Embryo survival was significantly reduced at ≥75 mg/Kg/day, with a DR increase in the percent resorption/implants and a decrease in the number of live fetuses/pregnant female. *This treatment related effect appeared to correlate with an increase in the incidence of vaginal bleeding observed GD 16-28.* A significant *peri-implantation* implantation loss also occurred at 300 mg/Kg/day. Based on the results of the study, the sponsor recommended dose levels of 10, 25, and 50 mg/Kg/day for the developmental toxicity study in rabbits.

7.3.1.iii. ORAL DEVELOPMENT TOXICITY STUDY IN RABBITS [Vol. 1.23; p. C-269]

Study: TT#95-704-0
Compound: L-748,731, lot L-748,731-000R009
Formulation: Suspensions prepared daily in control vehicle.
Route: Oral gavage at 4 mL/Kg
Dose Levels: Group- 1 2 3 4
mg/Kg/day- 0 10 25 50 from GD7 through GD20
Strain: NZW, approx. 25 weeks old, 2757 to 3857 body weight at initiation.
Number: 18F/group
Control Treatment: 0.5% (w/v) methylcellulose in deionized water
Study Site: Merck Research Laboratories, West Point, PA
Date: 9 January 95 - 14 June 95
GLP/QAU Statements: Both present with signatures.

The object of the study was to determine the teratogenic potential of L-748,731 in rabbits following oral administration from GD7 through GD20. Body weights were recorded D0, 7, 9, 11, 13, 15, 17, 19, 21, 24, and 28. Food consumption was recorded nine times during the study. Females were observed daily. The rabbits were killed GD28. Gross examination was done on the thoracic and abdominal viscera of all F_0 females. Selected tissues were processed for histopathology.

RESULTS AND DISCUSSION

F_0 DATA

- no treatment related deaths or early sacrifices-
- abortions: 2 in G1 in 1 G 3 - not treatment related-
- no treatment effect on F_0 body weight-
- food consumption: a slight drop GD15-28 in G3 and even slighter in G1 and G2 GD28-
- no treatment effect on physical signs-
- F_0 necropsy:

G1- (control) no gross or histologic lesions-
G2- 1 with dark red focus in *stomach* mucosa (focal congestion-agonal hemorrhage)-
1 with reddening of stomach fundic mucosa (agonal hemorrhage in subepithelial lamina propria)-
G3- 1 with red focus in fundus-
1 with dark focus in duodenum (erosion, focal, moderate)-
G4- 1 with raised reddened area (no lesion seen on histologic examination)-

EMBRYONIC AND FETAL DATA

- *summary of external examination data – There was a single fetus in the high dose group which was found to have omphalocele [M] and tail malformation [fetal incidence of 1/141 or 0.7%]. Reported fetal incidence of omphalocele in historical controls was a mean of 0.04 ± 0.2% [maximum fetal incidence of 1.47%]. The reported fetal incidence of tail malformation ranged from 0.0-0.015% [mean] with a maximum incidence range of 0 – 0.7% depending on the malformation observed.*[1] *The mean values are comparable to those submitted for Sponsor historical controls.*

[1] Historical Control Data [1992-1994] for Developmental and Reproductive Toxicity Studies using the New Zealand White Rabbit [1996]. Compiled by Middle Atlantic Reproduction and Teratology Association [MARTA] and Midwest Teratology Association [MTA].

- summary of visceral, skeletal, and fetal ossification data

Type and # of Fetal Alterations (%, LM)*	G1	G2	G3	G4
Ventricular septal defect (malformation)	1 (0.67)			
Aortic stenosis (malformation)	1 (0.67)			
Fused kidney (malformation)				1 (2.8)
Retrocaval ureter (malformation)		3 (2.1)	2 (1.5)	2 (1.2)
Gallbladder malformation (malformation)		1 (0.69)		
Hypoplastic lungs (malformation)	1 (0.95)	1 (2.8)		
Agenesis of diaphragm (malformation)		1 (2.8)		
Hydrocephalus (malformation)	1 (2.2)			
Ureter variation (variation)			1 (0.69)	
Gallbladder reduced in size (variation)		1 (0.56)		
Gallbladder variation (variation)			1 (0.57)	
Variation in lung lobation (variation)	5 (4.9)	3 (4.2)	12 (14)	6 (4.1)
Cerebral ventricles enlargement (variation)	1 (2.2)			
Craniostenosis (malformation)				1 (0.69)
Atlas malformation (malformation)		1 (0.69)		
Axis malformation (malformation)		1 (0.69)		
Cervical vertebra malformation (malformation)		1 (0.69)		2 (5.6)
Thoracic vertebra malformation (malformation)		2 (1.8)		3 (4.0)
Lumbar vertebra malformation (malformation)			2 (6.9)	
Missing vertebra (malformation)	1 (0.67)			1 (0.62)
Fused rib (malformation)		1 (0.69)		1 (0.56)
Hypoplastic rib (malformation)				1 (0.62)
Sternebral malformation (malformation)		2 (3.5)		1 (2.8)
Sacral vertebra variation (variation)	2 (1.9)	5 (3.5)	8 (6.5)	6 (4.1)
Cervical rib (variation)		5 (3.3)		5 (3.9)
Reduced 13th rib (variation)	33 (27)	23 (14)	35 (26)	29 (18)
Sternebral variation (variation)		1 (0.69)		
Litters with incomplete ossification (%)	8 (53)	10 (56)	12 (75)	16 (89)
Fetuses with incomplete ossification (%, LM)*	23 (19)	29 (19)	37 (33)	50 (37)
Incomplete ossification of thoracic vertebra				2 (5.6)
Incomplete ossification of sternebra	7 (5.4)	9 (5.5)	20 (16)	12 (8.2)
Incomplete ossification of metacarpal	17 (15)	21 (14)	18 (18)	36 (26)
Incomplete ossification of pelvic bone	1 (1.1)	1 (0.69)	2 (1.2)	2 (1.2)
Incomplete ossification of talus/calcaneus	2 (1.4)	3 (1.9)	3 (7.6)	

