3.9.3. L748,731: Twenty-Seven-Week Oral Toxicokinetic Study in Mice [Vol. 1.40: p. E-1981]

Study Identification: TT# 96-606-0
Site: Laboratoires Merck Sharp & Dohme-Chibret, Centre de Recherché, Riom, France
Study Dates: Feb. 26, - Aug 26, 1996
Formulation: Test article - L748,731
Batch No.: [Batch #032] in 0.5% aqueous methylcellulose
Certificate Analysis Submitted: No (X) Sponsor indicates that "assays for drug concentration and uniformity were within acceptable limits" but do not indicate when these analyses were conducted.
Final Report (X) Jan. 9, 1997
GLP and QA Assurance Statements Signed: Yes (X)
Objective: "To monitor plasma level of L-748,731 when given orally to mice after repeated administration under the condition of the concomitant mouse carcinogenicity study."

Test Material/ Group Designation	Dose*				Sex	N	Species/Strain
	mg/kg	ml/kg	Route	# days dosed			
Group 1 - L-748,731	60	10	oral, gavage	F and M = 30 or 182-183	M	30	-Crl:CD-1® [IBR] BR albino mice - F - 18.0 to 24.6 g; M - 23.2 - 31.7 g 6 weeks at study start
Group 2 - L-748,731	100						
Group 3 - L-748,731	300						

Parameter Evaluated	Time Point(s)
Mortality check	daily
Body weights	once in Drug Week 1, 2X/week for Drug Week 2-13, the 1X/wk until termination
Plasma concentrations - vena cava from 5 nonfasted anesthetized mice/sex/drug-treated group	Drug Weeks 5 and 26 - 0.5 and 10 hours

Results - The following animals were found dead and were discarded without necropsy:[1] 3, 2, and 2 males at 60, 100 and 300 mg/kg/day, respectively.

Body Weight - No drug-related changes in body weight or body weight gain were observed in males. There was a mild decrease in both body weight gain [16%] and body weight [5%] in the 300 vs. 60 mg/kg/day group.

Pharmacokinetic Data - The table below delineates the plasma concentration in males and females during Drug Weeks 5 and 27 at 0.5 and 10 hours post dosing.

Dose [mg/kg/day]	Plasma Concentration [µg/ml ± SEM]							
	Week 5				Week 27			
	0.5 hours		10 hours		0.5 hours		10 hours	
	F	M	F	M	F	M	F	M
60	2.37 ± 0.19	3.67 ± 0.21	0.75 ± 0.13	1.32 ± 0.26	2.41 ± 0.30	3.30 ± 0.23	1.00 ± 0.38	1.67 ± 0.55
100	4.92 ± 0.45	5.36 ± 0.40	1.32 ± 0.46	2.39 ±0.42	2.36 ± 0.25	3.82 ± 0.21	0.74 ± 0.17	2.35 ± 0.40
300	5.64 ± 0.61	7.83 ± 0.54	3.56 ± 1.03	4.67 ± 1.35	4.09 ± 0.36	6.62 ± 0.41	1.54 ±0.83	2.96 ± 0.46

Reviewer's Comment (Study Design and Data Presentation) - Inadequate time points were obtained to determine AUC. These data would have provided a basis for comparison to human exposure. This is less problematic since the Sponsor is basing the doses in the mouse carcinogenicity study on maximum tolerated dose [MTD]

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

1. Male mice generally had plasma drug concentrations greater than those observed in females.

2. There was no evidence of drug accumulation based on plasma drug concentrations comparing Drug Weeks 5 and 27.

Reviewer's Comment - The Reviewer concurs.

3.10. TOXICOKINETIC STUDIES – MULTIPLE DOSE RAT STUDIES

3.10.1. Title: L-748,731: Sixteen-Day Oral Toxicokinetic Study in Male Rats [Vol. 1.16; p. B-2863]

Study Identification: TT #95-022-0
Site: Merck Research Laboratories, West Point, PA
Study Dates [In-Life]: Apr. 26 - May 11, 1995
Formulation and Lot No.: Test article - L-748,731-000R015;
Vehicle - 0.5% aqueous methylcellulose
Certificate Analysis: No (X) - assayed for purity on Day 14, assayed for concentration on Drug Days 3 and 14
Final Report (X) Oct. 30, 1995
GLP and QA statements signed: Yes (X)
Objective: "To determine the effects of different feeding regimens on the plasma and gastrointestinal toxicokinetics and gastrointestinal toxicity of L-748,731 when administered to male rats by oral gavage"

Test Material/ Group Designation	Dose*				Sex	N#	Species/Strain
	mg/kg	ml/kg	Route	# days dosed			
Control 1 - *ad libitum*	-	5	oral	15	M	4	Crl:CD®(SD)BR - Sprague Dawley Rats
Control 2 - measured	-		gavage			4	
Group 3 - L-748,731, *ad libitum*	10		SID			24	Charles River Labs; Raleigh, NC
Group 4 - L-748,731, measured	10						87 days at start of study
Group 5 - L-748,731, *ad libitum*	50						340 - 465 g
Group 6 - L-748,731, measured	50						individually housed

*Rats were fed either ad libitum or 24 gm/day @ 2-2.5 hours post-dosing
#4 animals/group for gross necropsy/histopathology on Day 16; 16/ drug-treated group for plasma and GI drug level determinations; 4/group – spare

Parameter Evaluated*	Time Point(s)
Physical Examination/mortality check	daily
Food consumption	average daily - 2X/week on a 3-day consumption period for *ad libitum* consumption estimated 2X/week - daily inspection for measured
Body weights	Drug Days 3, 6, 9, 12, and 14
Necropsy - examination of thoracic and abdominal viscera in situ, 4 animals/group	
Histopathology - GI tract, 4 animals/group	
Toxicokinetic parameters* - plasma levels - 4/animals/drug treated group - anesthetized	Drug days 1 and 5 - 0.5, 1, 2, 4, 6, 10, and 24 hours post dosing
- GI levels - stomach, duodenum, jejunum, ileum, cecum, and colon - 4/animals/drug treated group	Drug Days 15 - 16 - 2, 4, 10, and 24 hours postdosing

*methyl-tert-butyl ether extraction.

