index#	92	79	70*
% perimplantation loss [litter mean]	5.1 [8.6]	18.5 [34.0]	48.2 [52.0]
Implants/pregnant female	17.8 [16.5]	14.3 [11.6]	9.0 [8.4]
% resorptions/implants [litter mean]	5.4 [5.9]	15.7 [18.3]	38.2 [38.2]
% dead fetuses/implants [litter mean]	0 [0]	1.3 [1.0]	2.6 [1.4]
% post implantation loss [litter mean]	5.4 [5.9]	16.9 [19.2]	40.8 [39.6]
Live fetuses/pregnant female	16.8 [15.6]	11.9 [9.3]	5.5 [4.9]

#averages for GD 15 and 21
*indicates statistical significance, statistical analysis was not conducted on all parameters

Reviewer's Comments [Study Design and Data Presentation] – For the stated objective, study design and data presentation were adequate.

Sponsor's Conclusions [numbered] and Reviewer's Comments

1. Maternal toxicity following administration of L-748,731 was characterized by GI toxicity [ulceration/peritonitis] associated with clinical signs and mortality [≥10 mg/kg/day], and decreased body weight [30 mg/kg/day]. Fecundity/fertility indices were significantly decreased at 30 mg/kg/day. **Reviewer's Comment** – Although the change in fecundity/fertility was not statistically significant at 10 mg/kg/day, the decrease was considered a treatment-related effect.

2. Embryonic/fetal survival was decreased following administration of L748,731 prior to mating and through GD 7 at ≥10 mg/kg/day.

Additional Reviewer's Comment – The NOAEL for maternal and embryo-fetal toxicity was not determined but was <10 mg/kg/day.

7.1.6: L-748,731: Oral Fertility Study in Male Rats [Vol. 1.30; p. C-2786]
Study Identification: TT #95-734-0
Site: Merck Research Laboratories, West Point, PA
Study Dates: Oct. 16 - Dec. 8, 1995
Formulation and Lot No.: L-748,731-000R009;
Vehicle - 0.5% methylcellulose
Certificate of Analysis Submitted: No (X) Assayed for uniformity Drug Week 1; assayed for concentration Drug Weeks 1, 6, and 8; all results were within acceptable limits, according to the Sponsor
Final Report: Jun. 6, 1996
GLP and QA Statements Signed: Yes (X)
Objective: "To evaluate the effects of oral administration of L-748,731 on the fertility of F_0 male rats".

Test Material/ Group Designation	Dose and Regimen#				N	Sex	Species/ Strain
	mg/kg	ml/kg	Route	# of doses			
Group 1 -VH Control	-	5	oral gavage	once daily X 4 wks prior to cohabitation; during cohabitation until sacrifice [app. 9 weeks]	32*	M	Sprague-Dawley - [Crl:CD®(SD)BR] App. 11[6] wks at study start for M[F] 284-360 g at start of study for M 206-274 at start of cohabitation for F
Group 2 - L-748-731	10						
Group 3 - L-748-731	30						
Group 4 - L-748-731	100						

#females fed *ad libitum*, males fed approximately 24 g/day
*25 males cohabited, cohabitation limited to 10 nights

Parameter Evaluated	Time Point(s)
Clinical observations -Males -Females **Mortality checks**	prior to and 1 hour post dosing from start to sacrifice body weight days daily
Body weight - Males - Females	2X/weeks premating, GD 0, 7, 15
Food consumption	observation
Female reproductive parameters - pregnancy status, no. of corpora lutea, no. of implants, live and dead fetuses, and resorptions	GD 15-17
Necropsy - abdominal and thoracic viscera *in situ*	Drug Week 8
Organ weights - left epididymis, combined testes	Drug Week 8
Histopathology - right epididymis, testes	Drug Week 8
Male reproductive parameters - epididymal sperm head quantitation, sperm motility [N=16]	Drug Week 8

Results

Mortality - There were no deaths during the study.

Clinical Observations - There were no treatment-related effects.

Body Weight - There were no treatment-related effects.

Food Consumption - There were no treatment related effects.

Mating Performance and Fertility - There were no treatment-related effects.

Embryonic/Fetal Survival - There were no treatment-related effects.

Sperm Analysis - There were no treatment-related effects.