*LM = liter mean

- no treatment reduction in embryo or fetal survival-
- resorptions increased slightly: 9G1, 14G2, 16G3, 13G4-
- resorptions/implant increased slightly: 0 6.7%G1, 9.7%G2, 10.5%G3, 7.7%G4-

The NOAEL for maternal toxicity was 50 mg/kg/day. The NOAEL in this study, *according to the Sponsor,* was 25 mg/Kg/day for *developmental* toxicity, as there was a slight treatment-related increase in incomplete ossification of metacarpal bones at 50 mg/Kg/day and in the previous oral range-finding study there were no gross lesions reported at 25-300 mg/Kg/day. Fetal effects consisted of an increase in the number of litters and fetuses in the 25 and 50 mg/Kg/day groups with incomplete ossification at thoracic vertebra, sternebra, talus, and metacarpal bones *when compared to controls. Therefore, a more conservative NOAEL would be 10 mg/kg/day based on an increase in overall incomplete ossification. The Sponsor considers this finding to be of "minimal toxicological significance since the average number of*

sacrocaudal vertebrae, an indicator of overall fetal ossification, were similar across all groups". The number of ossified sacrocaudal vertebrae was 19.4 –19.6 ± 0.4 S.D. for all groups.

Based on recommendations of the Reproduction Toxicity Committee, vertebral malformations, including axis and atlas malformation, were combined for further analysis. The fetal [litter] incidence for all vertebral malformations was 1/115[1], 2/136[2], 2/126[2], and 5/141[4] at 0, 10, 25, and 50 mg/kg, respectively. Statistical analysis [Cochran-Mantel-Haenszel method], conducted by Dr. Baldeo Taneja, indicated that there was no trend for an increase in the incidence of vertebral malformations based either on fetal incidence [p=0.415] or on litter incidence [p=0.603]. The Sponsor was requested to supply additional information with respect to the nature of the vertebral malformations. This was supplied in submission dated April 13, 1999. The Sponsor states that "the spectrum of malformations is different for each fetus and when broken down by incidence, according to region and description,...all values are within MARTA historical control range reference. The MARTA historical control range of vertebral malformations for related alterations is as follows: Cervical [including bipartite, fused, hemicentra, hypoplastic or misshapen] is 0-1.96%, Thoracic [including agenesis, bipartite, fused, hemicentra, hypoplastic or misshapen] is 0-2.65% and Lumbar [including bipartite or fused] is 0 – 2.63%". Based on these considerations, the evidence is not conclusive with respect to a drug-related effect. Additional information regarding historical controls for the Sponsor's lab has been requested.

Fused kidney was seen in 1/141 [0.7%]. The MARTA data base reports a fetal incidence of fused kidney as 0. The mean fetal incidence of this finding in Sponsor submitted historical controls was 0.02%. Since it was observed in only a single high dose fetus, however, the relationship to drug treatment is questionable. Retrocaval ureter was observed at a low frequency [≤2.2%] in treated only. A dose-dependent relationship was not observed in fetal incidence. The historical control data for retrocaval ureter in the MARTA data base was a mean 0.324 ± 0.87% [maximum of 4.27%] and the mean of the Sponsor submitted historical controls [submission date of March 19, 1999] was 1.28%.

Craniostenosis was observed in 1/141[0.7%] of the high dose fetuses. The historical control data for craniostenosis in the MARTA data base was a mean 0.008 ± 0.08% [maximum of 7.9%] and the mean of the Sponsor submitted historical controls was 0.04%. Therefore, this finding was considered incidental.

Other findings occurred sporadically, did not show a dose dependent relationship, were comparable to concurrent controls, or within MARTA historical controls.

There were minor differences in the figures presented in the table for fetal examinations and those cited in the Results text. These differences did not modify the assessment of this study.

7.3.2 Developmental Studies - Rat

7.3.2.i. ORAL RANGE-FINDING REPRODUCTION STUDY IN FEMALE RATS [Vol. 1.24; C-396]

Study No.: TT#94-733-5
Compound: L-748,731, Lot L-748,731-000R009[redacted]
Formulation: Suspension prepared daily in 0.5% methylcellulose (w/v) in deionized H_2O
Route: Oral gavage on GD 6 through LD 20
Dose Levels:

Group-	1	2	3	4	5	6	7
mg/Kg/day-	0	25	25WD*	125	250	500	500WD* at 5 mL/Kg

* treatment was withdrawn from GD 21 to LD 0, inclusive

Strain: Sprague-Dawley [Crl:CD®(SD) BR, 10 weeks old, 260 to 345 g body weight
Number: 10 mated F/group
Control Treatment: 0.5% methylcellulose at 5 mL/Kg
Study Site: Merck Research Laboratories, West Point, PA
Date: 23 August 1994 to 06 January 1995
GLP/QAU Statements: Not present.

The study was done to determine the dose levels of L-748,731 for the developmental toxicity (TT#94-733-0) and female fertility (TT#95-705-0) studies in rats. The dose selection was based on the findings in the 14-Week Oral Toxicity Study in Rats (TT#94-615-0). In the present study, only G 1 (control) and G 2 (25 mg/Kg) survived to scheduled termination; these groups were dosed to LD 19, 20, 21, or 22, depending on day of mating.

Females were housed with untreated males until a plug and/or sperm was found. Animals were observed twice a day for physical signs. Body weight was determined GD 0, 6, and every other day to GD 24 and on LD 0, 3, 7, 10, 14, 17, and 21. Blood samples were collected on GD 14 about 24 hours after dosing for limited hematological analysis (9 parameters) and serum biochemical analysis (16 parameters). Parturition and length of gestation were also determined. Due to mortality, G3-7 were sacrificed prior to the scheduled termination. G1 and 2 were sacrificed LD 20-23. Surviving F_1 pups were observed daily. Body weight was determined PND 0, 7, 14, and 21. Sacrifice was D21. A gross examination of the thoracic and abdominal viscera in situ was conducted on G1 and G2.

