Parameter Evaluated	Time Point(s)
Physical examination/mortality	Daily
Body weight	pretest, 1X/ Drug Week 1, 2X/Drug Week 2
Food consumption	3-day period, 1X/week for Drug Weeks 1 and 2
Necropsy -10/group; GI tract only	Day 16
Histopathology - 10 group; stomach, small and large intestine including cecum, gross GI changes	Day 16
Special procedures - 6/group; PG levels [PGE_2, 6-keto PGF_1] CO activity, L-748,731 concentration - samples conducted 2 hours post-dosing	Day 16

Results -

Mortality - No unscheduled deaths or premature sacrifices.

Physical signs - On Drug Day 15, one Group 3 animal had urine staining. This has been associated with L-748,731 animals with GI lesions. This animal did not undergo necropsy, and therefore, it is not possible to rule out this sign as treatment related.

Body weight - Transient body weight loss and/or decreased body weight gains were observed in both Groups 2 and 3 compared to control values. Decreases in body weight gain were associated with GI lesions observed at necropsy in several animals.

Food consumption - A decrease in food consumption was sometimes associated with a decrease in body weight gain.

Necropsy - The following observations were made in Group 3 animals [1 each]: [1] distended, firm distal jejunum/ileum adhering to other intestinal segments; [2] slightly thickened area of cecal mucosa.

Histopathology - The following histopathological changes were observed in the 2 Group 3 animals with gross necropsy findings: [1] small intestinal ulcer and moderate focal acute peritonitis; and [2] cecal ulcer. Histopathological lesions observed in Group 2 included [1] pyloric erosions; or [2] slight focal subepithelial inflammation of the pylorus in 3 rats; and [3] very slight focal area of cecal inflammation in 1 animal.

Jejunal levels of L-748,731 – Mean jejunal concentration of L-748,731 at 2 hours following dosing was 23.98 ± 10.31 μg/g [range of 4.70 –68.40 μg/g]

Special procedures - Ibuprofen resulted in large decreases in basal PG levels and CO activity [approximately 85-97%]. Similar results were not seen with L-748,731. There was only a slight decrease in basal PG levels compared to controls.

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

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

1. COX1 activity and basal PG levels in the intestinal tract were not significantly modified by treatment with L-748,731 especially compared to the effects of Ibuprofen.

2. These data suggest that factors other than COX1 inhibition induce the GI toxicity observed with L-748,731.

Reviewer's Comments - The Reviewer concurs. It was interesting to note that GI toxicity was observed at 100 mg/kg/day in this study, but not at comparable doses and higher in studies of similar duration.

[Note: A second study: TT #95-069-0: L748,731, Ibuprofen: Exploratory fifteen-Day Cyclooxygenase Inhibition Study in Female Rats [Vol. 1.17; p. B-3066] was conducted as a confirmatory assay. This study will only be summarized. The PG and COX1 activity in the animals administered L-748,731 were decreased generally by 20-30% which was considerably less than the decrease observed with ibuprofen [60-90%].]

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

4.3.4. Fourteen-Week Oral Range-Finding Study in Rats:[Vol. 1.15; B-2400 and Vol. 1.16; B-2612]

Study Number: TT# 95-018-0
Compound: L-748,731, Batch L-748,731-000R014 and L-748,731-000R015, >99% analysis of each batch, *uniformity tested Drug Week 1, concentration tested Drug Weeks 1, 6, and 14*
Formulation: Suspension in 0.5% aqueous methylcellulose.
Control Treatment: 0.5% aqueous methylcellulose
Route: Oral, by gavage at 5 mL/Kg
Dosage Groups: 1 2 3 4 5 6
mg/Kg/day: 0 125 250 500 1000 2000
Total No. of Doses: M 92, F 92*
* F at 250 mg/Kg and higher were terminated Week 8 due to treatment-related mortality
Females and males were fed 17 and 24 gm/day, respectively
Strain: [Crl:CD®(SD)BR], 37 days old, body wt. M 133 to 207 g, F 109 to 160 g
Number of Animals: 10/sex/group
Control Vehicle: 0.5% aqueous methylcellulose
Study Site: Merck Research Laboratories, West Point, PA
Date of Study: 23 Mar 95 to 31 Aug 95
GLP/QAU Statements: Both present and signed.

This study was done to determine the toxicity of high dose levels of L-748,731 in order to support dosage level selection for the carcinogenicity study.

The study includes physical examinations (daily), body weight (pretest, once during W1 and twice/week thereafter), food consumption (twice/week during W1-6, three times/week during W7, and daily during W8-14), ophthalmoscopic examination (G1 and 6 W5, and W11 on controls and remaining G6 males and G2 females), hematology*/serum biochemistry** (all remaining animals W6 and 10, and on 5-6 early sacrificed animals), and urinalysis*** (all remaining animals W6 and 10). Blood was collected from 2-3 per sex in the treatment groups at W10 (0.5, 1, 2, 4, 6, 10, and 24 hours) for drug level determinations. Complete necropsy was done on all males, females in the control and G2 as well as females that died W8. Microscopic examinations were performed on G1 2F, 6M and all that died/sacrificed prior to study termination. Toxicokinetic parameters evaluated were AUC, Cmax, and time (Tmax) to achieve Cmax.