Results -

Mortality – There were no treatment-related deaths. One high dose, *ad libitum* rat was found dead Drug Day 11, but the cause of death was undetermined.

Physical examination - No treatment-related effects

Body weight - The *ad libitum* rats were larger than the measured feed rats at the start of the study [e.g. avg. for the 3 *ad libitum* groups was 421 gm and for measured feed rats the avg. was 366 gm]. The mean weight gain in the *ad libitum* control, 10 and 50 mg/kg groups was 62, 58, and 53 gm, respectively. The average weight gain in the measured feed control, 10, and 50 mg/kg groups was 23, 24, and 24 gm, respectively.

Food consumption – There were no treatment-related effects. The total mean food consumption [gm/day] was 33.7, 31.5, and 30.9 for control, 10, and 50 mg/kg *ad libitum* groups, respectively. This translates to approximately 7-7.5 gm/100 gm body weight. Average food consumption was approximately 6-7 gm/100 gm body weight for the measured feed groups.

Necropsy – There were no treatment-related effects.

Histopathology – There were no treatment-related effects.

Toxicokinetics - The tables below demonstrate the plasma and tissue drug toxicokinetics.

TABLE C-13. L-748,731: 16-DAY ORAL TOXICOKINETIC STUDY IN MALE RATS. TT 695-022-0

Summary of Mean Plasma Toxicokinetic Parameters of L-748,731 in Male Rats Following *Ad Libitum* and Measured Feeding at 10 and 50 mg/kg/day

Ad Libitum

Parameters	10 mg/kg/day DD 1	10 mg/kg/day DD 15	50 mg/kg/day DD 1	50 mg/kg/day DD 15
AUC [(0-24 hr) (µg•hr/ml)]	19.83	16.11	53.79	47.45
C_{max} (µg/ml)	4.66	1.56	5.07	4.54
T_{max} (hr)	4	2	4	4

Measured

Parameters	10 mg/kg/day DD 1	10 mg/kg/day DD 15	50 mg/kg/day DD 1	50 mg/kg/day DD 15
AUC [(0-24 hr) (µg•hr/ml)]	16.82	17.66	40.94	43.35
C_{max} (µg/ml)	1.27	1.55	3.08	4.12
T_{max} (hr)	6	2	4	2

DD = Drug Day

TABLE C-14. L-748,731: 16-DAY ORAL TOXICOKINETIC STUDY IN MALE RATS. TT 695-022-0

Summary of Mean Intestinal Tissue Toxicokinetic Parameters of L-748,731 in Male Rats Following *Ad Libitum* and Measured Feeding at 10 and 50 mg/kg/day

Ad Libitum

Parameters	10 mg/kg/day Jejunum	10 mg/kg/day Ileum	50 mg/kg/day Jejunum	50 mg/kg/day Ileum
AUC [(0-24 hr) (µg•hr/g)]	33.58	30.42	93.92	83.54
AUC [(0-4 hr) (µg•hr/g)]	9.88	16.50	29.24	27.56
C_{max} (µg/g)	3.64	6.73	10.32	12.19
T_{max} (hr)	2	2	2	4

Measured

Parameters	10 mg/kg/day Jejunum	10 mg/kg/day Ileum	50 mg/kg/day Jejunum	50 mg/kg/day Ileum
AUC [(0-24 hr) (µg•hr/g)]	34.38	49.16	77.90	157.96
AUC [(0-4 hr) (µg•hr/g)]	13.46	38.96	43.90	75.44
C_{max} (µg/g)	5.56	18.78	19.84	26.94
T_{max} (hr)	2	2	2	2

Parameters were similar for both feeding regimens, although AUC was slightly greater in the *ad libitum* groups on Drug Day 1 compared to the measured feed group. Absorption was considered slow and prolonged. Increase in AUC and C_{max} were not dose proportional.

For rats fed *ad libitum*, on Drug Day 15, jejunal levels at T_{max} ranged from 2.33 to 5.24 µg/g and 4.19 to 15.7 µg/g at 10 and 50 mg/kg/day, respectively. Values were considerably more variable for the ileum ranging from 0.748-20.3 µg/g and 1.23 to 20.8 µg/g at 10 and 50 mg/kg/day, respectively. A similar pattern was observed in the measured food group although jejunal values exhibited more variability ranging from 2.29 to 12.4 µg/g and 5.83-33.1 µg/gm at 10 and 50 mg/kg/day, respectively. Increase in C_{max} and AUC were not dose proportional.

Reviewer's Comment (Study Design and Data Presentation) - For the stated objective, these were adequate.

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

1. There was no treatment-related toxicity observed in any group.
2. Feeding regimen did not appear to alter [a] absorption; [b] plasma toxicokinetics; or [c] gastrointestinal toxicokinetics. Although the ileal concentrations in the measured feed group was approximately 89% greater than that in the *ad libitum* group at 50 mg/kg, interpretation of this is complicated by the large inter-animal variability.
3. Tissue concentration in the jejunum and ileum tended to be several fold higher than respective systemic concentrations
4. Increase in systemic and tissue exposure was not dose proportional.

Reviewer's Comment – It is not known whether the inter-animal variability observed in the GI drug levels reflected true differences in biodistribution of the drug or whether this variability was due to the analytical process used.

Reviewer's comment - The Reviewer concurs.