RESULTS AND DISCUSSION

F_0 Generation Females:

- mortality: died/sacrificed- 1 G3, 4 G4, 3 G5, 3 G6 (due to peritonitis/ulcers in small intestine)-
- physical signs: animals that died- nasal or ocular discharge, brown discharge around mouth, urine stained fur-
- body weight: no treatment related effects, except in animals that died or were sacrificed-
- hematology: no treatment related changes-
- serum biochemistry: no treatment related changes-*[1-2 females at ≥125 mg/kg which died –[not including the 500 mg/kg withdrawn group] - had ↓glucose, protein, albumin and/or chloride, ↑BUN]*
- reproductive performance: no treatment related effects other than slight increase in gestation length in all drug groups (22.6 to 22.9 D vs. 22.2 D in G1)-
- no evidence of external developmental toxicity in pups or in pup weight-
- *↑in stillborn pups and dead pups between PND 1-3: 0.5% in control vs. 4.2 -23% in the treated groups*
- *no treatment related physical signs or preweaning weights effects in F_1 pups*

Maternotoxicity was seen at 25 mg/Kg/day. Other than a slight increase in the length of gestation, the reproductive performance data indicated no treatment-related effects. *There was an increase in the incidence of stillborn/found dead pups between post lactation days 1-3 at all doses when compared to controls.* A high dose of 50 mg/Kg/day was *selected by the Sponsor* for the rat developmental toxicity study (drug administration from GD 6 to LD 20) and 300-500 mg/Kg/day for the rat female fertility study (drug administration up to GD 7).

7.3.2.ii. ORAL DEVELOPMENTAL TOXICITY STUDY IN RATS [Vol 1.24: p. C-597 and Vol. 1.25: p. C-806]]

Study No.: TT #94-733-0
Compound: L-748,731, lot No. L-748,731-000R009
Formulation: Suspension in 0.5% (w/v) methylcellulose in deionized water.
Route: Oral gavage from GD 6 through GD 20 for C-sectioning and from GD 6 through the LD 7 or to GD 23 for F that were not pregnant.

Dose Levels: Group	1	2	3	4
	0	10	25	50 mg/Kg/day at 5 mL/Kg

Strain: Sprague-Dawley Crl:CD®(SD)BR, ≈10 weeks old, 185 - 308 g body wt
Number: 44/group
Control Treatment: 0.5% (w/v) methylcellulose in deionized water at 5 mL/Kg.
Study Site: Merck Research Labs., West Point, PA
Date: 02 Nov 94 to 19 Apr 95

GLP/QAU Statements: Both present with signatures.

The purpose of this study was to evaluate the developmental toxicity of L-748,731 when administered to F_0 female rats. The intended evaluation of the lactational and post weaning phases were terminated early due to excessive maternal toxicity.

C-sections were done on 23 G1, 22 G2, 22 G3, and 19 G4 on GD 21. Each F was housed with one M until a copulatory plug or vaginal sperm was observed (D = 0). Animals were observed daily. Body weights were recorded GD 0, 6, 8, 10, 12, 14, 16, 18, 20, 21, 22, 24, and LD 0. Food consumption was measured GD 3 to 5, 6 to 8, 10 to 12, 14 to 16, 18 to 20 (2 day periods), and LD 1 to 5. Observation of parturition and length of gestation were observed. Females intended for natural delivery were euthanized between LD 1- 8 and examined grossly (thoracic and abdominal viscera *in situ);* microscopic examination was done on the stomach, small and large intestine, and gross lesions. Fetuses were examined, weighed, sexed, and dissected for visceral and skeletal examination.

The F_1 generation was observed daily. Body weight was recorded PND 0 and 7. Pups were counted PND 0 and 10, sexed, and reduced to 4 M and 4 F PND 3. All F_0 females and surviving pups were terminated during or near the first week of lactation due to excessive maternal toxicity.

RESULTS AND DISCUSSION

- mortality: <u>pregnant females</u>

  2G3 and 2G4 died during gestation
  1G3 and 3G4 sacrificed during gestation
  1G3 and 2G4 died/sacrificed during parturition
  18G1, 22G2, 17G3, and 16G4 postmortem death/early sacrifice
  21G1, 20G2, 19G3 and 19G4 cesarean sectioned

  <u>nonpregnant females</u>
  5G1, 2G2, 5G3, 2G4 sacrificed

- females with live pups/pregnant females: 100% in all groups-
- signs - dams: diarrhea, urine-stained fur, red nasal or eye discharge - all observed in G3 and 4-
- body weight gain: ↓ (* 6%) in G4 during gestation - treatment related-
  ↓(10.6%) in G4 during lactation - treatment related-
- food consumption: ↓ treatment related - G4 14%-
- embryo survival: no treatment related effects - dead pups ↑ in G3 due to 1 F that died D21-
- reproductive performance: no treatment related effects-
- live fetal wt: no significant changes-

Treatment Group:	G1	G2	G3	G4
Live fetuses/litters examined	306/20	309/20	273/18	280/19
Dead fetuses/litters examined	1/1	1/1	14/1	0
Fetuses with malformations (%, LM)*	1 (0.45)	0	2 (0.58)	2 (0.73)
Litters with malformations (%)	1 (5.0)	0	1 (5.3)	2 (11)
Fetuses with variations (%, LM)	0	0	0	0
Litters with variations (%)	0	0	0	0
Type and No. of fetal alterations (%, LM)				
Exencephaly (malformation)	0	0	0	1 (0.40)
Microphthalmia (malformation)	0	0	1 (0.31)	0
Micrognathia (malformation)	1 (0.45)	0	0	0
Protruding tongue (malformation)	1 (0.45)	0	0	0
Omphalocele (malformation)	0	0	0	1 (0.33)
Tail malformation (malformation)	0	0	1 (0.31)	0

*LM = litter mean

- visceral examinations:

TREATMENT GROUP:	G1	G2	G3	G4
Thoracic and abdominal examination				
Live fetuses/litters examined	160/20	161/20	141/18	146/19
Dead etuses/litters examined	1/1	1/1	6/1	0
Fetuses with malformations (%, LM)*	1 (0.56)	1 (0.62)	0	0
Litters with malformations (%)	1 (5.0)	1 (5.0)	0	0
Fetuses with variations (%, LM)*	1 (0.62)	0	3 (2.2)	0
Litters with variations (%)	1 (5.0)	0	3 (16)	0
Coronal examination				
Live fetuses/litters examined	158/20	161/20	140/18	144/19
Fetuses with malformations (%, LM)*	0	0	0	0
Litters with malformations (%)	0	0	0	0
Fetuses with variations (%, LM)*	0	0	0	0
Litters ith variations (%)	0	0	0	0
Type and No.of fetal alterations (%, LM)*				
Agenesis of kidney (malformation)	0	1 (062)	0	0
Situs inversus (mlformation)	1 (0.56)	0	0	0
Ureter Variation	1 (0.62)	0	0	0
Hemorrhage focus on liver (variation)	0	0	3 (2.3)	0

* LM = litter mean

- skeletal examination: no treatment related skeletal malformations or alterations - slight increase seen in % of litters with variations (G1 60%, G2 70%, G3 53%, G4 74%) - slight increase in % of litters with incomplete ossification [G1 0 (0%), G2 2(10%), G3 5(28%), G4 3(16%) *and % fetuses with incomplete ossification [G1 0 (0%), G2 2(0.75%), G3 7(2.5%), G4 3(1.1%)*

F_1 Rats from birth to termination:

- F_1 mortality: % of total pups PND 0 (LM): G1 6(2.2%), G2 10(3.1%), G3 41(18.5%), G4 32(13.4%)- % of total pups PN Day 1-3 (LM): G1 2(0.7%), G2 9(2.9%), G3 5(2.9%), G4 28(13.7%), includes 1 pup that died after live weighing Day 0-
  % of total pups PND 4-7 [LM]: G1 1 [0.7 ± 2.9], G2 2 [1.1 ± 5.3], G3 4 [8.6 ± 24.9], G5 8 [7.1 ± 9.4]
- physical signs among F_1 pups during lactation: none-
- pup body weight: ↓ PND 0 M/F G3(3-4%), G4 (6-7%)-
  ↓ PND 7 M/F G3 (13%), G4(10%)-
- external examination on PND 0:

Treatment group	G1	G2	G3	G4
Pups				
Number delivered (dead pups)	273 (6)	306 (10)	222 (38)	239 (32)
Number intrauterine examined	0	0	0 (1)	10 (2)
Number with malformations	0	0	0	0
Number of malformations	0	0	0	0
Number with variations	0	0	0	0
Number of variations	0	0	0	0
% live pups with variation - litter mean	0.0	0.0	0.0	0.0
Litters				
Number examined	18	22	18	18
Number with malformations	0	0	0	0
% with malformations	0.0	0.0	0.0	0.0
Number with variations	0	0	0	0
% with variations	0.0	0.0	0.0	0.0

- F_0 necropsy: treatment related peritonitis due to intestinal perforation was seen in all but one of the rats that died - 0/44, 2/44, 15/44, and 18/44 in G1-4, respectively - adhesions - ulcers (4 in G3 and 8 in G4)- extramedullary hematopoiesis in spleen of 1 F G3-

Maternal toxicity was seen at 25 and 50 mg/Kg/day. Reduction in body weight gain, reduced food consumption, altered clinical signs, and mortality occurred at 25 mg/Kg/day. Acute or chronic peritonitis occurred in all drug treated groups. The reproductive performance was not reduced nor was fetal development impaired; however, the drug was toxic to the pups, producing an increase in the number of stillborn and intrauterine dead pups at 25 and 50 mg/Kg/day. Malformations (exencephaly and omphalocele) occurred in two fetuses, one in each of two high dose litters. *The incidence for either finding was 1/280 [0.4%]. The mean historical control incidence submitted by the Sponsor [Submission dated March 19, 1999] was 6/3248 [0.02%] for either finding.* The NOAEL was < 10 mg/Kg for maternal toxicity *based on histopathological lesions* and < *10* mg/Kg/day for *embryofetal* toxicity.

7.4 Gestation/Lactation Study

The current Pharmacology/Toxicology Reviewer reviewed the following studies.

7.4.1. L-748,731 Fostering/Cross-Fostering Study in Rats [Vol. 1.26: p. C-1437 and Vol. 1.27: p. C-1590]

Study Identification: TT #95-730-0
Site: Merck Research Laboratories, West Point, PA
Study Dates [In-life]: July 9 – Aug. 25, 1995
Formulation and Lot No.: L-748,731-000R009; [redacted]
Vehicle - 0.5% methylcellulose
Certificate of Analysis Submitted: No (X) Assayed for uniformity and concentration at the start of dosing; assayed for concentration at another time point during dosing; assays were within acceptable limits, according to the Sponsor
Final Report: (X) Jan. 22, 1996
GLP and QA Statements Signed: Yes (X)
Objective: "The purpose of this study was to determine if the effects on F_1 pup mortality observed in previous studies with a postnatal phase were due to treatment of the dam during gestation and/or lactation interval".