**RBC and WBC count, differential, Hb, Hct, MCV, MCH, MCHC, platelet count*
***gluc. BUN, creat., total protein, alb., A/G ratio, chol. triglycerides, AST, ALT, AP, Na, K, Cl, P, Ca*
****urine vol., spG, pH, protein, gluc., bili., occult blood, ketones, urobilinogen, microscopic exam of sediment*

RESULTS AND DISCUSSION

mortality:	mg/Kg/day	Number	Week	No. of doses	Cause of death	Moribund
related	125	1	11	77	GI toxicity	yes
to	250	3	8	49, 49, 51	"	"
treatment		1	12	81	"	"
	500	1	8	51	"	"
	1000	1	8	51	"	"
		1	8	55	"	"
	2000	1	14	91	"	"

- mortality unrelated to treatment: 2 M at 2000 mg/Kg died prior to bleeding W10: considered to be due to anesthesia overdose-
- remaining G3 F and above were terminated W8 due to excessive GI toxicity-
- signs: brownish/reddish ocular and nasal discharge, brownish/reddish staining around mouth, pallor, distended abdomen, urine and fecal staining, and black or decreased/soft feces-
- body weight: no treatment related changes - wt loss in 3 F G3 and 1 MG 6 ranged from 6-23 g, *1 male at 500 mg/kg/day lost 47 gm between predose and Drug Week 7-*
- food consumption: reduced consumption in animals sacrificed due to GI toxicity.
- ophthalmoscopic exam: no treatment-related changes-
- hematology: ↓ RBCs (2-6%), Hb (1-5%), and Hct (2-5%) in all F and M G5 and 6- *anisocytosis, polychromasia, hypochromia was observed in the majority of the animals sacrificed early, these findings indicate a regenerative anemia*
  - ↑platelets in early sacrifices and occasionally in other animals-
  - ↑(%) neutrophils (42%) and neutrophil count (50%) in early sacrifices, *some demonstrated PMN basophilic cytoplasm, hypersegmented neutrophils, and band cells-*
  - ↓lymphocytes (12% G3W6), other F groups ↓ 4-6%-
  - ↑monocytes (29%) F G3 and 4, W6-
- serum biochemistry: ↑urea nitrogen (13-33%) in M all G and early sacrifices *[3/6 ranging mg/dL]-*
  - ↑ creatinine 2 M *[2 early sacrifices with ↑BUN]-*
  - ↓ albumin in a few M/F G3 (slight); large ↓ in early sacrifices-
  - ↓ total protein, slight; large ↓ in early sacrifices-
  - ↑ AP in early sacrifices-
  - ↑ ALT and AST 1 F G3-
  - ↓ glucose in most animals in early sacrifices-
  - ↑ triglycerides F all G -
  - changes in K, phosphorus, Ca, and Na in one or two G3-
- urinalysis: no treatment related changes-
- gross findings: intestinal tract lesions- peritonitis, ascites, fibrinous adhesions, focal thickening and/or ulceration of jejunum and cecum-
- organ weights: no treatment related changes-

- histopathology:

Number Of Animals With Lesions
Males(Females)

	mg/Kg					
	control	125	250*	500*	1000*	2000*
examined	10(10)	10(10)	10(3)	10(0)	10(1)	10(0)
Stomach:						
pylorus-erosion	(0)	(0)	(2)	(0)	(0)	(0)
nonglandular mucosa-ulcer	(0)	(0)	(1)	(0)	(0)	(0)
Small intestine:						
ulcer	0(0)	1(1)	1(2)	1(0)	1(1)	1(0)
peritonitis, chronic	0(0)	1(1)	1(3)	1(0)	0(1)	1(0)
lymphoid tissue-giant cell formation	0	0	1	1	0	0
Large intestine:						
Cecum-ulcer	0(0)	2(0)	2(0)	5(0)	3(0)	6(0)
peritonitis, chronic	0(0)	2(1)	3(1)	2(0)	0(1)	2(0)
Lymph node						
giant cell formation	1(0)	5(0)	7(0)	6(0)	5(0)	6(0)
Kidney:						
tubular basophilia	1(0)	7(2)	7(1)	8(0)	8(1)	8(0)
cellular infiltration	1(0)	1(2)	5(1)	4(0)	7(1)	7(0)
Liver:						
focal necrosis	0(0)	2(1)	3(0)	3(0)	4(0)	3(0)
vacuolation	0(0)	0(1)	0(0)	0(0)	0(0)	4(0)

* all remaining rats in this study were euthanized and discarded without examination-

In addition to the above findings, chronic peritonitis was seen in the stomach, liver, pancreas, adrenals, kidney, urinary bladder, uterus, and spleen of treated animals. Lymphoid atrophy and or necrosis occurred in one or two animals in G2, 3, and 5. Extramedullary hematopoiesis occurred in the spleen and liver of one or two of all the treated groups.

In males, the incidence of large intestine ulceration [approximately 20-60% in distal jejunum and cecum] was greater than that observed in the small intestine [approximately 10% for all dose groups]. In females examined, ulceration was observed only in the small and not the large intestine.

toxicokinetics:

The limit of quantitation/detection was 0.0200 µg/mL using 250 µL of rat plasma. The mean percent absolute recovery of L-748,731 in maternal rat plasma was approximately 94.1% over the concentration range of 0.02000 to 10.0 µg/mL.

Summary of Mean Plasma Toxicokinetic Parameters at 125 and 250 mg/Kg/day

	125 mg/Kg/day		250 mg/Kg/day	
	Male	Female	Male	Female*
Cmax (µg/mL)	6.60	8.46	7.43	NA
Tmax (hr)	6.0	6.0	6.0	NA
AUC (µg hr/mL)	72.48	104.88	108.25	NA

* NA = not applicable, female group terminated prior to scheduled bleeding

The following table shows the toxicokinetic data from W10 of this study compared with those of drug Day 1 from the following single oral dose toxicokinetic study (TT#95-027-0) in rats.

Week 10 and Day 1 Mean Plasma Toxicokinetic Parameters at 125 and 250 mg/Kg/day

	125 mg/Kg/day				250 mg/Kg/day			
	Male		Female		Male		Female	
	Day 1	Week 10	Day 1	Week 10	Day 1	Week 10	Day 1	Week 10*
Cmax (µg/mL)	9.41	6.60	12.56	8.46	8.56	7.43	11.67	-
Tmax (hr)	6.0	6.0	6.0	6.0	6.0	6.0	6.0	-
AUC (µg hr/mL)	95.56	72.48	147.36	104.88	113.84	108.25	184.61	-

* Terminated prior to scheduled bleeding

A slow absorption of the drug is seen in the data, requiring 6 hours to attain Cmax. It was suggested that dissolution-limited rate of absorption may be one factor responsible for slower absorption. Both AUC and Cmax values for females were higher than observed in males. This was also the case in study TT#95-027-0.