3.10.2. L-748,731: Sixteen-Day Oral Toxicokinetic Study in Female Rats [Vol. 1.16; p. B-2940]

Study Identification: TT # 95-022-1
Site: Merck Research Laboratories, West Point, PA
Study Dates [In-Life]: May 24 - Jun 8, 1995
Formulation and Lot No.: Test article - L-748,731-000R015;
Vehicle - 0.5% aqueous methylcellulose
Certificate Analysis: No (X) - assayed for purity on Day 13, assayed for concentration on Drug Days 3 and 14
Final Report (X) Nov. 30, 1995
GLP and QA statements signed: Yes (X)
Objective: "To determine the effects of different feeding regimens on the plasma and gastrointestinal toxicokinetics and gastrointestinal toxicity of L-748,731 when administered to male rats by oral gavage"

Test Material/ Group Designation	Dose*				Sex	N#	Species/Strain
	mg/kg	ml/kg	Route	No. days dosed			
Control 1 - *ad libitum*	-	5	oral gavage SID	15	F	4	Crl:CD®(SD)BR - Sprague Dawley Rats Charles River Labs; Raleigh, NC 87 days at start of study 218-294 g individually housed
Control 2 - measured	-					4	
Group 3 - L-748,731, *ad libitum*	10					24	
Group 4 - L-748,731, measured	10						
Group 5 - L-748,731, *ad libitum*	50						
Group 6 - L-748,731, measured	50						

*rats were fed either ad libitum or 17 gm/day @ 2-2.5 hours post-dosing
#4 animals/group for gross necropsy/histopathology on Drug Day 16; 16/drug-treated group for plasma and GI drug level determinations; 4/group - spare

Parameter Evaluated*	Time Point(s)
Physical Examination/mortality check	daily
Food consumption	average daily - 2X/week on a 3-day consumption period for *ad libitum* consumption estimated 2X/week - daily inspection for measured
Body weights	Drug Days 3, 6, 9, 12, and 14
Necropsy - examination of thoracic and abdominal viscera in situ, 4 animals/group	
Histopathology - GI tract, 4 animals/group	
Toxicokinetic parameters* - plasma levels - 4/animals/drug treated group - anesthetized	Drug days 1 and 5 - 0.5, 1, 2, 4, 6, 10, and 24 hours post dosing
- GI levels - stomach, duodenum, jejunum, ileum, cecum, and colon - 4/animals/drug treated group	Drug Days 15 - 16 - 2, 4, 10, and 24 hours post dosing

*Methyl-tert-butyl ether extraction

Results -

Physical examination - Signs, were observed in 1 *ad libitum* rat at 50 mg/kg/day starting Drug Week 2. These signs included abdominal distention, palpable intestinal loop aggregation, slight weight loss, decreased food consumption, dehydration, and urine staining. This animal died prematurely.

Mortality - One animal died on Drug Day 15 due to drug-induced intestinal perforation and peritonitis.

Body weight - The *ad libitum* rats were larger than the measured feed rats at the start of the study [e.g. avg. for the 3 *ad libitum* groups was 265 gm and for measured feed rats the avg. was 234 gm]. The mean weight gain in the *ad libitum* control, 10 and 50 mg/kg groups was 18, 22, and 18 gm, respectively. The mean weight gain in the measured feed control, 10, and 50 mg/kg groups was 1, 7, 6 gm, respectively.

Food consumption – There were no treatment-related effects. The total mean food consumption [gm/day] was 24.0, 21.8, and 21.3 for control, 10 and 50 mg/kg groups, respectively. This translates to approximately 8 gm/100 gm body weight. Average food consumption was approximately 7 gm/100 gm body weight.

Necropsy – Two animals in the high dose group exhibited "peritonitis with intestinal contents and adhesions in the peritoneal cavity".

Histopathology - Perforations were not demonstrated histologically, although the change is compatible with this lesion.

Toxicokinetics - The tables below demonstrate the plasma and tissue drug toxicokinetics.

Summary of Mean Plasma Toxicokinetic Parameters of L-748,731 in Female Rats Following *Ad Libitum* and Measured Feeding at 10 and 50 mg/kg/day

	AD LIBITUM			
	10 mg/kg/day		50 mg/kg/day	
Parameters	DD 1	DD 15	DD 1	DD 15
AUC [(0-24 hr) (μg•hr/ml)]	35.12	31.33	93.11	70.85
C_{max} (μg/ml)	2.32	2.46	6.98	5.83
T_{max} (hr)	4	4	6	6

	MEASURED			
	10 mg/kg/day		50 mg/kg/day	
Parameters	DD 1	DD 15	DD 1	DD 15
AUC [(0-24 hr) (μg•hr/ml)]	31.82	30.40	89.07	78.00
C_{max} (μg/ml)	2.57	2.43	6.99	6.24
T_{max} (hr)	6	4	6	2

DD = Drug Day

Summary of Mean Intestinal Tissue Toxicokinetic Parameters of L-748,731 in Female Rats Following *Ad Libitum* and Measured Feeding at 10 and 50 mg/kg/day

	AD LIBITUM			
	10 mg/kg/day		50 mg/kg/day	
Parameters	Jejunum	Ileum	Jejunum	Ileum
AUC [(0-24 hr) (μg•hr/g)]	42.00	31.14	194.22	442.04
AUC [(0-4 hr) (μg•hr/g)]	11.48	12.32	48.47	399.14
C_{max} (μg/g)	3.94	4.59	32.65	196.30
T_{max} (hr)	2	2	4	2

	MEASURED			
	10 mg/kg/day		50 mg/kg/day	
Parameters	Jejunum	Ileum	Jejunum	Ileum
AUC [(0-24 hr) (μg•hr/g)]	43.18	30.60	167.98	256.86
AUC [(0-4 hr) (μg•hr/g)]	13.81	13.87	91.05	214.44
C_{max} (μg/g)	5.16	5.68	39.40	103.58
T_{max} (hr)	2	2	2	2

Parameters were similar for both feeding regimens on both Drug Days 1 and 15. Absorption was considered slow and prolonged. Increase in AUC and C_{max} were not dose proportional.