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	GD 6 - LD 20 SID	50	F	Sprague-Dawley - [Crl:CD®(SD)BR] [redacted] App. 10-11 wks at study start 217-279 g
Group 2 - L-748-731	25						

#fed *ad libitum*

The following fostering/cross-fostering groups were assessed [N=15/group], "crossovers were made on the day of birth as soon after delivery as possible"; 5 pups per sex were randomly selected for fostering/cross-fostering; culled to 4/sex on PND 3:

Control dams/control pups [CxC]
Control dams/treated pups [CxT]
Treated dams/control pups [TxC]
Treated dams/treated pups [TxT]

Parameter Evaluated	Time Point(s)
Clinical observations	mortality daily 1X on GD 0 GD 6-20 - prior to and 1-5 hours post-dosing
Body weight	GD0, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 LD0, 1, 3, 7, 10, 14, 17, and 21
Food consumption	GD 3-5, 6-8, 10-12, 14-16, 18-20 LD 1-5, 8-12
Parturition/Gestation length	GD 21 until completion of delivery - 4-5X/day
Necropsy-reproductive status	premature death, unscheduled and scheduled sacrifice
Reproductive parameters - implants/female, % postimplantation survival, % live pups on PND 0, live pups per litter	
F_1 Analysis	
Clinical observations - including mortality	daily
Body weight	PND 0, 7, 14, 21
External examinations -malformations, variations	PND 0

Results -

I. F_0 Generation Females

Mortality - There were 8 and 10 treatment-related deaths during the gestation and lactation periods, respectively. Four additional treated females were sacrificed during the lactation period because they had no surviving pups [3 dams] or one surviving partially cannibalized pup [1 dam].

Clinical Observations - Oculonasal discharge, urine staining, unkempt coat, and/or salivation was observed for only a few days primarily in the premature decedents. One animal was pale, lethargic, and assumed a hunched position. Other findings were considered incidental by the Sponsor.

Body Weight - There were no treatment-related effects for GD 6-20 or during lactation. The slight decrease in average maternal body weight [-3%] or body weight gain [-8%] in the treated group was attributed to 3 females which lost weight and subsequently died or were prematurely sacrificed.

Food Consumption - A slight decrease [10-12%] was observed during the gestational period in the treated dams.

Reproductive Performance and Fertility - There were no treatment-related effect on average implants per female or gestation length.

II. F_1 Observations

F_1 Mortality During Lactation - There was a slight treatment-related increase in pup mortality incidence on the day of birth in the treated [93.3 ± 9.9% live pups] vs. control [99.3 ± 2% live pups]. During PND 1-7, there was a treatment related increase in pup mortality for pups exposed during gestation only [CxT] and during both gestation and lactation [TxT]. The mortality in the TxC group was slightly increased compared to CxC pups. During PND 8-21, there was an increase in pup mortality in pups exposed during lactation only [TxC], with pup mortality comparable in the other 3 groups.

The table below delineates the treatment-related effects on pup postnatal mortality

Pup Deaths [% Pup deaths ± SD] - Litter means	Fostering/Cross Fostering Groups			
	CxC	TxC	CxT	TxT
Postnatal days 1-7	1 [0.7 ± 2.6]	5 [3.3 ± 6.2]	16 [10.7 ± 26.0][b]	19 [12.7 ± 22.8][c]
Postnatal days 8-21	1 [0.8 ± 3.2]	18 [15 ± 34.8][a]	1 [0.8 ± 3.2]	1 [0.8 ± 3.2]
Postnatal days 1-21	2 [1.3 ± 3.5]	23 [15.3 ± 27.2]	17 [11.3 ± 25.9]	20 [13.2 ± 22.9]

[a]all pups [8/litter] in 2 litters and 1 pup/litter in 2 litters died
[b]all 8 pups in 1 litter; 1 pup/litter in 3 litters; and 2 or 3 pups/litter in 1 litter each died
[c]all 8 pups in 1 litter; 1 pup/litter in 5 litters; and 2 pups/litter in 3 litters died

F_1 Clinical Observations - There were no treatment-related effects.

F_1 Body Weight - The decrease in average body weights PND 7-21, which was noted by the Sponsor in the TxT and TxC groups, could be attributed, in part, to low body weights in 2 and 3 litters, respectively.

External Examinations - 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. The increase in postnatal death is due to both prenatal and postnatal factors. "Gestational exposure did not accentuate the effects of postnatal drug exposure".

2. Decreases in postnatal pup weights are due to drug administration to the dam during lactation.

3. The comparable decreases in average pup weights in the TxT and TxC groups indicate that "gestation exposure does not accentuate the postnatal effects of drug exposure to the offspring".

Reviewer's Comment – In general, the Reviewer concurs with the Sponsor's conclusions. However, the data should be interpreted cautiously due to the high incidence of mortality [e.g. toxicity] in the treated dams. This level of toxicity may be a confounding factor. Not unexpectedly, there appears to be a shift in time at which mortality is observed dependent on time of exposure of the pup. Those pups exposed to drug during gestation tended to die at an earlier time point than those exposed during lactation.

7.5 LATE GESTATION – INCLUDING LACTATION PERIOD AND POSTWEANING DEVELOPMENT

The following studies were reviewed by the current Pharmacology/Toxicology Reviewer

7.5.1. L-748,731:Oral Length of Gestation and Parturition Study in Female Rats [Vol. 1.27; p. C-1682]
Study Identification: TT #96-719-0
Site: Merck Research Laboratories, West Point, PA
Study Dates: May 19 - Oct. 17, 1996
Formulation and Lot No.: MK-0966-00R027[redacted]
Vehicle - 0.5% methylcellulose
Certificate Analysis: No (X) Assayed for uniformity at the start of dosing; assayed for concentration at the start and near the end of dosing; assays were within acceptable limits, according to the Sponsor
Final Report: Oct. 22, 1996
GLP and QA Statements Signed: Yes (X)

Objective: "The objective of this study was to accurately assess the potential effect of MK-0966 on the length of gestation and duration of parturition in F_0 rats, and to assess F_1 viability during this 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	GD 15 to LD 6 SID	20	F	Sprague-Dawley - [Crl:CD®(SD)BR] App. 10 wks at study start 205-332 g
Group 2 - L-748-731	1						
Group 3 - L-748-731	5						
Group 4 - L-748-731	15						

#fed *ad libitum*
litters were reduced to 4/sex/group on PND 3, sacrificed on LD 7

Parameter Evaluated	Time Point(s)
Clinical observations	daily and 1-5 hours post-dosing
Body weight	GD0,7, 15, 17, 19, 20, 22, 23, 24 LD0, 1, 3, 7
Parturition/Gestation length	GD 21 until completion of delivery -q1hr
Necropsy-reproductive status	LD 7
Reproductive parameters -no. of metrial glands, litter size	LD7
F_1 Analysis	
Clinical observations - including mortality	daily
Body weight	PND 0, 3, 7
External examinations -malformations, variations	PND 0

Results -

I. F_0 Generation Females

Mortality - There was one treatment-related death at 15 mg/kg/day on LD 6. One dam in the 15 mg/kg/day group with no surviving pups was sacrificed prematurely on LD 4.

