[89.2]% and 100.1[100]% at 96-hours following iv and oral administration, respectively in the rat [dog]. Excretion of radioactivity was predominantly in the feces [66.3 (65.7)% and 71.5(79.3)% following iv and oral administration, respectively in the rat (dog)] with lesser amounts in the urine [26.1(23.5) % and 28.6(20.7)% following iv and oral administration, respectively].

- ***In Vivo* Metabolism** – L-755,190 and its glucuronide conjugate were identified as the major radioactive components in the urine of the rat and dog. No MK-0966 was detected in urine of either species.

-***In Vitro Metabolism*** – *In vitro* results supported the *in vivo* findings. Biotransformation of L-775,190 to MK-0966 was an NADPH-dependent reaction occurring in liver and intestinal cytosol of dog, monkey, and humans. This reaction occurred in liver but not intestinal cytosol of rats.

-Plasma Protein Binding-L-755,190 was highly protein bound with 87, 87, and 94% binding for rats, dogs, and humans.

-Erythrocyte Partitioning – The blood:plasma ratio was 0.8 indicating blood clearance is slightly greater than plasma clearance.

3.14.2. Biliary Excretion of L-755,190 in Rats Following Administration of [^{14}C]L-755,190 at 2m/kg I.V. or 5 mg/kg P.O. [Vol. 1.45; p. G-348]

Site: Merck Research Laboratories; West Point, PA

Formulation and Lot No.: Test article - [^{14}C] L-755,190-001K002 and 3 with ^{14}C incorporated at the C-4 position of the furanone ring

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

Certificate Analysis: No (X)
Final Report (X) Jan. 29, 1998
QA statements signed: Yes (X)

Study Design – Bile-cannulated, fasted male Sprague-Dawley rats [N=3] were administered radiolabeled L-755,190 at 2 mg/kg iv or 5 mg/kg po. Bile was collected every hour for the first 6 hours, and then 6-24 hours. Urine was collected for the 24-hour interval. Total radioactivity in the bile and urine was determined conducted on bile samples. Samples were evaluated both before and after β-glucuronidase treatment.

Results – Radioactivity recovery in the bile was 69.5 ± 10.9% and 61.3 ± 13.3% following the iv and po doses, respectively. Radioactivity recovery in the urine was 8.6 ± 1.8% and 11.0 ± 5% following the iv and po doses, respectively. Two metabolites but no parent compound were identified in the bile following drug administration by either route. Incubation of the bile with β-glucuronidase resulted in hydrolysis of the major metabolite [β-O-glucuronide conjugate of L-755,190] to the form the minor metabolite [L-755,190, the 5-OH furanone metabolite of MK-0966]. The glucuronide and aglycone metabolite constituted ≥80% and 4-14% of the total radioactivity, respectively.

3.14.3. The Effect of Bile Flow on the Recirculation of MK-0966 and L-755,190 in Rats Following Intravenous [2mg/kg] and oral [5 mg/kg] Administration of [^{14}C]L-755,190 [Vol. 1.45; p. G-388]

Site: Merck Research Laboratories; West Point, PA

Formulation and Lot No.: Test article - [^{14}C]L-755,190-001V001, 002, and 003 with ^{14}C incorporated at the C-4 position of the furanone ring

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

Certificate Analysis: No (X)
Final Report (X) Dec. 16, 1997
QA statement signed: Yes (X)

Study Design – Fasted, male Sprague Dawley rats, with or without bile cannulation, [N=3 for cannulated or 4 intact rats] were administered 2 or 5 mg/kg via the iv or po routes, respectively. Bile and

blood was collected at 0, 0.25, 0.5, 1, 2, 4, 6, 8, 10, and 24 hours. MK-0966 and L-755,190 concentrations were analyzed

Results – There was a rapid increase in the plasma concentration of MK-0966 regardless of dosing regimen. In the intact animals, MK-0966 decreased rapidly to below quantifiable limits at 0.5 hours. This was followed by an increase in concentration of MK-0966 beginning at 4 hours and reaching a 2nd C_{max} at 10 hours. In the cannulated animals, MK-0966 was measurable until 2-4 hours and 6 hours following iv and oral administration, respectively. The reason that MK-0966 could be detected for a longer duration in the cannulated animals following the initial C_{max} is not known. The table below compares mean pharmacokinetic parameters in the cannulated vs. intact rats.

	Intravenous Route[a]		Oral Route	
	Intact	Cannulated	Intact	Cannulated
MK-0966				
Partial AUC - ng•hr/ml[b]	184 ± 101	25.3 ± 17.6[c]	376 ± 171	37.2 ± 13.0[d]
L-755,190				
Partial AUC - ng•hr/ml	1042 ± 79.9	1255 ± 334	1018 ± 155	514 ± 109

[a] 2 mg/kg for iv and 5 mg/kg for po
[b] 0-24 interval for AUC
[c] partial AUC calculated on 0-2 hour interval
[d] partial AUC calculated on 0-6 hour interval

Note: The Sponsor did not provide individual data.