Organ Weights - There were no treatment-related effects.

Necropsy and Histopathology - There were no treatment-related effects.

Reviewer's Comments [Study Design and Data Presentation] – For the stated objective, study design and data presentation were adequate. Based on the fact that there was no toxicity [e.g. GI lesions] observed in this study, there is concern that adequately high doses were not used. This dose represents a 20[7] multiple of the human exposure based on AUC.

Sponsor's Conclusion [numbered] and Reviewer's Comment
1. There were no effects on male fertility [mating performance, fertility indices, embryonic/fetal survival, sperm count and motility, testicular/epididymal organ weights, histopathology] at doses up to 100 mg/kg/day. **Reviewer's Comment** – The Reviewer concurs.

7.2 IMPLANTATION STUDY

7.2.1. L-748,731: Oral Implantation Study in Rats [Vol. 1.26; p. C-1270]

Study Identification: TT #95-716-0
Site: Merck Research Laboratories, West Point, PA
Study Dates [In-life]: April 2-20, 1995
Formulation and Lot No.: L-748,731-000R009; [redacted]
Vehicle - 0.5% methylcellulose
Certificate of Analysis Submitted: No (X) Assayed for uniformity during Drug Week 1; assayed for concentration Drug Weeks 1 and 2; assays were within acceptable limits, according to the Sponsor
Final Report: Oct. 11, 1995
GLP and QA Statements Signed: Yes (X)
Objective: "The objective of this study was to evaluate the effects of L-748,731 on embryonic/fetal survival following administration of the compound to F_0 female rats during the pre/peri-implantation period".

Test Material/ Group Designation	Dose and Regimen#				N	Sex	Species/ Strain
	mg/kg	ml/kg	Route	# of doses			
Group 1 -VH Control	-	5	oral gavage	Initiated after ovulation/fertilization GD1-7	24	F	Sprague-Dawley - [Crl:CD®(SD)BR] [redacted] App. 10 wks at study start 219-320 g
Group 2 - L-748-731	10						
Group 3 - L-748-731	30						

#fed *ad libitum*

Parameter Evaluated	Time Point(s)
Clinical observations	daily during treatment - prior to and 1-5 hours post-dosing
Body weight	GD 0, 1, 3, 5, 7, 8, 12, and 15
Necropsy-thoracic and abdominal viscera *in situ*	GD 15
Fertility/Implantation - no. corpora lutea, no. of implants, live and dead fetuses, resorptions	GD 15
Ovary weights and histopathology	GD 15

Results-

Mortality - The cause of death in 1 female at 30 mg/kg/day was undetermined.

Clinical Observations - There were no treatment-related effects.

Body Weight - There was a statistically significant decrease in mean body weight gains observed from GD 8-15 at ≥10 mg/kg/day. The control value was 54 ± 11 g compared to 46 ± 9 gm and 42 ± 9 g at 10 and 30 mg/kg/day. This decrease may, in part, be a function of the increase in embryonic/fetal loss in the treated groups.

Embryonic/Fetal Survival - The number of corpora lutea were comparable in all groups. The percentage of pre/peri-implantation loss was significantly increased in the 30 mg/kg/day with an associated decrease in implants/pregnant female. There was a statistically significant decrease in the number of live fetuses and a statistically significant increase in the percentage of postimplantation losses at ≥10 mg/kg/day.

The table below provides the values for the parameters that demonstrated changes.

Parameter	Dose [mg/kg/day]		
	0	10	30
% pre/peri-implantation loss [litter mean]	10.3	10.8	18.3*
Implants/pregnant female	16.2	16.3	15.0
% resorptions/implants [litter mean]	8.2	29.0	53.1
% postimplantation loss [litter mean]	8.2	29.5*	53.7*
Live fetuses/pregnant female	14.9	11.6*	7.2*

Necropsy - Vaginal mucosa hyperemia and the presence of a red crusty material [dried blood] in the high dose female that died prematurely were supportive of fetal resorption. Peritonitis was observed in 2 of the females dosed at 30 mg/kg/day. A thick, dark red-to-black fluid in the vagina was associated with embryonic/fetal loss. This finding was seen 4/23 surviving females at 30 mg/kg/day.