Clinical Observations - Three animals in the high dose group exhibited the following signs: unkempt coat, dorsolateral abdominal indentation, and no apparent nursing. One animal also exhibited nasal discharge and subsequently died.

Body Weight - There were no treatment-related effects.

Reproductive Performance: Gestation and parturition length – There was a dose-dependent increase in duration of gestation. The maximum increase at 15 mg/kg/day was 0.3 days from a control value of 22.12 days to 22.45 days. A slight increase of 19-24 minutes in parturition duration was observed at ≥5 mg/kg/day. Due to the magnitude of the increase, this change is of questionable biological significance. However, prostaglandin inhibitors have been associated with an increase in both gestation and parturition duration. The table below indicates the duration of parturition for the various groups.

Duration Hours	Female with Parturition Completed – Dose [mg/kg]			
	0	1	5	15
1	2	4	0	1
2	12	8	10	9
3	3	8	7	6
4	2	0	3	3

II. F_1 Observations

F_1 Mortality - On PND 0 there was a dose dependent increase in pup mortality compared to the controls at ≥1 mg/kg/day. The incidence of pup mortality reached statistical significance at ≥5 mg/kg/day.

Although not statistically significant, the increase was 3X the control value at the low dose. This increase in pup mortality was associated with a mild decrease in percent postimplantation survival and percent live pups delivered at ≥5 mg/kg/day. On PND 3 and 7, pup mortality was significantly increased in the 15 mg/kg/day group. Although not statistically significant, there was a 2.7X increase in pup mortality on PND 3 for the 5 mg/kg/day group compared to the controls.

External Examination - There were no treatment-related effects.

Clinical Observations - There were no treatment-related effects.

Body Weight - There were statistically significant decreases in average pup weight on PND 0 at ≥5 mg/kg/day and in all treatment groups on PND 3 and 7. The Sponsor states that the changes in pup weight at ≤5 mg/kg/day are unrelated to treatment for several reasons including [1] the litter averages are within the 95% confidence limit of historical controls for their lab; [2] the decrease was <%7; and [3] similar findings were not observed in Studies TT #95-706-5 and TT #95-706-0. With the exception of pup weights on PND 3 and PND 7, the Reviewer would agree. There was variability from litter to litter.

The table below delineates the treatment-related effects on pup postnatal mortality and body weights.

Parameter	Dose [mg/kg/day]			
	0	1	5	15
% Postimplantation survival to Day 0 [LM#] ± S.D.	92.4 ± 7.7	92.1 ± 9.1	88.2 ± 15.5	85.8 ± 15.3
Dead pups [%,LM] – PND 0	4 [1.19]	12 [3.44]	21 [7.23]*	27 [8.41]*
Pup deaths [% pup deaths ± SD] [LM] -PND 1-3	3 [0.9 ± 3]	5 [1.5 ±4.1]	8 [2.4 ± 4.3]	13 [4.8 ± 9.9]*
Pup deaths [% pup deaths ± SD] [LM] -PND 4-7	0	0	0	10 [6.6 ± 23.0]
Pup weight (gm) [LM] ± SD PND 0	6.3 ± 0.5	6.3 ± 0.4	6.2 ± 0.4*	6.1 ± 0.5*
Pup weight (gm) [LM] ± SD PND 3	9.6 ± 0.9	9.1 ± 0.9*	9.2 ± 0.9*	8.3 ± 1.3 *
Pup weight (gm) [LM] ± SD PND 7	17.9 ± 1.2	17.2 ± 1.4*	17.2 ± 0.8*	16.7 ± 2.1*

#LM = litter mean
*denotes statistical significance

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. MK-0966 at ≤15 mg/kg/day did not prolong the duration to initiation or completion of delivery. **Reviewer's Comment** – There was a slight increase in both parameters at ≥5 mg/kg/day. Due to the magnitude of the increase, the biological significance is not known.

2. Fetotoxicity [e.g. pup mortality] was seen at ≥5 mg/kg/day. **Reviewer's Comment** – The biological significance of the 3X increase in pup mortality observed on PND 0 at 1 mg/kg/day is not known. Postimplantation survival and percent live pups delivered at this dose [92% and 97%, respectively] were comparable to that observed for control animals [92% and 99%, respectively]. The NOAEL for this study is considered to be ≤1 mg/kg/day.

7.5.2. L-748,731: Oral Range-Finding Late Gestation and Lactation Study in Rats [Vol. 1.28; p. C-1937]

Study Identification: TT #95-706-5
Site: Merck Research Laboratories, West Point, PA
Study Dates: Jan. 23 - Mar 13, 1995
Formulation and Lot No.: L-748,731-000R014
Vehicle - 0.5% methylcellulose
Certificate of Analysis Submitted: No (X)
Final Report: June 13, 1995

GLP and QA Statements Signed: No (X) - non-GLP range finding study
Objective: "To determine dose levels of L-748,731 for a subsequent late gestation and lactation study with postweaning evaluation in 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	GD15 - LD 20	10	F	Sprague-Dawley - [Crl:CD®(SD)BR] App. 10 wks at study start 219-319 g
Group 2 - L-748-731	1						
Group 3 - L-748-731	5						
Group 4 - L-748-731	25*						
Group 5 - L-748-731	50*						

#fed *ad libitum*, on PND 0, pups [5/sex/litter] were selected for evaluation, on PND 3 culled to 4/sex/litter
*sacrificed LD 13-15 due to unacceptable maternal toxicity