3.15 Summary of Pharmacokinetics and Toxicokinetics -

Pharmacokinetics – Absorption in the rat following oral administration was approximately quantitative. The drug was eliminated primarily in the bile in rats. Following oral administration of 5 mg/kg of radiolabeled L748,731, approximately 58 – 76% was recovered in the bile and approximately 3 – 15% in the urine. Studies also indicated that [1] enterohepatic recirculation played a role in the pharmacokinetics of both the parent compound and the primary metabolites [e.g. 5-OH furanone and its glucuronide] in the rat and [2] metabolic conversion of these metabolites to parent compound occurred in the lower GI tract beginning at the level of the cecum; and [3] conversion to the 5-OH-furanone metabolite was fairly rapid. Bioavailability in the dog was approximately 30-40%. In the dog, the drug was eliminated in the feces [approximately 76% based on radioactive elimination] and in the urine [approximately 17% based on radioactive elimination]. However, in the dog, due to low bioavailability, only 27% of the drug, based on radioactive elimination, was eliminated in the bile. Feeding in dogs tended to increase the pharmacokinetic parameters, although there was considerable interanimal variability. There was mean increase in $AUC_{0\text{-}24\ hr}$ of 30%, a 13% increase in C_{max}, and a 2X increase in T_{max} when drug was administered to fed dogs. Although there was some conversion of the parent compound from the 5-OH furanone metabolite, it was less in dogs [<3%] than that observed in rats. In humans, the primary route of excretion is primarily by the kidney.

Protein binding was comparable across species based on results of equilibrium dialysis with 85.3%, 87.3%, and 89.1% of the drug bound in rats, dogs, and humans, respectively. Similar results were obtained with centrifree micropartition system for the dog and humans, but protein binding was increased in the rat using this methodology to 93%. Based on this latter method, there would be approximately 50% less free drug in rats than in humans. MK-0966 did not appear to effect protein binding of salicylic acid. There was no indication of covalent protein binding. Either methodology is acceptable, but the differences in protein binding in the rat may reflect differences in methodology. A dose-response was incorporated into the study in which protein binding was evaluated by centrifree micropartition system. Generation of multiple points increases the level of confidence in the data.

Following iv administration of L748,731 at 2 mg/kg, maximum mean concentration was achieved in most tissues at 5 minute. The exceptions included [1] fat, liver, prostate, skin, urinary bladder at 30 minutes and [2] small intestine at 2 hours. With the exception of the small intestine, large intestine,

stomach [including contents and wash]; liver; and kidneys, tissue levels by 24 hours were ≤1 µg equivalents/g of tissue. The tissue:plasma ratio was generally ≤1. This was not the case for the adrenals, kidneys, liver, and small intestine. Following an oral dose of L748,731, elimination profile from the brain and the CSF was similar to plasma elimination. The brain:plasma ratio was generally 0.4. The level of drug in the brain at 24 hours was 9.6 ng ± 1.9 ng/g tissue. With respect to GI distribution, following a single oral dose [5 mg/kg], the amount of radioactivity recovered from the tissues and lumenal contents was approximately 60-70%, with the majority of the radioactivity associated with lumenal contents. Radioactivity tended to be highest in the jejunum and ileum, and increased in the cecum and large intestine with time. At the early time points [e.g. 1 hour], the majority of the radioactivity was associated with parent compound in the jejunum and ileum. However, at 6 hours post dosing the 5-OH furanone metabolite and its glucuronide accounted for the entire radioactivity. At 6 hours in the cecum, the majority of the radioactivity was attributed to parent compound, supporting the Sponsor's hypothesis that the cecum is the site for the conversion of metabolite to parent compound.

Metabolism – *In vitro* metabolism was qualitatively similar but quantitatively dissimilar in humans, non-induced rats, dogs, and rhesus monkeys. The oxidative pathway was important in the rat, but in humans the reductive pathways were predominant. The table below provides a comparison of the *in vitro* metabolic profile for the various species. Values are expressed as % of initial substrate. In general, these findings are consistent with *in vivo* metabolism in the various species. Both the reductive and oxidative activities were associated primarily with the cytosolic fractions. In human liver preparations, the oxidation of MK-0966 was predominantly a function of CYP3A4 and 1A2 and a non-P450 oxidase activity. The enzymes involved in the reductive metabolism of MK-0966 were not identified. Studies indicated that MK-0966 was not an inhibitor of human CYP isoforms. In the rat, however, liver CYP3A was induced at a concentration of 50 µM of MK-0966. The conditions under which MK-0966 was incubated were determinants of which process [e.g. oxidative vs. reductive] predominated.