4.3.5. Fourteen-Week Oral Toxicity Study in Rats: [Vol. 1.11; p. B-1000; Vol. 1.12; p. B1184]

Study: TT#94-615-0
Compound: L-748,731 (L-748,731-000R), batch No. 009, purity [redacted]
Formulation: Suspension in 0.5% aqueous methylcellulose
Route: Oral, by gavage at 5 mL/Kg body wt
Dose Levels:
Group: 1 2 3 4
0, 10, 100, 300 mg/Kg/day-7 days/week for 91-92 days
Strain: Sprague-Dawley Crl; CD®(SD) BR, 6 weeks old
Body Wt: M 192 to 253 g, F 162 to 200 g
Number: 15/sex/group
Control Treatment: 0.5% methylcellulose
Study Site: Laboratoires Merck Sharp & Dohme-Chibre, Centre de Recherche, Riom, France
Date: April - November 1994
GLP/QAU Statements: Both present and signed.

This study evaluated the toxicity and toxicokinetic profile of L-748,731. The suspension was prepared daily. Body wt was determined once or twice per week. Food consumption was measured weekly. Ophthalmologic examinations were done Weeks 4, 8, and 11 in the control and high dose group. Hematology* and blood chemistry** parameters were measured post dose, Week 5, 8, and 11. Urinalysis*** was evaluated Week 8 and 11. All rats were necropsied and histopathology done on all control and high dose animals. PK parameters were determined Week 12 *[0.5, 1, 2, 4, 6, and 24 hours].*

**RBC and WBC count, differential, Hb, Hct, MCV, MCH, MCHC, platelet count*
***gluc. BUN, creat, total protein, alb., A/G ratio, chol. Triglycerides, AST, ALT, AP, Na, K, Cl, P, Ca*
****vol., spG, pH, protein, gluc., bili., occult blood, ketones, urobilinogen, microscopic exam of sediment*

Results of an "Exploratory Cyclooxygenase [COX] Immunohistochemistry" evaluation were appended to this report in Section D. The purpose was to determine the levels of COX-1 and COX-2 in cervical and mesenteric lymph nodes in 16 rats administered L-748,731[300 mg/kg/day] and in 8 control rats.

Results and Discussion

-signs: none reported
-deaths: 1 M G4D72 (anesthetic overdose during orbital bleeding)
-body wt: all groups gained uniform weight - no drug related changes in body wt gain
-food consumption: group averages were surprisingly similar
-ophthalmologic exam: no drug related changes stated (no data)

-hematology: average values
RBC/Hb/Hct *[app. 4%]*: M/F tendency to ↓ with ↑ dosage at all time points
leukocytes: F ↓ G4[W5(20%), W8(27%), W11(9%)]
lymphocytes (%): F ↓ G4(slight, 1.3-3.2%)
M ↑ G4 (slight, 3-5%)
lymphocytes (cells/mm^3): F ↓ G4[W5(23%), W8(28%), W11(11%) *[comparable decrease in G3]*
M ↓ G4 (slight, 4-6%)
neutrophils (%): F ↑ G4(10-37%)
M ↑ G4 (42-49%)
neutrophils (cells/mm^3): F ↑ (12-16%) *[↑in all dose groups, with greatest ↑in G2 - 13-50%]*
M ↑ (39-46%) *[↑in all dose groups]*
-serum chemistry: average values
BUN: F slight DR ↑ G4 (4-10%)
M DR ↑ G4 (25-34%)
-urinalysis: no drug related changes
-necropsy: no gross changes were reported

-organ wt		absolute	relative (% brain wt)
liver:	F	DR↑ (1.7-14%)	DR↑ (5.5-10%)
	M	DR↑ (0.8-6.2)	DR↑ (1.4-4%)
ovaries:		↑G4(1.3-11.5%)	↑G4(8.8%)
heart:	F	↑G4(8.9%)	↑G4(4.7%)
	M	↑G4 (2%)	
kidneys:	M	↑G4(8%)	↑G4(5.8%)
prostate:		DR ↑G4(10%)	DR ↑G4 (7%)

-histopathology:
liver: centrilobular hepatocellular hypertrophy G4F (2/15 - *also had ↑liver weights*)
kidney: tubular basophilia G3M (5/15), G4[M(8/15) F(2/15)], *[pelvis dilatation - 1 G1M, 2 G4 M]*
small intestine: ulcer G4[M(1/15)
cervical lymph nodes: granulomas G1F(1/15), G3M(2/15), G4[M(4/15) F(1/15)] [*Table on p. 1410 indicates incidence of granulomas in control animals is 0, both summary and individual animal tables indicate that 1 control female had cervical lymph node granuloma]*
pancreas: cellular infiltration G4M(1/15)
testis: seminiferous tubule, unilateral atrophy G3(1/15), G4(1/15)
-pharmacokinetic parameters: (mean values)

Dose	C_{max} (µg/mL)		T_{max} (h)		$t_{½}$ (h)		AUC_{0-24h} (µg h/mL)	
mg/Kg/day	M	F	M	F	M	F	M	F
10	2.42	4.52	4	4	3.2	4.0	22.6	40.0
100	6.86	12.69	2	2	11.7	8.1	81.9	129.4
300	8.26	15.87	2	2	9.8	12.9	103.2	195.7

Immunohistochemical evaluation of COX-1 and COX-2 in cervical and mesenteric lymph nodes: COX-1 staining of blood vessels in the cervical and mesenteric lymph nodes was comparable in dosed and control animals. COX-2 staining of macrophages in the mesenteric lymph nodes of both treated and control rats was comparable. There was an increase in the number of COX-2 positive macrophages in the cervical lymph nodes of rats administered 300 mg/kg/day when compared to control rats.