Parameter Evaluated	Time Point(s)
Clinical observations Mortality checks	1X/daily - GD 0-7, GD 15-LD21 and 1-5 hours post dosing daily
Body weight	GD0, 7, 15, 16, 18, 20, 22, 24 LD0, 3, 7, 10, 14, 17, 21
Parturition/Gestation length	GD 21 until completion of delivery -4X/day weekdays
Necropsy-thoracic and abdominal viscera *in situ*	scheduled sacrifice
Histopathology - stomach, small and large intestine	premature deaths, scheduled and unscheduled necropsy
Reproductive parameters -no. of metrial glands, % live pups	LD7
F_1 Analysis	
Clinical observations - including mortality	daily
Body weight	PND 0, 7, 14, 21
Necropsy - unselected pup - thoracic and abdominal viscera *in situ* - dead pups - high dose group - visceral alterations, trachea	PND 3
Histopathology - unselected pup - representative regions of the GI tract and kidneys	PND 3

Results -

I. Maternal Parameters

Mortality - Three rats at both 25 and 50 mg/kg/day prematurely died or underwent unscheduled sacrifice between LD 11-14 and LD 6-11, respectively. In addition, 1 rat at 25 and 50 mg/kg/day were sacrificed with ≤1 surviving pup. The remaining animals in these two dose groups were sacrificed prematurely LD 13-15 due to excessive maternal toxicity.

Clinical Observations - Signs were observed for several days in the premature decedents and included urine-stained fur, unkempt coat, and/or red/brown nasal discharge. All other findings were considered incidental by the Sponsor.

Body Weight - At 50 mg/kg/day, there was 42% decrease in body weight gain compared to controls for PND 0-7. A comparable change was observed at 1 mg/kg/day but not at 5 or 25 mg/kg/day.

Reproductive Performance - There were no treatment related effects on average implants/female, average length of gestation, and % females with live pups. There was a mild decrease in % postimplantation survival on PND 0 at ≥5 mg/kg/day: 94.8 ± 5.4, 95.6 ± 4.0, 87.0 ± 7.7, 74.6 ± 24.4, 89.6 ± 13.2% at 0, 1, 5, 25, and 50 mg/kg/day.

Necropsy - Peritonitis and/or small intestinal ulceration was observed in 5/10 and 8/10 females at 25 and 50 mg/kg/day, respectively.

II. F_1 -Birth to termination

Mortality - There was a trend for an increase in percent dead pups at ≥5 mg/kg/day. The increase in percent dead pups was associated with a concomitant decrease in percent live pups on PND 0 at ≥25 mg/kg/day. Pup mortality increased at 25 and 50 mg/kg/day on PND 1-3 and PND 4-21, respectively and was considered to be treatment related. The Sponsor states that there was a correlation between litters with the greatest number of dead pups and those dams noted to have gross GI lesions. The table below delineates these findings.

Parameter	Dose [mg/kg]				
	0	1	5	25	50
Dead pups [%, LM]	3 [1.89]	0	7 [4.61]	16 [12.5]	15 [12.82]
% live pups on PND 0[LM] ± S.D.	98.1 ± 3.1	100 ± 0	95.4 ± 5.5	87.5 ± 19.8	87.2 ± 28.1*
Live pups/litter ± SD	16.0 ± 2.0	15.0 ± 1.9	14.3 ± 2.3	12.3 ± 3.7	14.2 ± 5.5
Pup deaths [% pup deaths ± SD] LM-					
PND 1-3	1 [0.5 ± 1.7]	2 [1.3 ± 2.7]	3 [2.4 ± 5.3]	16 [16.4 ± 31.0]	4 [2.9 ± 4.3]
PND 4-21	0	1 [1.2 ± 4.0]	1 [1.2 ± 4.0]	1 [1.2 ± 4.0]	5 [6.9 ± 12.7]

Clinical Observations - There were no treatment-related effects.

Preweaning Body Weights - Male pup weights tended to decrease at ≥25 mg/kg/day with the magnitude of the difference between treated and control increasing with time. The decrease ranged from 5-18% at the various time points.

Fetal Examinations - There were no treatment-related external or visceral variations, alterations or malformation.

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

1. Maternal toxicity [mortality, GI, toxicity, decreased body weight gain] was observed at ≥25 mg/kg/day. **Reviewer's Comment** – The NOAEL for maternal toxicity was 5 mg/kg/day.

2. There were no treatment-related effects on reproductive performance. **Reviewer's Comment** – The significance of the decrease in percent postimplantation survival in the treated groups is not known.

3. Pup deaths were increased and body weights were decreased at ≥25 mg/kg/day on PND 0, 7, and/or 14. Determination of the relationship of the changes in pup mortality and body weight to drug treatment is confounded by the present of moderate to severe GI toxicity in dams. **Reviewer's Comment** – The Reviewer concurs.

4. Doses of 5, 10, and 15 mg/kg/day are recommended for the late gestation and lactation study in rats since excessive toxicity was observed at ≥25 mg/kg/day. **Reviewer's Comment** – The Reviewer concurs.

<u>7.5.3. L-748,731: Oral Late Gestation and Lactation Study in Rats with Postweaning Evaluation [Vol. 1.28; p. C-2084; Vol. 1.29; p. C-2318]</u>
Study Identification: TT #95-706-0
Site: Merck Research Laboratories, West Point, PA
Study Dates: April 16 - Sept. 2, 1995
Formulation and Lot No.: L-748,731-000R009[redacted]
Vehicle - 0.5% methylcellulose
Certificate of Analysis Certificate: No (X) Assayed for uniformity and concentration at start of dosing, concentration assayed at 2 additional time point; all results were within acceptable limits, according to the Sponsor
Final Report: Oct. 14, 1996

GLP and QA Statements Signed: Yes (X)
Objective: "The objective of this study was to evaluate the effects of L-748,731 administration to F_0 female rats on growth, behavior, reproductive performance, and fertility of the F_1 generation".