Species	Oxidized Metabolites[a]		Reduced Metabolites[b]		M + 18 [Major Peak Only][c]		Total Metabolism		Reduced/Oxidized	
	4 hr	24 hr	4 hr	24 hr	4 hr	24 hr	4 hr	24 hr	4 hr	24 hr
S9-Fraction										
Human	4.0	3.6	8.0	44.8	2.2	2.9	14.2	51.4	2.0	12.4
Monkey	11.4	12.6	9.8	53.7	9.2	13.4	30.4	79.7	0.9	4.3
Dog	3.1	8.8	-	18.3	10.8	33.4	13.9	60.5	-	2.1
Rat	18.9	20.2	23.8	56.1	1.0	9.5	43.8	86.4	1.3	2.8
Cytosol										
Human	-	-	13.3	56.8	-	-	13.3	56.8	-	-
Monkey	-	-	27.0	79.6	0.4	1.4	27.3	81.0	-	-
Dog	-	1.1	6.0	37.1	10.3	26.0	35.8	64.2	-	-
Rat	3.0	3.1	20.3	66.9	0.2	1.6	23.6	71.6	-	-

[a]L-755,190
[b]*cis*-dihydrohydroxy acid + *trans*-dihydrohydroxyacid + *cis*-dihydrolactones + *trans*-dihydrolactones
[c]dihydro analog of L-755,190

In vivo metabolism was qualitatively similar but quantitatively different in humans and animals. The schematic below outlines the metabolites of MK-0966 which have been identified and in which species they were present.

In the rat, the primary metabolite is the glucuronide of the 5-hydroxy furanone metabolite [L-755,190] which involves an oxidation pathway. As noted above, the primary route of elimination was in the bile. This metabolite is also the predominant metabolite found in the urine of rats as well as small quantities of the 3', 4'- *trans*-dihyrodiol metabolite, parent compound, the sulfate conjugate of 4'-phenol derivative, and the dihydrohydroxy acid of MK-0966. Similar metabolites were observed in the urine of mice.

The metabolism in the dog was more complex with at least 14 peaks identified but not all characterized. As in the rat, L-755,190 was the most abundant metabolite in the bile but was a minor metabolite in urine. The *trans*-dihydro metabolite, which constituted 14-22% of the total radioactivity recovered in urine, was identified in the dog. No parent compound was found in the urine or bile.

The major metabolite in humans was the dihydrohydroxy acids.

Toxicokinetics – These studies provided a basis for the comparison of exposure in animals to humans. In general, exposure based on AUC in dogs, rats, and mice were not dose proportional, although a linearity was observed over restricted dose ranges. The exposures on Day1 were comparable to those observed following repeat dosing.

Although the plasma toxicokinetics in pregnant vs. non-pregnant rats was similar, the pregnant rats appeared to have increased sensitivity to the GI toxicity induced by MK-0966 compared to non-pregnant rats. Placental transfer of parent compound was high in both rats [90-100%] and rabbits [40-70%]. In addition, a study in lactating rats indicated that at 1 and 10 mg/kg, the mean milk:plasma ratio was 0.52 and 1.27, respectively.

4. Toxicology:

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

4.1 Single Dose Studies

4.1.1. Acute Oral and Intraperitoneal Studies in Female Mice and Rats [*Vol. 1.7; p. A-21*]:

Study Numbers: TT#94-2639, TT#94-2640, TT#94-2641, TT#94-2642
Compound: L-748,731, identified as L-748,731-000R, Lot No. 9
99.7% pure
Formulation: 6% suspension in 0.5% aqueous methylcellulose
Route: Oral (gastric intubation) and IP - *Mice were fasted for 2 hours and rats were fasted overnight prior to oral drug administration*
Dosage: 2000 mg/Kg only
Strain - Mice: Crl:CD-1®(ICR) BR, 6-7 weeks old, 22.5 -24.9 g
Rats: Crl:CD®(SD) BR, 6-7 weeks old, 142-174 g
Number: 3/females/group
Study Site: Merck Research Labs., West Point, PA
Date: April - July 1994
GLP/QAU Statement: Both present and signed.

Body weights were obtained pretest and Days 7 and 14

Results and Discussion

Mice:

No signs or deaths in po or ip administration at 2000 mg/Kg over 14 days. No significant change in body weight in po or ip administration at 2000 mg/Kg over 14 days. LD_{50} >2000 mg/Kg.

Rats:

No signs or deaths at 2000 mg/Kg po over the 14 days. Distended abdomen D7-D14 in one at 2000 mg/Kg. No significant change in body weights. LD_{50} >2000 mg/Kg.

Conclusion: *"The approximate lethal dose$_{50}$ for L-748,731 is >2000 mg/kg when administered as a single oral or intraperitoneal dose to female mice and rats."*

4.2 Repeat Dose Studies - Dogs

4.2.1. Exploratory 15-Day Oral Toxicity Study in Dogs[*Vol. 1.9;B-125*]:

Study: TT# 94-020-0
Compound: L-748,731, batch L-748,731-000R004
Formulation: Suspension in 0.5% aqueous methylcellulose, 20 mg/mL
Route: Oral, gavage at 5 mL/Kg

Dosage: Group: 1 2 3
0, 10, 100 mg/Kg/day x 14
Strain: Beagle, 37-39 weeks old, M - 9.8-13.5 Kg, F - 8.9-11.8 Kg
Number: 4/sex/group
Control Treatment: 0.5% aqueous methylcellulose
Study Site: Merck Research Labs., West Point, PA
Date: February 7, 1994 to May 6, 1994
GLP/QAU Statements: Not present.