The dosages used in this study produced no clinical signs, drug related deaths, changes in food consumption, body weight changes, ophthalmologic changes, or changes in the urinalysis parameters. Slight decreases in RBC, Hb, and Hct in both male and female rats may indicate anemia as a toxicity associated with this drug. Other changes include increases in neutrophils and increases in BUN. Organs showing toxicity mid *[male]* and high *[male and female]* dose kidney (very slight/slight tubular basophilia), small intestine (ulceration in one high dose male), and cervical lymph node granulomas (mid *[male]* and high *[male and female]* dose groups). *Slight centrilobular hepatocellular hypertrophy was observed in the high dose females. This response is considered to be an adaptive response since there are*

no other biochemical changes or histopathological lesions associated with the liver. Slight cellular infiltration in the pancreas of one high dose male and slight and moderate unilateral seminiferous tubule atrophy in one mid and one high dose rat, respectively, may also be drug related toxicities. *The NOAEL based on histopathological changes [tubular basophilia, cervical lymph node granulomas] is 10 mg/kg/day.*

With respect to the PK parameters, female exposure tended to be greater than that for males. Neither AUC or C_{max} values exhibited a linear or dose proportional increase over this dose range.

COX-1 and COX-2 staining was comparable between rats administered either control or 300 mg/kg/day with the following exception. COX-2 staining was increased in rats dosed with L748,731 when compared to controls. Control staining was negative to very slight. Staining in treated rats ranged from minimal to slight. Of the 8 rats with slight staining, 5 also exhibited cervical lymph node granulomas. The relationship between these findings is not known.

The current Pharmacology/Toxicology Reviewer reviewed the following study.

4.3.6 L-748,731: Fourteen-Week Oral Toxicity Study in Rats with a Seven-Week Interim Necropsy and a Recovery Period: [Vol 1.13: p. B-1512]

Study Identification: TT #95-614-0

Site: Laboratoires Merck Sharp & Dohme-Chibret, Centre de Recherche, Riom, France

Study Dates [In-life phase]: April 18, 1995 - Jan. 16, 1996

Formulation and Lot No.: L-748,731-000R014; [redacted] preparations prepared daily

Certificate of Analysis Submitted: No (X) assayed for uniformity Drug Weeks 1 and 7; assayed for concentration Drug Weeks 1, 7, and 13; Sponsor indicates the results were within acceptable limits.

Final Report (X) May 2, 1996

GLP and QA statements signed: Yes (X)

Objective: "To reproduce and assess the reversibility of target organ toxicity observed in a previous 14-week oral toxicity study with L-748,731, a cyclooxygenase-2 inhibitor, when given orally to rats."

Test Material/ Group Designation	Dose* mg/kg	ml/kg	Route	# days dosed	Sex	N#	Species/Strain
Group 1 - Vehicle control	-	5	oral, gavage	M and F - 42 or 91 doses	M	60	-Crl:CD® [SD] BR- Sprague Dawley rats, [redacted]
Group 2 - L-748,731	300				F	60	- ~ app. 7 wks days at study start -F – 155 to 241 g; M – 236 to 323 g -individually housed

*females and males were fed app. 17 and 24 gm/day, respectively
#15/sex/group were sacrificed Drug Week 7 and 14, Recovery Weeks 14 and 27

Parameter Evaluated	Time Point(s)
Clinical observations/mortality	Daily during treatment, weekly during recovery period
Body weight	1X/Drug Wk 1; 2X/wk - Drug wks 2-13; 1X/wk thereafter
Food Consumption	estimated 2X weekly
Necropsy - limited to brain, GI tract, liver, kidneys, cervical and mesenteric lymph nodes	Drug Week 7 and 14, Recovery Weeks 14 and 27
Organ Weights - brain, liver, kidneys	Drug Week 7 and 14, Recovery Weeks 14 and 27
Histopathology - stomach, small and large intestine, kidneys, cervical and mesenteric lymph nodes	Drug Week 7 and 14, Recovery Weeks 14 and 27

Results -

Physical signs - There were no treatment-related effects.

Mortality - There was only one unscheduled death in a control male [Day 105] At necropsy, this animal was found to have a renal hemangiosarcoma.

Body weights - There were no treatment-related effects.

Food consumption - There were no treatment-related effects. However, food consumption was only estimated.

Necropsy - There was 1 female with 2 jejunal/ileal focal mural thickenings [app. 0.5 and 1.5 cm in length].

Organ weights - There was a mild increase in absolute [relative] liver weights in male and female rats and a mild increase in absolute [relative] kidney weights administered drug compared to controls at the Drug Week 7 and 14 scheduled necropsies, respectively. The maximum change was approximately ≤15%.

Histopathology - The treatment related effects are outlined in the Sponsor provided table below.

	Females 300 mg/kg/day	Males 300 mg/kg/day
Drug Week 7 interim necropsy:		
Kidney : very slight tubular basophilia	none	5/15
Cervical lymph node: increased number of multinucleate giant cells	3/15	1/15
Drug Week 14 necropsy:		
Jejunum / ileum: ulcer present	1/15	none
Liver: very slight hypertrophy of the centrilobular hepatocytes	1/15	none
Kidney : very slight to slight tubular basophilia	3/15	11/15
very slight unilateral papillary necrosis	none	1/15
Cervical lymph node: increased number of multinucleate giant cells	4/15	4/15
Recovery Week 14 necropsy:		
Cervical lymph node: increased number of multinucleate giant cells	1/15	none
Recovery Week 27 necropsy:		
All drug-related changes were absent after a 6-month recovery period.		

These lesions were not observed in control animals.

Reviewer's Comments - Study Design and Data Presentation - For the stated objective, these were adequate. Inclusion of hematology and serum chemistry [BUN, creatinine, AST, ALT, AP as a minimum] would have provided a more complete picture.