At 10 mg/kg/day, jejunal and ileal values were similar for both feeding regimens on Drug Days 1 and 15. At 50 mg/kg/day, values tended to be greater in rats fed *ad libitum* on both Drug Days 1 and 15. On Day 15 jejunal levels at T_{max} ranged from 2.93 to 6.32 μg/g and 4.90-110 μg/g for 10 and 50 mg/kg/day, respectively. The values for the ileum at T_{max} ranged from 1.65 - 9.28 μg/g and 2.54-422 μg/g] for 10 and 50 mg/kg/day, respectively. Due to the large inter-animal variability, the values were "disproportionately greater than the 5-fold increase in the dose

Reviewer's Comment (Study Design and Data Presentation) - For the stated objective, these were adequate.

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

1. Feeding regimen did not appear to alter [a] absorption; [b] plasma toxicokinetics; or [c] gastrointestinal toxicokinetics. The ileal values appeared to be greater in the *ad libitum* rats than in the measured feed rats, but the Sponsor considered this effect to be a reflection of the large inter-animal variability. **Reviewer's Comment** – It is not known whether this inter-animal variability reflected true differences in biodistribution of the drug in the gut or whether this variability was due to the analytical process used.
2. Tissue concentration in the jejunum and ileum tended to be several fold higher than respective systemic concentrations at 50 mg/kg/day. **Reviewer's comment** - This increase was due to a single animal each for the jejunum and ileum that amplified the apparent tissue exposure compared to systemic exposure. For the other animals, the values for tissue concentration were only slightly greater than plasma values at the respective time points. It is of note, that based on these studies, although the exposure based on plasma AUC levels tended to be greater in females than in males, the exposure of the intestinal tract tended to be comparable.
3. Increase in systemic and tissue exposure was not dose proportional. **Reviewer's comment** - The Reviewer concurs.

3.10.3. L-748,731: Twenty-Seven-Week Oral Toxicokinetic Study in Rats: [Vol. 1.36; p. E-613]

Study Identification: TT#95-076-1
Site: Merck Research Laboratories, West Point, PA
Study Dates [In-life]: Nov. 22, 1995 – May 23, 1996
Formulation: Test Article - L-748,731 Vehicle - 0.5% aqueous methylcellulose
Lot No. L-748,731-000R015; Weeks 1-11; L-748,731-000R027; Weeks 11-27; 99.9% purity mixed daily, "has been previously documented to be stable under the conditions of use in this study"
Certificate Analysis: No (X) assayed for uniformity Drug week 1; assayed for concentration Weeks 1, 7, 12, 24, and 27 with results within acceptable limits
Final Report (X) Nov. 12, 1996
GLP and Quality Assurance Statements: Yes (X)
Objective: "To determine the plasma drug levels of the test article when administered orally to rats for approximately 14 and 27 weeks."

Test Material/ Group Designation	Dose*				Sex	N	Species/Strain
	mg/kg	ml/kg	Route	# days dosed			
Group 1 - L-748,731	2	5	oral, gavage	183	M	10	-Crl:CD® [SD] - Sprague Dawley
Group 2 - L-748,731	5				F	10	– 38 days at start of study -F – 103-142 g; M – 122-177
Group 3 - L-748,731	8						-individually housed

*administered daily, female and male rats were fed approximately 17 and 24 gm of PMI Certified Rodent diet, free access to drinking water.

Parameter Evaluated	Time Point(s)
Clinical observations/mortality	Daily
Body weight	pretest, 1X/week for Week 1, then generally 2X/week through Week 13, then weekly thereafter
Plasma drug levels *– blood collection from 5 nonfasted rats/sex/group – AUC_{0-24}, C_{max}, T_{max}	Drug Weeks 14 and 27 – 1, 2, 4, and 24 hours

Results

Mortality – There were no unscheduled sacrifices or deaths.

Body weight - There were no treatment-related effects on body weight gain.

Pharmacokinetic Parameters – The table below outlines the AUC_{0-24}, C_{max}, and T_{max} for females and males administered L748,731 for 14 and 27 weeks.

Dose	Females						Males					
mg/kg/day	Week 14			Week 27			Week 14			Week 27		
	T_{max} [hrs]	C_{max} [µg/ml]	AUC_{0-24} [µghr/ml]	T_{max} [hrs]	C_{max} [µg/ml]	AUC_{0-24} [µghr/ml]	T_{max} [hrs]	C_{max} [µg/ml]	AUC_{0-24} [µghr/ml]	T_{max} [hrs]	C_{max} [µg/ml]	AUC_{0-24} [µghr/ml]
2	2	0.687	5.90	2	0.595	6.51	2	0.549	4.80	1	0.426	4.63
5	2	1.67	19.9	2	1.49	18.6	2	1.26	13.9	2	1.06	12.1
8	2	2.56	29.2	2	2.33	27.8	2	1.73	20.2	2	1.45	19.3

Reviewer's Comment –Study Design and Presentation – These were adequate.

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

1. Rapid absorption in male and female rats following oral administration of L-748,731 is evidenced by a T_{max} of approximately 2 hours.