All dogs were observed daily. Body weight was measured pre-study, once in Week 1, and twice in Week 2. Food consumption was measured four times in Week 1 and twice in Week 2. Serum biochemical parameters were measured prior to study initiation and Day 10. Drug plasma levels were measured Day 14. Only the livers, kidneys, and GI tracts were examined at necropsy. Organ weights were determined for liver and kidney. Histopathologic examination was done on liver, kidney, stomach, small and large intestine from control and high dose dogs. *Histopathology was also performed on the intestinal sections from the low dose group.*

Results and Discussion

-clinical signs: none reported-
-mortality: all dogs survived to necropsy-
-body weight: mean wt change was (+) 0.2, 0.6, 0.5 in G1, 2, 3 from Day (-)1 to Day 13-
-food consumption: no unusual changes-
-clinical chemistry: slight ↑ M/F in 100 mg/Kg BUN *but not creatinine – There was app. 20% increase in mean value*
-organ wts: very slight ↑ in combined M/F relative and absolute liver wt (4% in G3)- *Increase was primarily due to slight ↑ in M liver weights*
-necropsy: reddened (hemorrhagic) intestinal contents and ulcers in small intestine of 1 MG3
-small intestine: slight focal acute jejunal enteritis 1 MG2-
ulcerations 1 each M/F G3-
-kidney: very slight acute pyelitis 1 MG2 [*kidneys evaluated in only 1 low dose animal]*-
-plasma level(average μM):

	1 Hour	6 Hour
10 mg/Kg:	8.6	3.5
100 mg/Kg:	18.7	7.0

GI toxicity was observed in the small intestine of animals dosed at 10 and 100 mg/Kg (200 and 2,000 mg/M^2). Ulceration was seen in the high dose and was described as full thickness loss of the small intestinal mucosa. The low dose animal was seen with focal jejunal enteritis. The slight acute pyelitis that was observed in one low dose dog may be of incidental occurrence. A hint of an e*levation* in serum urea nitrogen and slight liver weight increases at 100 mg/Kg were the only other notable changes. *Increase in BUN without concomitant increase in creatinine does not suggest renal toxicity. Potential causes of the observed increase in BUN include dehydration and GI bleeding.*

4.2.2. Fourteen-Week Oral Toxicity Study in Dogs; [*Vol. 1.9; B-183 and Vol 1.10*]:

Study: TT#94-040-0,
Compound: L-748,731: L-748,731-000R009
Formulation: Suspension in 0.5% aqueous methylcellulose
Route: Oral, gavage at 5 mL/Kg
Dosage: Group 1 2 3 4
0, 2, 10, 50 mg/Kg/day x 91 in M and x 92 in F
Strain: Beagle, 39-43 weeks old, M 9.9-12.8 Kg, F 7.7-10.6 Kg
Number: 4/sex/group
Control Treatment: 0.5% aqueous methylcellulose
Study Site: Merck Research Labs., West Point, PA
Date: April 18, 1994 to November 18, 1994
GLP/QAU Statements: Both present and signed.

All animals were observed daily. Body wt was determined pretest, once Week 1, twice weekly through Week 9, and once weekly thereafter. Food consumption was measured two/four times/week. Ophthalmoscopic examinations were done pretest and Weeks 6 and 12. Hematology and serum chemistry parameters were determined pretest and Weeks 4, 8, and 12 on all dogs. Urinalyses were performed pretest and Weeks 8 and 12 on all dogs. EKGs were taken on all dogs pretest and Weeks 4, 8, and 12. Plasma drug levels were determined Day 1 and Week 13. Necropsy examinations were done on all dogs on study.

Body wt and weights of the following organs were recorded: adrenals, brain, heart, liver, ovaries/testes, kidney, liver, pituitary, spleen, prostate, and thyroid. Extensive organ and tissue histopathology was done.

For the PK data, three groups of 4 and 4 were given daily oral doses of 0, 2, 10, or 50 mg/Kg/day of L-748,731 in 0.5% aqueous methylcellulose for approximately 14 weeks. The control received vehicle. Blood samples were taken at 0.5, 1, 2, 4, 6, and 24 hours post administration and Week 13.

Results of immunohistochemical evaluation for the presence of both COX-1 and COX-2 in the gastrointestinal tract was appended to this study report. Immunohistochemistry was conducted on 2 control and 4 high dose dogs. The following tissues were evaluated: [1] jejunum; [2] ileum; [3] duodenum; [4] fundus; [5] pylorus; and [6] colon. Competitive control sections [COX-1 or COX-2 human peptide added to the primary antibody] and positive control tissues [prostate for COX-1 and placenta for COX-2] were included.