Ovarian Weights and Histopathology - There was 9.8% and 12.9% decrease in ovarian weights in the 10 and 30 mg/kg/day groups, respectively, compared to controls. There were no treatment related histopathological changes.

Reviewer's Comments [Study Design and Data Presentation] – For the stated objective, study design and data presentation were adequate.

Sponsor's Conclusions [numbered] and Reviewer's Comment

1. Treatment of rats at ≥10 mg/kg/day during the pre/peri-implantation period of gestation resulted in decreased embryonic/fetal survival. **Reviewer's Comment** – The Reviewer concurs. The NOAEL for maternal toxicity [GI ulceration/peritonitis] was 10 mg/kg/day. The embryo-fetal toxicity NOAEL [increase in pre/peri-implantation and post implantation losses] was <10 mg/kg/day.

7.3. DEVELOPMENTAL STUDIES –*The following studies were reviewed by the initial Pharmacology/Toxicology Reviewer, Dr. Will Coulter. Additional comments by the current Pharmacology/Toxicology Reviewer are in italics.*

7.3.1 Developmental Studies - Rabbits

7.3.1.i. ORAL RANGE-FINDING STUDY IN NON-PREGNANT RABBITS [Vol. 1.23; p. C-142]

Study: TT #94-737-6
Compound: L-748,731, lot L-748,731-000R009
Formulation: Suspensions prepared daily in 0.5% methylcellulose (w/v) in deionized water
Route: Oral, gavage at 4 mL/Kg based on recent body weight
Dose Levels: Group 1 2 3 4 5
mg/Kg/day 0 10 30 90 270 for 14 days
Strain: NZW, approx. 24 weeks old, 2916 to 3660 g body weight a initiation
Number: 6F/group
Control Treatment: 0.5% methylcellulose at 4 mL/Kg
Study Site: Merck Sharp & Dohme Research Laboratories, West Point, PA
Date: 5 Oct 94 - 20 Dec 94
GLP/QAU Statements: Not present.

The purpose of this study was to determine the dose levels for a subsequent range-finding study in pregnant rabbits. Animal observation was twice daily. G5 was improperly dosed D1 and replaced. Body

weight was recorded D1, 3, 5, 7, 9, 11, 13, and 15. Hematology and serum biochemical parameters were evaluated D15, 24 h after the last dose. All animals were killed D15 without examination.

RESULTS AND DISCUSSION

- no deaths during study-
- mean weight gain was greater in treated groups-
- ↓G4 (5/6) RBCs, Hb, Hct (10%, 9.2%, 8.5%, respectively) - no change in other groups-
- ↑1/6 G4 in SUN, creatinine, AST, ALT (55%, 24%, 96%, 91%, respectively) - other groups appeared ok-

The high dose showed a slight decrease in the erythroid parameters and perhaps an increase in some of the serum parameters; other than that, the 270 mg/Kg/day level was well tolerated. Doses of 25, 75, 150, and 300 mg/Kg/day were recommended for the study (TT#94-737-5) in pregnant rabbits.

7.3.1.ii. ORAL RANGE-FINDING STUDY IN PREGNANT RABBITS [Vol. 1.23; p. C-183]

Study: TT#94-737-5
Compound: L-748,731, lot L-748,731-000R009[redacted]
Formulation: Suspension in 0.5% methylcellulose (w/v) in deionized water
Route: Oral, gavage at 4 mL/Kg/day on GD7 through GD20
Dose Levels: Group 1 2 3 4 5
mg/Kg/day: 0 25 75 150 300
Strain: NZW, approx. 28 weeks old, 3196 to 4307 g body weight
Number: 10F/group
Control Treatment: 0.5% methylcellulose at 4 mL/Kg/day
Study Site: Merck Research Laboratories, West Point, PA
Date: 9 November 1994 - 28 December 1994
GLP/QAU Statements: Not present-

The purpose of this study was to determine the dose levels to be used in a subsequent developmental toxicity study. Animals were observed daily. Body weight was recorded GD 0, 7, 9, 11, 13, 15, 17, 19, 21, 24, and 28. Food consumption was measured GD 4, 8, 9, 10, 12, 16, 20, 24, and 28. Hematology and serum biochemical parameters were determined on GD 21, approximately 24 h after dosing.. All animals were killed GD 28 and gross necropsy conducted on the thoracic and abdominal viscera. Fetuses were weighed and examined externally. Statistical methods used were analysis of variance or covariance and trend analysis.