2. Toxicokinetics were "approximately linear ... over the dose range of 2 to 8 mg/kg/day" at both Week 14 and 27.

3. There was a slightly higher exposure to drug in females than males at both time points evaluated.

4. The toxicokinetics for Weeks 14 and 27 were comparable.

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

3.11 TOXICOKINETIC STUDIES – MULTIPLE DOSE DOG STUDY

3.11.1. MK-0966: Sixteen-Day Oral Toxicokinetic Study in Dogs [Vol. 1.49; p. Q-129]

Study Identification: TT #98-049-0
Site: Merck Research Laboratories, West Point, PA
Study Dates [In-Life]: April 23-May 8, 1998
Formulation and Lot No.: Test article - L-748,731-000R069;
Vehicle - 0.5% aqueous methylcellulose
Certificate of Analysis Submitted: [X] No - assayed for uniformity Drug Week 1, assayed for concentration Drug Weeks 1 and 2; all assays were within acceptable limits according to the Sponsor
Final Report: July 17, 1998
GLP and QA statements signed: [X] Yes
Objective: "To determine the synovial fluid and plasma toxicokinetic profiles of MK-0966 when administered orally to dogs for approximately 2 weeks.

Test Material/ Group Designation	Dose*				Sex	N	Species/Strain
	mg/kg	ml/kg	Route	# days dosed			
Group 1 – L-748,731	50	5	oral gavage	15	M/F	11	Beagle dogs – 43-51 weeks at study start Females – 8.6-10.7 kg Males – 9.7-14.3 kg

*dogs were fed 350 g/day

Parameter Evaluated	Time Point(s)
Physical Examination/mortality check	daily
Body weights	For dose calculation, 1X/week
Food consumption	4 days/week
Toxicokinetic parameters [N=1-4/timepoint]* – blood and synovial fluid [anesthetized]	2, 6, 12, and 24 hours post dosing

*A total of 9 and 11 males and females were evaluated.

Results

Mortality - All animals survived to study termination

Clinical Observations – According to the Sponsor, there were no treatment-related effects.

Food Consumption – There were no treatment-related effects

Plasma and Synovial Fluid Drug Levels - The table below outlines these results.

Toxicokinetic Parameter	Plasma [P]		Synovial Fluid [S]		Ratio [S/P]	
	Male	Female	Male	Female	Male	Female
AUC [µg•hr/ml]	20.7	21.4	6.99	7.47	0.338	0.349
C_{max}	3.43	3.47	1.05	1.21	0.306	0.349
T_{max}	2	2	2	2	NA	NA

Reviewer's Comment [Study Design and Data Presentation] – The N was small and only 1 male was evaluated at 24 hours. However, for the stated purposed, the study design and data presentations were adequate.

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

1. T_{max} for this study was 2 hours, although this was the earliest time point evaluated.

2. "The qualitative toxicokinetic profile in plasma and synovial fluid was similar". The exposure for synovial fluid, however, was approximately 30-35% that observed in the plasma.

Reviewer's Comment - The Reviewer concurs.

3.12 TOXICOKINETIC STUDIES – REPRODUCTION TOXICOKINETICS IN RATS

3.12.1. L748,731: Oral Toxicokinetic Study in Pregnant and Nonpregnant Rats [Vol. 1.29; p. C-2627]

Study Identification: TT #95-709-0, TT #95-709-1, TT #95-709-2
Site: Merck Research Laboratories, West Point, PA
Study Dates [In-Life]: TT #95-709-0; Feb. 13-Mar 9, 1995
TT #95-709-1; Feb. 27-Mar 14, 1995
TT #95-709-2; Mar. 15-Mar 22, 1995
Formulation and Lot No.: Test article - L-748,731-000R009;
Vehicle - 0.5% aqueous methylcellulose
Certificate Analysis: No (X) Assayed for uniformity Drug Week 1; assayed for concentration Drug Week 1 and at the end of the study; results were within 15% of the desired concentration
Final Report (X) Oct. 13 1995
GLP and QA statements signed: Yes (X)
Objective: "The purpose of this study was to determine the concentrations of L-748,731 in plasma and gastrointestinal [GI] tissues from pregnant and nonpregnant rats as well as in fetal plasma, and to compare the GI toxicity between pregnant rats after repeated oral administration."

Test Material/ Group Designation	Dose and Regimen#				N*	Sex	Species/ Strain
	mg/kg	ml/kg	Route	# of doses			
Group 1 -Vehicle Pregnant Nonpregnant	-	5	oral gavage	GD 6-20 15 days for NP*	12 [P**] 16 [NP] 36 [P] 40 [NP]	F	Sprague-Dawley - fCrl:CD®(SD)BR] TT #95-709-0 - app. 10 wk; 206-286 g TT #95-709-1 - app. 12 wk; 231-309 g TT #95-709-2 - app. 12 wk; 205-247 g
Group 2 - L-748-731 Pregnant Nonpregnant	10						
Group 3 - L-748-731 Pregnant Nonpregnant	50						

#Diet - all pregnant females and half of the nonpregnant were fed *ad libitum*, the remaining half of the nonpregnant rats were fed approximately 17 gm/day
*NP = nonpregnant
**P = pregnant

Protocol nonadherence - All nonpregnant females fed 17 gm/day were euthanized and discarded prior to scheduled sacrifice. The Sponsor states that this decision was based on the fact "that the time of feeding was not standardized for these animals and this variable could potentially influence GI tract toxicity and/or the pharmacokinetic profile of the compound".

Parameter Evaluated*	Time Point(s)
Clinical observations	GD0, first day of dosing through sacrifice, 1-4 hours postdosing
Mortality	daily
Body Weight	GD 0, 6, 8, 10, 12, 14, 16, 18, 20 NP - Drug Day 1, 3, 6, 9, 12, 15
Food consumption	Timepoints not provided
Plasma kinetic profile [C_{max}, t_{max}, AUC_{0-24}] - anesthetized, retro-orbital bleed/inferior vena cava - P - 4 females/time point**	GD 6, 14, and 20 at 0.5, 1, 2, 4, 6, 10, and 24 hours post dosing
- fetal blood - umbilical vein, pooled samples	GD 20 - 2 and 4 hours post dosing
- NP - 4 females/time point***	After the first and last dose at 0.5, 1, 2, 4, 6, 10, and 24 hours post dosing
GI tissue collection for drug concentration - jejunum, ileum	GD 20 - 2, 4, 10, and 24 hours post dosing NP - Drug Day 15 - 2, 4, 10, and 24 hours post dosing
Necropsy -thoracic and abdominal viscera *in situ* - 4 female/group	GD 7, 15, and 21 [all 24 hours post dose - 1, 9, and 15 doses, respectively] NP - 24 hours following the first and last dose All premature deaths, unscheduled sacrifices
Histopathology - 4 female/group, GI tract	GD 7, 15, and 21 [all 24 hours post dose] NP - 24 hours following the first and last dose