Results and Discussion

-signs: loose stools and salivation on occasion in all groups-
-mortality: no premature deaths-
-body wt: mean wt change of +0.7(G1), +0.6(G2), +0.5(G3), and +0.3(G4) Kg between pretest period -1 and wt in W13 - one M 10 mg/Kg dog began loosing wt W6 and by W13 had lost 7.7% of W6 body wt - two F G4 dogs lost about 10% of their highest weight by W13-
-food consumption: decrease in animals that showed wt changes-
-eye exams: no treatment related changes were said to occur, but no data presented-
-hematology: (changes from mean values)
RBC: ↓ G4 (11% from predose, 5% from G1)
Hb: ↓ G3-4 (4%-16%)
Hct: ↓ G3-4 (3%-13%)
protime: ↓ G4W12(2.5%)
PTT activated: - ↑ slightly in G3 and G4 (10%)
platelets: ↑ G4 over duration of study 30% by W12
leukocytes: ↑ G4W12(28%) - *4 animals unchanged, 1 female ↑55%, 3 males ↑28, 55, and 100% from predose*
neutrophils %: ↑ G4(13%)
neutrophils: ↑ G4W12(*45%*) - *counts ranged from approximately 1-3X pretest values, increases observed in same animals with leukocytosis*
eosinophils: ↑ G4W12(39%) *[in controls - ↑28% compared to predose]*
monocytes: ↑ G4W12(43%)
-serum chemistry: (changes from mean pretest values)
BUN: ↑ 3G4 (1.5 to 1.9x)
albumin: DR ↓ G4(17.6%), A/G ratio: ↓ G4(36%)
triglycerides: ↑ G4W8(21%), G4W12(16%)- *similar increase in G2*
Ca: ↓ (5%) *–probably 2° to decrease in albumin*
-urinalysis: (change from mean pretest values)
volume: ↑ G4W8(19%), G4W12(14%)
leukocytes 3G4 with 6-10 vs G1 with 0-5
-organ wts: (changes from mean control)
spleen: DR↑ G2(3%), G3(4%), G4(7%) - rel. and abs.
liver: ↑ G3(5%), G4(6%) - rel. and abs.
adrenals: ↑ G4(14%) - rel. and abs.
ovaries: ↓ G3(13%), G4(28%) - rel. and abs.

-histopathology: (lesions not seen in control animals)

N = 4	G3		G4	
	M	F	M	F
small int: villus, focal loss (slt or small)	-	-	-	1*
ulcer	-	-	1	1*
kidney: unilateral focal *papillary* necrosis (very sl)	-	-	-	2*
testis: focal orchitis (very sl)	-	-	1	-
prostate: prostatitis (moderate)	-	-	1	-
skin: cellulitis (slight or moderate)	-	-	2	-
inflammation	1	-	-	-
ulcer	-	-	1	-
lung: artery, mural hypertrophy (moderate)	-	-	1	-
spleen: congestion	-	1	-	-
hemosiderosis (slight or small)	-	-	-	1
nodular lymphoid hyperplasia	1	-	-	-
lymph node: lymphoid hyperplasia (moderate)	-	-	1	-
thymus: interstitium, acute hemorrhage (marked)	-	-	-	1

** one animal exhibited all three lesions*

-pharmacokinetics:

Drug Day 1

Mean ± SEM n = 8 unless indicated

Dose (mg/Kg/day)	C_{max} (µg/mL)	T_{max} ▫ (h)	$T_{½}$ (h)	AUC (µg h/mL)
2	0.84 ± 0.07	1.5 ± 0.2	2.3 ± 0.3	4.94 ± 0.50
10	2.30 ± 0.56*	1.5 ± 0.2*	2.3 ± 0.2*	15.67 ± 5.07*
50	5.88 ± 1.61	2.0 ± 0.6	2.1 ± 0.3*	61.15 ± 28.43

Drug Week 13

Dose (mg/Kg/day)				
2	0.97 ± 0.14	1.8 ± 0.2	2.3 ± 0.2	5.89 ± 0.75
10	1.99 ± 0.12	1.4 ± 0.2	2.4 ± 0.2	12.53 ± 0.89
50	4.75 ± 1.15	2.0 ± 0.5	2.3 ± 0.2*	53.52 ± 15.31

▫ half-life calculated over the range T_{max} to 6 hrs.

* n = 6

Immunohistochemical Analysis of COX-1 and COX-2 in the GI tract - Endothelial staining for COX-1 was comparable in the control and high dose animals with the exception of the ulcerated areas in 2 high dose dogs. In these areas, COX-1 staining was decreased compared to non-ulcerated jejunum. Staining for COX-1 was slightly greater in the distal small intestine and/or colon of both control and treated dogs. COX-2 staining of macrophages was comparable between HD and control dogs and between ulcerated and nonulcerated jejunum.