RESULTS AND DISCUSSION

F_o DATA

- no deaths-
- no abortions-
- physical signs: *dose related increase in* blood staining in cage and pan of G3, 4, and 5 during mid/late pregnancy- *[Sponsor indicates that this finding tended to correlate with an ↑ in the number of resorptions]*
- body weight: ↓*4% in G4 and 5-
- food consumption: no treatment related changes-
- hematology: G5- 5.8% ↑ in RBCs, serum protein and albumin – [*Sponsor considered this to be 2° to dehydration]*
  G5- 16% ↑ lymphocytes
  G4 and 5 ↑ WBCs (5.9% and 23.9%, respectively) - treatment related-
  ↑ absolute neutrophils (14.4% and 40%, respectively)-
  ↑ basophils (10.8% and 66.2% respectively-

serum biochemistry:

Treatment Related Changes Compared to Control[a]

	75 mg/Kg/day	150 mg/Kg/day	300 mg/Kg/day
Urea	+ 30.8%	+ 30.8%	+ 69.2%
Creatinine	+ 7.7%	+ 7.7%	+ 30.7%
Protein		+ 7.1%	+ 10.7%
Albumin		+ 5.1%	+ 7.7%
Calcium	+ 4.7%	+ 7%	+ 6.2%
Phosphorus	+ 6.5%	+ 8.7%	+ 15.2%
Cholesterol	+ 71%	+ 93%	+ 186%

[a] no treatment changes in the 25 mg/Kg/day group

- gross lesions were not reported for F_0 females-

EMBRYO AND FETAL DATA

- summary of laparotomy data

GROUP	G1	G2	G3	G4	G5
Pregnant	9/10	10/10	10/10	10/10	10/10
Live litters	9	10	10	9	4
Resorbed or dead litters	0	0	0	1	6
% Peri-implantation loss	6.0	9.9	12.8	7.2	19.4*
Resorptions	2	5	33	40	69
% Resorptions/implants (litter mean)	2.3	4.9	37.4*	46.2*	85.7*
% Postimplantation loss (litter mean)	4.4	5.9	40.7	46.2	85.7
Live fetuses	77	84	46	46	13
Live fetuses/pregnant female	8.6	8.4	4.6*	4.6*	1.3*

% peri-implantation loss = (corpora lutea - implants / corpora lutes) x 100
% postimplantation loss = (resorptions + dead fetuses / implantations) x 100
* trend statistically significant (p 0.05) through the indicated dose

- summary of external and visceral examination of fetuses –*visceral examination was conducted in only a single fetus*

GROUP	G1	G2	G3	G4	G5
Live fetuses/litters examined	77/9	84/10	46/10	46/9	13/4
Dead fetuses/litters examined	2/1	1/1	1/1	0	0
Fetuses with malformations (%, LM)[a]	0	1 (1.1)	2 (5.3)	3 (4.4)	1 (8.3)
Litters with malformations (%)	0	1 (10)	2 (20)	2 (22)	1 (25)
Fetuses with variations (%, LM)[a]	0	0	0	1 (5.6)	0
Litters with variations (%)	0	0	0	1 (11)	0
Type and No. of fetal alterations (%, LM)[a]					
Microphthalmia (M)[b]	0	0	0	1 (1.6)	0
Gastroschisis (M)[b]	0	0	1 (3.3)	0	0
Caudal dysplasia (M)[b]	0	0	1 (3.3)	0	0
Tail malformation (M)[b]	0	1 (1.1)	0	0	1 (8.3)
Forelimb flexion (M)[b]	0	0	1 (2.0)	2 (5.6)	0
Petechial hemorrhage of skin (V)[c]	0	0	0	1 (5.6)	0
Local edema (V)[c]	0	0	0	1 (5.6)	0
Coronal Examination					
Live fetuses/litters examined	0/0	0/0	0/0	1/1	0/0
Fetuses with malformations (%, M)[b]	0	0	0	1 (100)	0
Litters with malformations (%)	0	0	0	1 (100)	0
Hydrocephalus (M)[b]	0	0	0	1 (100)	0

[a] LM = litter mean [b] M = malformation [c] V = variation