Sponsor's Conclusion (numbered) and Reviewer's Comments;

1. With the exception of an increase in cervical lymph node multinucleate giant cells, treatment-related histopathological lesions [renal tubular basophilia; jejunal/ileal ulcer; hepatic centrilobular hypertrophy] were reversed by 14 weeks after cessation of treatment. The lymph node lesions were reversed by 27 weeks after cessation of treatment. **Reviewer's Comment** - The Reviewer concurs with the following caveat. Only 1 animal exhibited GI ulceration. This is not an adequate number of affected animals to provide a basis for determination of reversibility of GI toxicity.

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

4.3.7. Twenty-Seven Week Oral Toxicity Study in Rats: [Vol. 1.13; B-1651, Vol. 1.14; B-1918; and Vol. 1.15; B-2259]

Study: TT#95-601-0
Compound: L-748,731 (L-748,731-000R), batch no. 014, *drug was assayed for uniformity in Drug Week 1 and concentration in Drug Weeks 1, 7, 19, and 27; results were within acceptable limits, according to the Sponsor*
Formulation: Suspension in 0.5% aqueous methylcellulose
Route: Oral, by gavage at 5 mL/Kg
Dose Levels Groups: 1 2 3 4 5
mg/Kg/day: 0 10 30 100 300 for 182 to 185 doses
Females and males fed approximately 17 and 24/gm/day, respectively
Control Treatment: 0.5% methylcellulose
Strain: Sprague-Dawley Crl:CD®(SD)BR, ≈ 5 weeks old, body wt M 104-200 g, F 114-171 g
Number: 30/sex/group
Study Site: Laboratoires Merck Sharp & Dohme-Chibret, Centre de Recherche, Riom, France
Date of Study: 06Jan95 to 26Sep95
GLP/QAU: Both present with signatures.

"The objective of this study was to evaluate the toxicity and toxicokinetic profile of L-748,731 when given orally at doses up to 300 mg/Kg/day for approximately 27 weeks. Initially, the study was scheduled for one year with a 27-week interim necropsy, but due to the observed mortality, the duration of the study was reduced and all surviving rats were killed in Drug W27."

All animals were examined daily. Body weights were determined pretest, once W1, twice from W2 through W13 and once /week thereafter. Food consumption was observed twice/week. Ophthalmic examinations were done during W11 and 24 on all animals in the control and high dose groups. Hematology* and serum biochemistry** were evaluated from 10/sex/group W4, 12/sex/group W12, and from 20/sex/group W25. Urinalyses*** were done during W12 and 25. Blood was collected Week 14 (0.5, 1, 2, 4, 6, 10, 24 hr) from 4-5/sex/group (control also) for determining plasma levels. Necropsy was done on all rats. Histopathology was conducted on thirty different tissues from all control and high dose rats, and rats found dead and moribund sacrifices. Organ weights were determined on adrenals, brain, heart, kidneys, liver, ovaries/testes, pituitary, prostate, spleen, and thyroids.

**RBC and WBC count, differential, Hb, Hct, MCV, MCH, MCHC, platelet count*
***gluc. BUN, creat, total protein, alb., A/G ratio, chol., triglycerides, AST, ALT, AP, Na, K, Cl, P, Ca*
****urine vol., spG, pH, protein, gluc., bili., occult blood, ketones, urobilinogen, microscopic exam of sediment*

RESULTS AND DISCUSSION

- mortality: a total of 44 were found dead or sacrificed early *beginning in Wks 9 and 10 at the 2 highest doses, and Wks 16-18 at 10 and 30-mg/kg/day; one rat was sacrifice at 300 mg/kg/day and, on necropsy, was found to have lymphoma*

L-748,731	-drug-related-				- incidental-			
mg/Kg/day	10	30	100	300	10	30	100	300
	2	1	11	6	-	-	-	1
	5	7	6	5	-	-	-	-
Total:	7	8	17	11				1

- signs: none up to W8 - signs not dose proportional - progressed through study beginning W9 at ≥100 mg/Kg and W18 at 10 mg/Kg and W16 at 30 mg/Kg, respectively - decreased food consumption, decreased body weight gain, slight abdominal distention, dark stools at times, skin pallor, occasional decreased activity-
- body weight: drug-related changes in animals showing GI lesions
- food consumption: ↓ in animals with treatment-related GI toxicity
- ophthalmic changes: none-
- hematology: dose/time dependent ↓ (5-6%) in RBC/Hb/Hct both sexes in all drug groups - ↑ thrombocytes *[primarily in males]* and neutrophil *[females 20-100%; males 20-190%]* associated with anemia-
- serum biochemistry: SUN- G2 (8.9%), G3 (25.3%), G4 (30%), G5 (22.9%), respectively -*maximum increase over controls during Drug Week 12 or 25-*
  protein and albumin ↓ in animals not surviving to study termination-
  AST and ALT- slight ↑ in individual animals in all groups-
- urinalyses: no drug-related changes
- necropsy: drug-related deaths- most of the 43 dead and early sacrificed animals had peritonitis, adhesions, and or fibrous deposits - ulceration of GI tract, often perforating the bowel *[most frequently ulcers were in jejunum, but were also found in the duodenum, ileum, cecum, colon and/or stomach]* - liver (subcapsular necrosis) - necrosis found in several tissues - hematopoiesis in spleen, liver, and bone marrow (granulopoiesis), *lymph node histiocytosis, thymic atrophy* - endocardial inflammation in heart - renal tubular basophilia-
- necropsy: at scheduled necropsy-10 mg/Kg group- 2 M 2 F G2 and 1FG4 with multifocal thickening of healed ulcers with adhesions between intestinal loops - enlarged mesenteric lymph nodes and spleen - small thymus and pale liver-
- organ weights: no drug-related changes-

- histopathology:

Summary of Histopathology											
L-748,731 (mg/Kg/day)		0		10		30		100		300	
		M	F	M	F	M	F	M	F	M	F
GI tract											
stomach	*ulcer/erosion*	1		2			4	6	4	4	1
	focal hyperkeratosis				1		1				
large intestine	*ulcer/erosion*			1			2	4	1	1	1
	focal congestion							1			
small intestine	necrotizing enteritis								1		
	ulcer			5	7	3	7	11	5	7	6
Liver											
metastatic lymphoma					3		1	2			1
extramedullary hematopoiesis		2	1			1	1				
fibrosis					1		3	6	2	1	1
increased mitotic activity		4		2	1	1	2	1	1	1	1
multifocal necrosis					2	1	4	7	1	6	1
subcapsular necrosis						1					
thrombosis				1	1	1	2	2		1	2
hepatocyte single cell necrosis											
Pancreas											
fibrosis		4	2	2	1		4	8	1	7	2
focal necrosis								1			
acinus, single cell necrosis					1			3	3	2	
Peritoneum											
multifocal peritonitis				5	7	1	7	11	6	6	5

Summary of Histopathology										
L-748,731 (mg/Kg/day)	0		10		30		100		300	
	M	F	M	F	M	F	M	F	M	F
Adrenal										
necrosis							1			
hemorrhage			1	2		4	1			1
cortex, degeneration				2			1		1	1
cortex, hypertrophy			1	1	1	4	3	3	2	1
medulla, degeneration									1	
Pituitary										
cyst						1				
Kidney										
tubular basophilia	1		5	4	14	8	22	14	23	12
cyst				1					1	
metastatic lymphoma									1	
nephritis	1					2	1		1	
pyelitis		5	2	2	1	2	2		1	5
cortex, necrosis							1			
papilla, necrosis							1		1	
pelvis, dilatation	5	2		2	1	2	1	3	1	1
Urinary Bladder										
cystitis	1	4				1			2	5
Testis										
focal hemorrhage									1	
congestion							1			
atrophy	3				1		1			
epididymis, spermatic granuloma					1					
Seminal Vesicle										
atrophy	1						1			
cyst	1									
Prostate										
atrophy							1			
focal necrosis							1			
metastatic lymphoma									1	
Mammary Gland										
cyst							1			
Heart										
endocardium, focal inflammation							2			
Vagina										
cyst						1				
Lung										
focal hemorrhage									1	
metastatic lymphoma									1	
focal pneumonia								1	1	
diffuse pleuritis							1			
Spleen										
lymphoid depletion			2	2		2		1	1	1
extramedullary hematopoiesis			2	3		4	7	2	5	1
plasma cell, increased number							1			
metastatic lymphoma									1	
necrosis						1			2	

Summary of Histopathology										
L-748,731 (mg/Kg/day)	0		10		30		100		300	
	M	F	M	F	M	F	M	F	M	F
Lymph Node										
focal necrosis							1			
lymphoid depletion						1				
multinucleate giant cell formation	2		4				1			3
histiocytosis			2	6	1	7	7	6	4	4
plasma cell, increased number		1	3	2		2	6	1		3
congestion	1								1	
cyst									1	
metastatic lymphoma									1	
lymphoid necrosis				1		1	3	2	2	
hemorrhage	1						1			
Thymus										
atrophy			2	3	1	7	11	3	7	5
lymphoid necrosis				1		2		1		1
Brain										
meninges, focal fibrosis					1					
focal hemorrhage									1	
Bone Marrow										
increased hematopoiesis			1	2		4	10	2	4	3
metastatic lymphoma									1	
Skeletal Muscle										
metastatic lymphoma									1	
focal myositis									1	
Eye										
traumatic panophthalmitis							1			
traumatic keratitis									1	
focal retinopathy									1	
cataract	1									
cornea, atrophy							1			
corneal deposit	1									

toxicokinetic parameters: the following mean toxicokinetic parameters are calculated from plasma concentrations of L-748,731 determined during W14.

	L-748,731 (mg/Kg/day)							
	Males				Females			
	10	30	100	300	10	30	100	300
C_{max} (µg/mL)	2.5	4.7	7.1	8.1	4.1	7.3	11.9	12.5
T_{max} (hr)	2.0	2.0	4.0	0.5	2.0	2.0	2.0	2.0
$AUC_{0\,24\,hr}$ (µg hr/mL)	23.7	45.9	75.6	121.5	39.7	77.0	125.5	172.4

AUC values were 1.7-fold higher in females at all dose levels-
C_{max} values higher in females-
non-linear increase in AUCs with increase in dose occurred; *there was a "trend towards a plateau at ≥100 mg/kg/day*-

SUMMARY

In the 27-Week study, drug related changes occurred in both sexes in all drug groups. This study started out to be a one-year study with an interim necropsy scheduled for Week 27. However, due to the extensive mortality, the study was terminated at Week 27. Drug related deaths occurred in all treatment groups from 10 mg/Kg to 300 mg/Kg. At the low dose, multifocal thickening of the intestinal wall (healed ulcers) and adhesions were seen in males and females. *Drug related mortality was not observed in rats dosed with 100 and 300 mg/kg/day X 14 weeks and only a single animal in each study at 300 mg/kg/day*

exhibited intestinal ulceration [Studies TT # 94-615-0 and 95-614-0]. In the 27-week study, however, mortality was observed by Week 9-10 of dosing with extensive GI lesions described. The reason that the animals appeared to be more sensitive to drug induced GI toxicity is not apparent. The studies were conducted in the same lab, with the same lot of L-748,731 and same vehicle, and a restricted diet was fed in all three studies. Cervical lymph node granulomas were not demonstrated in the 27-week study as in the two 14-week studies at the high doses. Although lymph nodes were evaluated, it was not indicated that the cervical lymph nodes were assessed. Multinucleate giant cell formation was observed sporadically in all groups including control with no apparent pattern. Centriblobular hepatic hypertrophy, which was described in the two 14-week studies, was not found in the 27-week study. The following findings were considered to be secondary to the GI toxicity: [1] decreases in RBC indices, increases in neutrophils and thrombocytes; [2] increase in the incidence of extramedullary hematopoiesis in the spleen, liver, and bone marrow; [3] hepatic subcapsular necrosis; [4] adrenal cortical hypertrophy; [5] thymic atrophy; [6] increase in BUN without an increase in creatinine; [7] a decrease in both albumin and total protein; [8] multifocal peritonitis; and [9] lymph node histiocytosis. There was an increase in pancreatic fibrosis in males at 100 and 300 mg/kg/day. In addition, kidney tubular basophilia was dose-related and occurred at ≥10 mg/Kg. Hence, a no-effect dose level was not reached in this study.