The NOEL in this study was 10 mg/kg. *Body weight gain was decreased in the high dose group with 3/8 dogs exhibiting weight loss.* In the 50 mg/kg/day group, hematology decreases of about 11-16% in RBC, Hb, and Hct were reported. There was also a 30% increase in platelets and a 28% increase in leukocytes (45% ↑ in neutrophils). Serum chemistry parameters that showed changes were an almost 2 fold increase in BUN, an 18% decrease in albumin, and 16-21% increase in triglycerides. Slight increases were also seen in the relative and absolute spleen (7%), liver (6%), and adrenal (14%) weights. At histopathologic examination, jejunal ulcers were seen occurring over Peyer's patches in one male and one female and focal loss of villi tips were present. In the kidney, unilateral focal necrosis of renal papilla was seen in two females. Mural hypertrophy of the pulmonary arterioles was reported in one male, but may not be treatment related. In addition, marked acute hemorrhage was observed in the thymus of one female. *The decrease in RBC indices and the increase in BUN without a concomitant increase in creatinine were*

probably secondary to GI toxicity. The decrease in albumin is probably secondary to the decrease in body weight gain and decreased food consumption and not to loss secondary to GI toxicity since globulins were apparently unaffected [e.g. there was a decrease in the A:G ratio]. A slight to moderate cellulitis was observed in 2/4 males at the high dose.

The pharmacokinetic parameters did not show any gross changes between Day 1 and Week 13. AUC values showed a non-proportional increase over the dose range. $T_{1/2}$ (plasma drug half-life) was between 2.1 and 2.4 hours, with T_{max} between 1.4 to 2 hours. A non-dose related C_{max} increase occurred. There were no gender differences.

The correlation or causal relationship between the decrease in COX-1 staining in the endothelial cells in the ulcerated regions of the jejunum compared to normal jejunum has not been established in this study. However, inhibition of COX-1 activity has been hypothesized to induce mucosal ulceration and bleeding [Seibert and Masferrer; 1994: Sponsor submitted reference]. An increase in COX-2 expression would be anticipated normally when there is evidence of ulceration/inflammation. However, COX-2 level was comparable between normal gut in control dogs and ulcerated gut in L-748,731 treated dogs. The significance of the ulcerations occurring over the Peyer's patches is not known.

4.2.3. L-748,731: Fifty-Three-Week Oral Toxicity Study in Dogs with a 27-Week Interim Necropsy: Final Report, TT # 95-003-0 *[Vol. 1.10: B-474 and Vol. 1.11: B-782]*

Study: TT #95-003-0
Compound: L-748,731-000R014 [redacted] a factor of 1.0 was used in all drug calculations.
Formulation: Suspension in 0.5 % methylcellulose.
Route: Oral, by gavage
Diet: Approximately 350 g of [redacted] Certified Canine Diet were provided daily.
Strain: Beagle, approximately 45 to 53 weeks of age and weighing approximately 8.0 to 14.6 Kg.
Number: 8/sex/dose

Treatment Groups:	1	2	3	4
Dose Levels:	0	3	10	30 mg/Kg/day

Control Treatment: 0.5% methylcellulose
Study Site: Merck Research Laboratories, West Point, PA
Date: February 1, 1995 - June 19, 1996
GLP/QAU Statements: Both submitted and signed.

The objective of this study was to evaluate the potential toxicity of L-748,731 when administered orally for approximately one year with a 27-week interim necropsy.

The study includes daily observations for mortality and clinical signs, body weight (pretest and weekly), and food consumption 3 to 4 times/week *on a* weekly *basis* from W1 to W13 and every four weeks, thereafter. Indirect and slit lamp ophthalmoscopic examinations were done pretest and during W12, 25, 39, and 52 on all surviving dogs. Hematological parameters (11*) and serum biochemical parameters (16**) were determined W4, 12, 25, 39, and 51. EKG examinations [*Leads I, II, III, aVR, aVL, aVF, V10, and CV5RL]* were performed pretest and during W12, 26, 38, and 51 on all surviving dogs. Urinalyses*** were done on all animals pretest and during W12, 25, 39, and 51. All dogs were necropsied. Brain, pituitary, spleen, heart, kidney, liver, adrenals, thyroid, testes/ovary, and prostate weights were recorded. Tissues from all control and G4 dogs were observed microscopically. Bone marrow smears were also prepared.

**RBC and WBC counts, differential, Hct, Hb, MCV, MCH, MCHC, ProT, APTT, platelet count*
***total protein, albumin, gluc., BUN, creat., A/G ratio, AST, ALT, AP, chol., triglycerides, Na, K, Cl, Ca, P*
****protein, bilirubin, gluc., pH, occult blood, spG, vol., urobilinogen, microscopic examination of sediment*