** for analysis of tissue. For plasma - linear over concentration of 0.02-5 µg/ml
For tissue - linear over concentration of 0.305 -76.3 and 0.250 - 62.5 µg/g of ileum and jejunum, respectively, ileum and jejunum, respectively. The rinsing procedure to remove drug present in the GI tract prior assaying the ileum and jejunum was considered adequate by the Sponsor

**Females were bled at 2 time points on GD6 and GD20, except for animals bled at the 24 hour time point. On GD 20 females were sacrificed after bleeding for GI tissue collection and/or necropsy. Females were bled at 2 time points on GD 14 and 15, euthanized and discarded except for GD 15 females bled at 24 hours post dose which were necropsied.

***Euthanized after blood collection for GI tissue collection and/or necropsy after the final dose

Results

Mortality - There were 1 and 5 premature sacrifices in the pregnant females at 10 and 50 mg/kg/day, respectively and 1 nonpregnant female at 50 mg/kg/day.

Clinical Observations – Treatment-related signs were observed for 1-2 days prior to premature sacrifice in 1 and 4 pregnant females at 10 and 50 mg/kg/day, respectively, and in 1 nonpregnant female at 50 mg/kg/day. The most common signs included urine -stained fur, no or soft feces, and or red oculonasal discharge. No other signs were described by the Sponsor for any group.

Body Weight - There were no apparent treatment-related effects on average body weight and body weight gain. However, there were 2 and 6 nonpregnant animals at 10 and 50 gm/kg/day, respectively, which exhibited a decrease in weight gain when compared to controls or an overall weight loss for the 15-day dosing period. [Note: Comparison is between 4 controls and 12 treated rats]

Food Consumption - There was a decrease in food consumption in 6/24 females GD 18-20 with values ranging from 1-17 g/day. The lowest control value was 27 g/day.

Necropsy -GI lesions were observed in [1] all animals prematurely sacrificed; [2] 3/4 pregnant rats at 50 mg/kg/day after 15 doses; and [3] 1/4 nonpregnant rats at 50 mg/kg/day after 15 doses. No gross lesions were observed after 1 or 9 doses.

Histopathology - Small intestinal erosions/ulcers, primarily in the posterior jejunum, and peritonitis were observed in all but 1 female in which gross lesions were seen. Slight gastric submucosal inflammation and nonglandular gastric ulcer was observed in 1 pregnant and nonpregnant female, respectively, at 50 mg/kg/day. No histopathologic lesions were observed after 1 or 9 doses.

Toxicokinetic Parameters - Plasma toxicokinetics - The toxicokinetics for pregnant and nonpregnant animals were, in general, similar and characterized by delayed absorption and a less than dose proportional exposure. There was approximately a 30% decrease in both C_{max} and AUC_{0-24} on Drug Day 15 as compared to Drug Day 1 in nonpregnant animals. L-748,731 readily crossed the placenta as demonstrated by fetal blood levels that ranged from 86-103% of maternal plasma levels at 10 and 50 mg/kg/day. These values are outlined in the table below.

SUMMARY OF MEAN PLASMA TOXICOKINETIC PARAMETERS OF L-748,731 IN PREGNANT AND NONPREGNANT RATS FOLLOWING ORAL DOSING AT 10 AND 50 MG/KG/DAY

PREGNANT

	10 mg/kg/day			50 mg/kg/day		
Parameters	GD 6	GD14	GD 20	GD 6	GD14	GD 20
AUC (µg • hr/ml)	30.92	43.25	36.80	116.29	108.56	112.97
C_{max} (µg/ml)	2.67	3.53	3.13	8.70	8.45	7.80
T_{max} (hr)	4	6	4	6	6	6

NONPREGNANT

	10 mg/kg/day		50 mg/kg/day	
Parameters	DD 1	DD 15	DD 1	DD 15
AUC (µg • hr/ml)	42.84	37.82	125.76	84.67
C_{max} (µg/ml)	3.07	3.15	10.19	7.20
T_{max} (hr)	4	2	6	4

FETAL (GD 20)

	10 mg/kg/day			50 mg/kg/day		
Parameters	Maternal	Fetal	Fetal/Maternal	Maternal	Fetal	Fetal/Maternal
2-hr concentration	2.75	2.45	0.89	6.57	5.98	0.91
4-hr concentration	3.13	2.68	0.86	7.19	7.37	1.03

GD = Gestation Day

- **Jejunal and ileal toxicokinetics** - Drug concentrations in the ileum and jejunum demonstrated variability but were approximately 2-3X that present in the plasma. There was also approximately a 2-3X increase in AUC and 5-12X increase in C_{max} in the jejunum compared to plasma. For both the ileum and jejunum, the exposure was roughly dose proportional. Based on AUC, the jejunal exposure at 50 mg/kg/day in pregnant females was considerably greater than that in the nonpregnant females. This was largely dependent on a single pregnant animal at a single time point [2 hr]. The mean concentrations at 4, 10, and 24 hours were comparable for both pregnant and nonpregnant animals.