Low RBC parameters and increases in PMNs compared to controls were not always associated with histopathological evidence of ulceration of the gastrointestinal tract. This does not, however, rule out the possibility that these changes were associated with GI toxicity/GI bleeding. In general, however, the animals exhibiting the greatest decreases in RBC parameters and increases in PMNs exhibited GI ulceration.

4.3.8. L-748,731: Fifty-Three-Week Oral Toxicity Study in Rats with a 27-Week Interim Necropsy:Final Report. TT #95-045-0

Study: TT #95-045-0
Compound: L-748,731-000R014 - [redacted] (factor 1.0) was used in calculations.
Formulation: Suspension in 0.5 % methylcellulose.
Route: Oral gavage at 5 mL/Kg.
Diet: Approximately 17 g for F and 24 g for M of [redacted] Certified Rodent Chow per day.
Strain: Crl:CD®(SD)BR, approximately 35 days of age, body weight M 119-173 g – F 83-139 g
Number: 30/sex/group - 10/sex/group underwent interim necropsy during W27.

Treatment Groups:	1	2	3	4
Dose Levels:	0	2	5	10 mg/Kg/day

Control Treatment: 0.5% methylcellulose
Study Site: Merck Research Laboratories, West Point, PA
Date: June 26, 1995 - November 25, 1996
GLP/QAU Statements: Both present and signed.

This study was done to determine the toxicity of L-748,731 in rats when administered orally for 53 weeks. The study also contains an interim necropsy at Week 27. The total number of doses administered to the interim animals was 183 to M - 184 to F and 364-365 to M- 365-366 to F in the study termination groups.

Rats were observed daily for mortality and clinical signs. Body weight and food consumption were determined pretest, once in W1, twice weekly through W13, and once a week thereafter. Indirect ophthalmoscopy and slit lamp ophthalmoscopic examinations were done during W12, 25, 39, and 52. Hematology (nine parameters*), serum biochemical (16 parameters**), and urinalysis (10 parameters***) determinations were obtained W4, 12, and 25 on 10 rats/sex/group and on all surviving rats at W39 and 51. All rats that died prior to the scheduled sacrifice were necropsied. Ten/sex/group were necropsied at W27 and all surviving animals were necropsied at W53. Histological examinations were evaluated on tissues from G1 and G4. Organ weights were determined for the brain, spleen, heart, kidneys, liver, adrenals, thyroid, pituitary, testes, prostate, and ovaries.

**RBC and WBC count, differential, Hb, Hct, MCV, MCH, MCHC, platelet count*
***gluc. BUN, creat, total protein, alb., A/G ratio, chol. triglycerides, AST, ALT, AP, Na, K, Cl, P, Ca*

***urine vol., spG, pH, protein, gluc., bili., occult blood, ketones, urobilinogen, microscopic exam of sediment*

RESULTS

- signs: G4 decreased activity, pallor, abdominal distention, reddish-brown ocular and nasal discharge *[2 males and 1 female, transient in the female, males found dead Drug Weeks 11 and 24]*
- mortality: **drug related:** 6G4 (during W11, 24, 39, 44, 46 and 48) due to peritonitis from intestinal ulceration and /or perforation- *3 males and 3 females*
  treatment unrelated: 1 MG2, 1 MG3 during W6 - 1 FG3 during W49-
- body weight: slight ↑ M G4 through W27-
- ophthalmic examinations: no treatment related changes reported-
- hematology: slight ↓ RBC, Hb, Hct G4 *males [Wks 39 and 51] and females [Wk 5, slight ↑ in anisocytosis and polychromasia in females: 0 vs 4 for control vs treated],* and moderate ↓ in M G2-
- serum biochemistry: slight ↓ in serum protein and albumin G4 W39 and W51 *in females-*
  slight ↑ in ALT and AST W25 in G4 W39 and W51 *[1 and 2 MD F and M and 2 HD F and M with significant ↑ in both AST and ALT Wk 51 and/or 39]*
- urinalysis: no treatment related changes reported-

Interim Sacrifice (Week 27)

- organ weights: body weight of M/F higher in treated groups compared to controls-
  absolute kidney ↑ 7.4 to 10.5% in treated groups-
  absolute liver ↑ 4.3 to 7.0% in treated groups - DR ↑ in M (G3 7.4%, G4 5.9%)-
  absolute wt of other F treated groups slightly higher -
- gross/histopathology: (1 = very slight, 2 = slight/small, 3 = moderate, 4 = marked, 5 = severe)
  - stomach: peritonitis (moderate) 1 MG4 (95-4012)-
  - small intestine: serosa, focal proliferative inflammation (very slight) 1 FG3-
    peritonitis 1 FG4 (95-4057), 2 MG4 (95-4012, 95-4028)-
    ulcer 1 FG3 (95-3987)-
    perforated ulcer 1 MG4 (95-4057), 1FG4 (95-4057)-
  - large intestine: ulcer 1 MG4 (95-4046)-
  - liver: focal necrosis 1 MG1, 2 FG4, 1 MG4 (all graded as very slight)-
  - spleen: extramedullary hematopoiesis 2 MG4 (very slight and moderate) *in same males with peritonitis-*
  - lymph node: reactive hyperplasia (moderate) 1 MG4 *in male with peritonitis-*
  - bone marrow: erythroid hyperplasia (moderate) 1 MG4 *in male with peritonitis -*
  - eyes: abscessed Harderian gland 1 MG3- retina, focal degeneration (very slight) 1 FG4 - phthisis bulbi 1 MG3-