RESULTS

- mortality: none treatment related - one sacrifice W49 due to trauma from fractured left canine tooth during W44 resulting in swelling, fluid accumulation and elevated (103°) body temperature-
- signs: blood in stools (drug related) W22 and W25 in 1G4 (94-0315) and in 2 other G4 dogs (*uncertain relationship to drug*) (94-0316, 94-0248) in W4 and W20- emesis and/or unformed liquid stools or yellowish/mucoid stools G1 and G4
- body weight: no treatment related changes-
- food consumption: no treatment related changes- *Body weight gain and food consumption tended to be decreased mildly in G2 and G4 F*
- ophthalmoscopic exam: no treatment related changes-
- EKG: no treatment related changes reported-
- hematology: slight ↓ in leukocytes in 2G4 (94-0268, 94-0283) , 1G3 (95-0246) , 1G2 (94-0307)-
- serum biochem: slight ↑ in BUN in 2G4 (94-0255, 94-0337) W4, 12, 25 and/or 39 *[<50% ↑]* - 1G3 (94-0276) slight ↑ W4, 12, and 25 *[app. 0.5 -2X↑]* - slight ↑ ALT 1G4W25 (94-0252) *[app. 130%↑ but normal W39 and 51]* , 1G3W25 (94-0309) *[app. 100%↑]*, 1G1 (94-0286) W12, 25 *[app. 35%↑]* - slight ↓ in glucose all groups - all serum changes were considered unrelated to treatment by study director-
- urinalysis: fine granular casts in 2G2 (94-0296, 94-0262), 1G3 (94-0264), 2G4 (94-0252, 94-0266) - considered treatment unrelated by study director-

Interim Sacrifice (Week 27)

- gross/histopathology:
  - 1M G2 (94-0310) with well-defined 1-2 cm reddened and slightly depressed foci in mucosa of jejunum and ileum - *moderate* multifocal enteritis consisting mostly of polymorphonuclear infiltration-*[since not dose dependent considered unrelated to treatment by the Sponsor]*
  - 1M G1 (94-0312) with dark red streaking in mucosa of the jejunum - *slight* segmental enteritis-
  - 1 M G4 (94-0248) killed W49 due to trauma from fractured left canine tooth-*[3 minute foci of small intestinal villous necrosis, 4 small scattered foci of mucosal atrophy - Sponsor attributes to stress and debilitated state-]*
  - liver: multifocal mononuclear cellular infiltration (very slight) 1M G1, 2 M G4, 1F G4- periportal polymorphonuclear cellular infiltration (very slight) 1M G1, 1M G4-
  - adrenal: zona reticularis, vacuolation (very slight) 1F G4-
  - lung: focal pneumonia (very slight) 1F G4-
  - thymus: squamous cyst 1M G4-
- organ weights: 1F G2 (94-0335) with splenomegaly, 1 G2M, 1 G2F - other changes did not appear to be related to treatment-

Final Sacrifice (Week 53)

- gross/histopathology
  - small intestine: mucosal multifocal atrophy (slight or small) 1M G4 (94-0248)-*this is the animal which was prematurely sacrificed*
    mucosal multifocal necrosis (very slight) 1M G4 (94-0248)-
  - liver: multifocal lipidosis (slight or small) 1M G4 (94-0266)-
  - pituitary: cyst 1F G1, 2 F G2, 3 F G4, 2 M G4-
  - kidney: chronic nephritis (very slight) 1 M G4 (94-0252), *tubular basophilia 1F G1, 2 F G4-*
  - urinary bladder: multifocal hemorrhage (very slight) 1 M G4 (94-0252)-
  - lymph node: reactive hyperplasia (moderate) 1 M G4- *[occurred in M with intestinal lesions and with a skin abscess]*
  - brain: mononuclear cellular infiltration (very slight) 1 M G4-
- organ weights: liver ↓ absolute M/F wt G4 (8.5%) *[↓18% for females but ↑4% increase for male, ↓ 18% G2F, ↓10% G3F]* - adrenals ↓ absolute M/F wt G4 (13.5%) *[comparable ↓ both males and females compared to controls, similar decrease in G2M]* - *↓16% in absolute testes weight G4M, primarily due to 1 animal -↓20, 12, and 26% in absolute ovary weight in G2, G3, and G4,*

respectively[necrotizing vasculitis of the ovary was observed in 1 G4 F with low ovary weight] - other weights did not appear to show treatment related changes- *these changes, in general, were not associated with any histopathological lesions.*

There was no early mortality in this study, nor was there any treatment-related change in body weight or food consumption. *There was one high dose male which sacrificed due to complications secondary to canine tooth fracture including pyrexia and tooth root abscessation. Other findings in this animal included lymph node reactive hyperplasia and small intestinal mucosal focal atrophy and necrosis. It is not possible to determine the impact of drug treatment on the outcome of this event, e.g. did the anti-inflammatory effects of L-748,731 alter the immunocompetence of this animal.* The ophthalmoscopic and EKG examinations were indicated as having no treatment related effects. At the interim sacrifice W27, the mucosa of the jejunum and ileum of one G2 animal was reddened with slightly depressed foci and segmental enteritis was indicated. *It is difficult to attribute this finding to drug administration due to a lack of a dose dependent relationship.* One high dose dog (30 mg/Kg/day) had bloody stools during W 20, 22, and 25, which was considered treatment related, but histopathology at W53 did not show any lesion. Two other high dose dogs had bloody stools during these weeks. After W25, no additional episodes of bloody stools were reported in any animal. Hematology and serum chemistry were uneventful, with the exception of a slight increase in BUN in two dogs in G4 and one dog in G3. Fine granular casts were seen in the urine of one or two dogs in each treatment group. Microscopic lesions (multifocal atrophy/multifocal necrosis) were reported in the small intestine of *the* high dose dog (30 mg/Kg/day) which was prematurely sacrificed, and one other high dose dog had very slight chronic nephritis and multifocal hemorrhage in the urinary bladder. The liver and adrenal weights were the only organ weights that showed slight decreases in absolute weight. The NOEL for this study was 10 mg/Kg/day. *Based on the occurrence of only minor toxicity, it is felt that the maximum tolerated dose [MTD] was not reached in this study.*