SUMMARY OF MEAN INTESTINAL TISSUE TOXICOKINETIC PARAMETERS OF L-748,731 IN PREGNANT AND NONPREGNANT RATS FOLLOWING ORAL DOSING AT 10 AND 50 MG/KG/DAY

PREGNANT

Parameters	10 mg/kg/day		50 mg/kg/day	
	Jejunum	Ileum	Jejunum	Ileum
AUC [(0-24 hr) (ug • hr/g)]	77.80	60.98	353.94	228.82
AUC [(0-4 hr) (ug • hr/g)]	37.43	16.33	211.64	54.29
C_{max} (ug/g)	15.76	10.13	94.17	41.35
T_{max} (hr)	2	4	2	4

NONPREGNANT

Parameters	10 mg/kg/day		50 mg/kg/day	
	Jejunum	Ileum	Jejunum	Ileum
AUC [(0-24 hr) (ug • hr/g)]	69.60	49.30	232.72	364.50
AUC [(0-4 hr) (ug • hr/g)]	22.19	14.24	103.28	176.41
C_{max} (ug/g)	8.50	6.02	41.43	80.35
T_{max} (hr)	2	4	2	2

Sponsor' Conclusions [numbered] and Reviewer's Comments

1. GI toxicity resulting in mortality was observed at ≥10 mg/kg/day in pregnant rats and at ≥50 mg/kg/day in nonpregnant rats. **Reviewer's Comment** – The data suggest that pregnant rats have an increased sensitivity to the toxic effects of L-748,731 compared to nonpregnant rats.

2. Pregnancy did not appear to alter the plasma toxicokinetics when compared to nonpregnant females. **Reviewer's Comment** – The Reviewer concurs.

3. Jejunal drug exposure tended to be greater in the pregnant vs. nonpregnant rats but this was due in large part to the 2-hr. value in a single pregnant animal. **Reviewer's Comment** – Considerable variability was also seen in Study TT # 95-022-1. As noted, it is not known whether this inter-animal variability reflected true differences in biodistribution of the drug in the gut or whether this variability was due to the analytical process used. In the study in pregnant and nonpregnant rats, the GI drug levels were greater than the plasma values. However, in Study TT # 95-022-1, plasma and tissue concentrations tended to be similar in females. The reason for this difference is not known.

4. L-748,731 readily crosses the placenta. **Reviewer's Comment** – This study indicates that the fetus will be exposed to plasma drug levels that are comparable to the dam.

<u>3.12.2. L-748,731: Oral Toxicokinetic Study in Lactating Rats [Vol. 1.30; p. C-2738]</u>

Study Identification: TT #96-712-0
Site: Merck Research Laboratories, West Point, PA
Study Dates [In-Life]: Feb. 22 - Mar. 21, 1996
Formulation and Lot No.: Test article - L-748,731-000R027;
Vehicle - 0.5% aqueous methylcellulose
Certificate Analysis: No (X) Assayed for uniformity and concentration Drug Week 2; results were within 15% of the desired concentration
Final Report (X) Oct. 14, 1996
GLP and QA statements signed: Yes (X)
Objective: "The purpose of this study was to determine the levels of MK-0966 on Lactation Day 3 in maternal plasma and milk following oral administration of drug on Gestation Day 15 through Lactation Day 3."

Test Material/ Group Designation	Dose and Regimen#				N	Sex	Species/ Strain
	mg/kg	ml/kg	Route	No. of doses			
Group 1 - L-748,731	1	5	oral gavage	GD 15 - LD 3	15	F	Sprague-Dawley - [Crl:CD®(SD)BR] app. 10 wks at study start 238-323 g
Group 2 - L-748,731	10						

#Diet - fed *ad libitum*

Parameter Evaluated*	Time Point(s)
Mortality	daily
Body Weight	GD 0, 6, 12, 15, 16, 18, 20,22; LD 0 and 3
Pup mortality	daily
Plasma kinetic profile# [C_{max}, t_{max}, AUC_{0-24}] - anesthetized, 4 rats/group, milk [after oxytocin]and blood	LD 3 -2 hours post dosing

*

**

milk: plasma - linear over concentration of 0.02-10 µg/ml for
. - linear over concentration of 0.096-24 µg/ml,

Results

Mortality - Maternal - There were no deaths during the study

- Pups - The number of live pups/female was comparable in both treatment groups. Between PND 0-3, there was an increase in pup death at 10 mg/kg/day [6 pups] compared to 1 mg/kg/day [1 pup].

Body Weight - There were no treatment-related effects except for a decrease in weight gain of 29 vs. 17 g at 1 vs. 10 mg/kg/day, respectively, in dams between LD 20-22.

Toxicokinetic Parameters - The increase in milk and plasma concentrations was greater than dose proportional for 1 and 10 mg/kg/day. The mean milk:plasma ratio was 0.52 and 1.27 at 1 and 10 mg/kg/day, respectively.

Sponsor's Conclusions -
1. L-748,731 readily passes into the milk resulting in significant exposure to the suckling rat.
Reviewer's Comment – The Reviewer concurs.

3.13 TOXICOKINETIC STUDIES – REPRODUCTION TOXICOKINETICS IN RABBITS

3.13.1. L-748,731: Oral Toxicokinetic Study in Pregnant and Nonpregnant Rabbits [Vol. 1.23; p. C-337]
Study Identification: TT #95-722-1,-1
Site: Merck Research Laboratories, West Point, PA
Study Dates [In-Life]: May 23-Jun 14, 1995
Formulation and Lot No.: Test article - L-748,731-000R009;
Vehicle - 0.5% aqueous methylcellulose
Certificate Analysis: [X] No - assayed for concentration at the initiation and termination of dosing interval; all assays were within acceptable limits
Final Report: Dec. 28, 1995
GLP and QA statements signed: [X] Yes

Objective: "To determine and compare plasma concentrations of L-748,731 in pregnant rabbits, and their fetuses, following oral administration for 14 days"

Test Material/ Group Designation	Dose*				Sex	N	Species/Strain
	mg/kg	ml/kg	Route	# days dosed			
Group 1 – L-748,731	25	4	oral gavage	GD 7-20	F	16 - Preg.	New Zealand White Rabbits; app. 6 mo. at study start; Pregnant - 2.8 - 4.3 kg/GD 0; Non-Pregnant - 3.3 - 4.0 kg/Day 1
Group 2 - L-748,731	150			Drug Day 1-14		12 Non-Preg.	