Final Sacrifice (Week 53)

- organ weights: body weights of M/F equivalent to controls, with G4 2.3- 2.7% higher-
  spleen: absolute wt MG4 ↑ 5.7%, FG4 ↑ 16% [1 F with 2.4x (1.33 g) the average wt]
  kidneys: absolute wt MG4 ↑ 4.3%, FG3 ↑ 11.6%-
  liver: absolute wt F G4 ↑ 4.5%-
  testes: absolute wt G4 ↓ 7.5%-
- gross/histopathology:
  - stomach: glandular mucosa, erosion (very slight) 1MG4 (95-4040)-
  - small intestine: peritonitis 1 FG4 (95-4005)-
    perforated ulcer 1 MG4 (954006), 4 FG4 (9504005, 95-4012, 94-4033, 95-4037)-
  - large intestine: ulcer 1 FG2 (95-3893), 2 MG3 (95-3960, 95-3976), 1 FG3 (95-3981)-
  - liver: focal basophilic cellular alteration (very slight) 1 MG4 (95-4040)-
    focal eosinophilic cellular alteration (very slight) 1 FG4 (95-4011)-
    hepatocellular adenoma 1 MG3 (95-3964)-
    disseminated focal necrosis (moderate) 1 FG3 (95-3982)-

- pancreas: atrophy (moderate) 1 MG4 (95-4006)-
- adrenal: cortical adenoma 1 FG2 (95-3917) - cortex hyperplasia (slight/small) 1 FG4 (95-4033)-
- thyroid: follicular cell adenoma 1 MG4 (95-4042)-
- kidney: hydronephrosis (severe) 1 FG3 (95-3951)-
  chronic nephritis (moderate) 1 MG2 (95-3888), 1 MG3 (95-3960)-
- testis: unilateral degeneration (slight/small) 1G4 (95-4010)-
- mammary gland: adenomatous hyperplasia (slight/small) 1 FG3 (95-3971)-
- lung: thrombosis 1 FG4 (95-4005)-
- spleen: extramedullary hematopoiesis (slight/small to moderate) 2 FG4 (95-4005, 95-4033)-
  lymphoid hyperplasia (moderate) 2 FG4 (95-4033, 95-4037)- *occurred in animals with small intestine perforated ulcer*
- lymph node: hyperplasia (moderate) 2 FG4 (95-4033, 95-4015)- *occurred in animals with small intestine perforated ulcer*
- bone marrow: hyperplasia (slight/small) 1 MG4 (95-4006), (moderate) 2 FG4 (95-4015, 95-4033))-
- eye: retinal, focal degeneration (moderate) 1 FG4 (95-4007)- phthisis bulbi 1 FG3 (95-3973)-

Drug related mortality occurred in six rats at 10 mg/Kg/day due to extensive peritonitis from intestinal ulceration and perforation. The drug produced decreased activity, pallor, and abdominal distention. No significant changes were seen in body weight, ophthalmic examinations, or urinalysis. There were slight decreases in RBC count, Hb, and Hct at 10 mg/Kg/day. Slight decreases were reported for serum protein and albumin and slight increases in ALT/AST occurred at 10 mg/Kg/day. In the interim sacrifice at Week 27, moderate peritonitis was seen in the stomach and small intestine of rats dosed at 10 mg/Kg/day. One male animal with a "broad" ulcer in the large intestine also had focal necrosis in the liver, and one female dosed at 5 mg/Kg/day developed a small ulcer that was associated with proliferative serositis of the small intestine. Kidney and liver absolute weights were higher than controls. In the final sacrifice at Week 53, drug related peritonitis, perforated ulcers, and stomach glandular mucosal erosion were present in the high dose. *It is surprising that the incidence of peritonitis was not the same as that for small intestine perforated ulcers.* Other lesions were present in a few animals, mostly in the high dose group. Absolute weight of spleen, kidney, and liver were higher than controls. The NOEL was <2 mg/Kg/day, as one female developed an ulcer in the large intestine.

The current Pharmacology/Toxicology Reviewer reviewed the following study.

4.3.9. L-748,731: Fifty-Three-Week Oral Gastrointestinal Toxicity Study in Rats [Vol. 1.19; p. B-3870]

Study Identification: TT #96-090-0
Site: Merck Research Laboratories; West Point, PA
Study Dates: Nov. 7, 1996 - Nov. 6, 1996
Formulation and Lot No.: L-748,731-000R014
Vehicle control - 0.5% aqueous methylcellulose
Certificate of Analysis Submitted: No (X) assayed for uniformity Drug Week 1, assayed for concentration Drug Weeks 1, 7, 19, 31, 43, and 52. With the exception of Drug Week 1 where concentration was approximately -20% of claim, all results were within acceptable limits - The Reviewer agrees with the Sponsor that this should not compromise the study
Final Report (X) Mar. 16, 1996
GLP and QA statements signed: Yes (X)
Objective: "To identify the potential gastrointestinal toxicity of MK-0966 in rats when administered daily by oral gavage for approximately 53 weeks."

Test Material/ Group Designation	Dose*				Sex	N	Species/Strain
	mg/kg	ml/kg	Route	# days dosed			
Group 1 - vehicle	-	5	oral gavage	M/F = 364	M	20	Crl:CD®(SD)BR strain - Sprague Dawley
Group 2 - MK-0966	0.2				F	20	
Group 3 - MK-0966	0.5						app. 39 days at study start
Group 4 - MK-0966	1.0						M - 140-198 g F - 107-146 g

*fed approximately 16 or 22 gm for females and males, respectively