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	GD15 - LD 20	25	F	Sprague-Dawley - [Crl:CD®(SD)BR] App. 10 wks at study start 198-293 g
Group 2 - L-748-731	5						
Group 3 - L-748-731	10						
Group 4 - L-748-731	15						

#fed *ad libitum*, 1-2 females in the control and low dose groups with decreased weight gain were inadvertently dosed on the first day and then replaced with substitute females

Parameter Evaluated	Time Point(s)
Clinical observations **Mortality checks**	1X/daily - GD 0-7, GD 15-sacrifice and 1-5 hours post dosing daily
Body weight	GD 0, 7, 15, 16, 18, 20, 22, 24 LD 0, 3, 7, 10, 14, 17, 21
Food consumption	GD 10-12, 15-17, 18-20 LD 1-5, 8-12
Parturition/Gestation length	GD 21 until completion of delivery -4X/day weekdays; 2X/weekends
Necropsy-thoracic and abdominal viscera, GI tract	LD 23-25
Histopathology - GI tract from 7 females*	LD 23-25
Reproductive parameters -no. of metrial glands, implants/female, live and dead pups/litter, % postimplantation survival	
F_1 Analysis**	
Clinical observations - including mortality	daily
Body weight	PND 0, 7, 14, 21
External examinations***	PND 0
F_1 postweaning evaluation	
Clinical observations - Physical signs Mortality	2X/wk daily
Body weights - males and females - female only	1X/wk - from weaning [PND 23-28] until sacrifice, except during cohabitation GD 0, 7, 14, 20, 24 LD 0
Developmental Signs Preputial separation - males Vaginal canalizatiaon	 PND 38-48, q48h PND 28 - 38, q48h
Ophthalmologic Examination	once between PND 35-56
Behavioral Assessment - 1/sex/litter Passive avoidance Auditory startle habituation Open field motor activity	 PND 35 ± 1, then 1 week later PND 63 ± 2 PND 70 ± 2
Mating-1/sex/litter [non-siblings] - 16 day mating period	Postnatal weeks 11-12
Parturition/Gestation length	GD 21 until completion of delivery -4X/day weekdays; 2X/weekends
Pregnancy status or metrial gland count - at sacrifice	F_1 females which delivered - LD 0-5 Mated females not delivering - GD 24 Non-mated females - 24 days after start of cohabitation
F_2 Analysis	
Body weight, sex, external examination, mortality	PND 0

*females with gross lesions
**5 pups/sex/litter were selected on PND 0, culled to 4/sex/litter on PND 4, culled to 2/sex/group on PND 21
***3 dead, externally malformed pups [PND 0] from the same dam administered 15 mg/kg/day were examined for visceral and skeletal alterations

Results

I. F_0 Generation Females

Mortality - Two and 5 treatment-related premature deaths/sacrifices occurred at 10 and 15 mg/kg/day, respectively, from LD 1-20. All 7 rats had gross evidence of peritonitis/intestinal ulceration.

Clinical Observations - Signs were observed in all premature decedents and in a few animals in the high dose group for approximately 1-3 days. These signs included diarrhea, abdominal distention, urine stained fur, paleness, ocular discharge, and decreased activity. All other signs were considered by the Sponsor to be incidental.

Body Weight - There was a decrease in average maternal weight gain LD 7 - 14 in the 15 mg/kg/day [9 gm] compared to controls [20 g] which resulted in an average body weight that was 6% lower than the control group body weight.

Food Consumption - There was a decrease of 11-13% in average maternal food consumption at 15 mg/kg/day on GD 20, LD 5, and LD 12.

Reproductive Performance - There was no treatment-related effect on percent females with live pups and implants/female, but there was a decrease in postimplantation and pup survival [discussed below under F_1 Results]. The Sponsor considered the statistically significant increase in average gestation length in all treatment groups to be unrelated to drug treatment since [1] delivery on GD 23 is within the normal range of control; [2] there is only a 0.5 day precision in this observation; and [3] increase in gestation length was not reproducible at higher doses in studies TT #94-733-0 and TT #95-706-5. Gestation length [days] was 22.3 ± 0.4, 22.6 ± 0.4*, 22.6 ± 0.5*, and 22.7 ± 0.4* at 0, 5, 10, and 15 mg/kg/day. However, mild increases were observed in studies TT #92-721-0 at 30 mg/kg [+0.6 days], TT #94-733-5 at ≥25 mg/kg/day [+0.4-0.7 days], and TT #96-719-0 at ≥5 mg/kg/day [+0.3 days].

Necropsy and Histopathology – All premature decedents had peritonitis/intestinal ulceration. The enlarged mesenteric lymph nodes occurred in females with peritonitis/ulceration. Histopathology revealed that the intestinal serosal foci were focal areas of ulceration and peritonitis. Treatment-related gross necropsy findings are outlined in the table below.

Lesion	Dose [mg/kg/day]		
	5	10	15
Intestinal serosal focus	1	2	4
Enlarged mesenteric lymph nodes	1	5	9
Peritonitis/Intestinal Ulceration	0	4	8

II. F_1 Results

Mortality - There was an increase in pup death at birth in all treatment groups with a concomitant, slight decrease in percent postimplantation survival and percent live pups. Pup deaths PND 1-3 increased at ≥10 mg/kg/day, reaching statistical significance at 15 mg/kg/day. There were no treatment-related effects on mortality after weaning. The table below provides the values for the changes in F_1 mortality.

Parameter	Dose [mg/kg/day]			
	0	5	10	15
% Postimplantation survival to Day 0 [LM] ± SD	93.3 ± 5.8	88.9 ± 10.0	87.8 ± 18.8	87.3 ± 10.8*
% Dead pups [% LM]	4 [0.87]	12 [2.77]	10 [4.20]	27 [8.37]
Live pups/litter ± SD	14.9 ± 1.8	14.4 ± 2.0	13.9 ± 3.4	13.4 ± 3.2*
Pup deaths [% pup deaths ± SD] [LM] - PND 1-3	4 [1.0 ± 2.9]	6 [1.6 ± 5.8]	8 [2.0 ± 5.2]	16 [8.5 ± 21.6]*