*rabbits were fed *ad libitum*

Parameter Evaluated	Time Point(s)
Physical Examination/mortality check	daily and 1-5 hours post dosing
Body weights	Pregnant - GD 0, 7, 9 ,11,13,14,17,19, and 20 Non-pregnant - Drug Days 1, 3, 5, 7, 9, 11, 13,14
Toxicokinetic parameters* - maternal and fetal plasma levels [4/group at each time point]	GD 20** - 0.5, 1, 2, 4, 6, 10, and 24 hours post dosing

*Methyl-tert-butyl ether extraction; linear over concentration of 0.02-10 µg/ml

**Collected from the medial ear artery or marginal vein at 0.5, 1, 6, and 10 hours; collected under isoflurane from the inferior vena cava at 2, 4, and 24 hours post dosing. Fetuses were then bled.

Results -

Clinical Observations - Three females dosed at 150 mg/kg/day had blood in their pans GD 18,19, or 20. This correlated with complete litter resorption. Blood in the pan was also observed in one female at 25 mg/kg/day. This finding was associated with a placental hematoma and live fetuses. The Sponsor considers this unrelated to treatment since "similar finding are occasionally noted in control does in this laboratory". Complete resorption was observed in 5/16 pregnant does at 150 mg/kg/day.

Body Weight Gain - The body weight gain from GD 7-20 for the high dose females was less [71 ± 109] than in the low dose females [172 ± 56]. This probably reflects litter resorption.

Toxicokinetic Results - Absorption was slow in pregnant does at both doses. The increase in exposure was greater than proportion to the increase in dose. This was apparently secondary to a decrease in clearance. The mean fetal: maternal plasma ratios were 0.63 and 0.57 at 2 and 4 hours and below quantifiable levels at 24 hours post dosing at 25 mg/kg/day. Fetal levels from the high dose does were not available for all time points due to complete litter resorption. The mean fetal:maternal plasma ratios were 0.39, 0.46, and 0.72 at 2, 4, and 24 hours post dosing at 50 mg/kg/day. The table below is a summary of the toxicokinetic data.

SUMMARY OF MEAN PLASMA TOXICOKINETIC PARAMETERS AND COMPARISON OF MATERNAL AND FETAL PLASMA LEVELS OF L-748,731 IN PREGNANT RABBITS FOLLOWING ORAL DOSING AT 25 AND 150 MG/KG/DAY

Maternal

Parameters	25 mg/kg/day	150 mg/kg/day
AUC (µg•hr/ml)	2.51	35.89
C_{max} (µg/ml)	0.47	3.25
T_{max} (hr)	4	2

Maternal/Fetal

	25 mg/kg/day			150 mg/kg/day		
	µg/ml			µg/ml		
Parameters	Maternal	Fetal	Fetal/Maternal	Maternal	Fetal	Fetal/Maternal
2-hr conc.	0.35	0.22	0.63	3.25	1.26	0.39
4-hr conc.	0.47	0.27	0.57	2.45	1.12*	0.46
24-hr conc.	0	0	ID	0.60	0.43	0.72

ID = Insufficient data available for calculation
*Not a mean value - result of one animal

Reviewer Comment -Study Design and Data Presentation - These were adequate for the stated purpose.

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

1. Placental transfer of L-748,731 is fairly high in rabbits at 25 and 150 mg/kg/day. **Reviewer's Comment** - The Reviewer concurs. The maternal:fetal plasma ratio was less in rabbits [approximately 40-70%] than in rats [approximately 90-100%].

3.14 PHARMACOKINETICS OF L-755,190, THE 5-HYDROXY FURANONE METABOLITE OF L-748,731

3.14.1. Absorption, Distribution, Metabolism and Excretion of L-755,190 in Rats and Dogs [Vol. 1.45; G-190]

Site: Merck Research Laboratories; West Point, PA
Formulation and Lot No.: Test article [^{14}C]L-755,190-001K001; [^{14}C] at 4-position of the furanone ring

Vehicle – DMSO for iv administration
- 0.5% aqueous methylcellulose for po administration

Certificate Analysis: No (X)
Final Report (X) April 1, 1996
GLP and QA statements signed: No (X)
Objective: To determine absorption, distribution, metabolism and excretion of L-755,190 in rats and dogs.

Study Design – Male Sprague Dawley rats [N=4] and Beagle dogs [N=4] were administered radiolabeled L-755,190 at 2 mg/kg intravenously or 5 mg/kg orally. Blood, urine, and feces samples were collected over a 96-hour interval. A crossover study was conducted in dogs with a 2-week washout period between doses. Total radioactivity in samples was determined L-755,190 and MK-0966 concentrations in plasma and were determined respectively. Only C_{max}, T_{max}, and apparent $t_{1/2}$ were determined due to reversible metabolism to MK-0966. *In vitro* and *in vivo* metabolism, protein binding, and erythrocyte partitioning were assessed.

Results – Absorption/Excretion - Plasma clearance in both rats and dogs was multi-exponential with an apparent $t_{1/2}$ of 8.4 [4.8] hours in the rat [dog] after iv administration and 10 hours for both species after oral administration. MK-0966 appeared rapidly in the plasma of the rat after either po or iv administration with two peaks occurring following oral administration at 5-15 minutes and after 4 hours. There were also two peaks in the dog, with the first occurring 15 minutes after dosing and at 8-24 hours. The initial C_{max} in dogs was approximately 5X greater than rats. Total radioactivity recovery was 92.4