4.2.4. Sixteen-Day Intravenous Toxicity Study in Dogs With L-748,731 [Vol. 1,20; P. 4232]

Study: TT#95-077-0
Compound: L-748,731, batch L-748,731-000R014
Formulation: Solutions in 0.8% PEG 400/normal saline at 5 µg/mL.
A factor of 1.0 was used in dosage calculations.
Route: Daily IV injections in the cephalic vein at approximately 40 mL/min.
Diet: 350 g of certified Canine Diet pellets/day.
Dose Levels:

Group	Dose Volume
1 Control (0.9% saline)	12 mL/kg
2 Control (vehicle)	12 mL/kg
3 20 µg/kg/day	4 mL/kg
4 40 µg/kg/day	8 mL/kg
5 60 µg/kg/day	12 mL/kg

Total Number of Doses: 14 in M and 15 in F
Strain: Beagle, approximately 31-35 weeks old, M 10.5-13.2 kg, and F 9.0-11.4 kg body weight.
Number: 4/sex/group
Control Treatment: G1 sterile 0.9% NaCl, G2 sterile 0.08% PEG 400 in 0.9% NaCl.
Study Site: Merck Research Laboratories, West Point, PA 19486
Date: 29 November 1995 to 25 April 1996
GLP/QAU Statements: Both present and signed.

The study was done to determine the toxicity of L-748,731 when administered by the intravenous route for 14 to 15 days. The dogs were observed daily for mortality and clinical signs. Body weight was determined prior to dosing and once W1 and W2. Food consumption was measured 3-4 days each week. Ophthalmoscopic examinations (slit lamp biomicroscopy and indirect ophthalmoscopy) were done pretest and W2. Hematology (erythrocyte count-Hct- Hb concentration-leukocyte count-differential leukocyte count-platelet count-activated partial thromboplastin time-mean corpuscular volume-mean corpuscular Hb- prothrombin time), serum biochemistry [total protein-glucose-creatinine-albumin-urea nitrogen- albumin to globulin ratio (A/G)-ALT-AST-alkaline phosphatase- triglycerides- Ca (total)-

cholesterol-Na+-Cl-phosphorus], and urinalyses (glucose-protein-bilirubin-occult blood-pH-specific gravity-ketone-urobilinogen-volume-microscopic examination of sediment) were done on all dogs prestudy and W2, with overnight fasting prior to bleeding. EKGs were recorded for all dogs prestudy and about 1.5-5.5 hours after dosing during W2. Necropsy was done on all dogs. Organ weights (adrenals-brain-heart-kidneys-liver-ovaries-pituitaries-prostate-spleen-testes-thyroid) were recorded. Histopathology examinations were done on 32 different tissues from all G2 and G5 dogs and on all grossly observed changes. Statistical analysis was not done.

RESULTS AND DISCUSSION

- mortality: all dogs survived-
- physical signs: no treatment related signs-
- body weight: no significant changes in any group-
- food consumption: 350 g/day was consumed by most dogs in all groups-
- ophthalmoscopic examinations: "no treatment-related changes" indicated-
- EKG: "no treatment-related changes" indicated-
- hematology: % neutrophils: slight↑increase (4%) G5 in W2-
  Eosinophils: slight ↓ of 7% in G5 in W2-
- serum chemistry: no treated changes-
- urinalysis: no treatment-related changes-
- organ weights: no treatment-related changes-
- gross changes: no treatment-related changes-
- histopathology: n = 4/sex
  injection site: perivascular cellular infiltration, perivascular fibrosis, vascular fibrosis, salivary gland: cellular infiltration 1MG5(510576)-
  pancreas: acinus atrophy 1 M G5 (No. 510573)-
  thyroid: cellular infiltration 1M G5 (No. 510576)-
  kidney: focal nephritis 1 G5 (No. 510574)-
  testis: degeneration 1G5 (No. 510574)-
  focal hypoplasia 1G5 (No. 510574)-
  prostate: focal chronic inflammation 1G5 (No. 510573)-
  lung: intravascular thrombosis 1FG5 (No. 510596)-
  brain: perivascular cuffing 1 FG5 (No. 510594)-

The intravenous administration of L-748,731 (0, 20, 40, or 60 µg/kg/day) to dogs for 14-15 days had no adverse effects on the animals. Most of the lesions at 60 µg/kg/day in the lung, kidney, testis, and prostate occurred in male No.510574. All of the above lesions were considered to be very slight. Further IV studies may confirm the G5 lesions as being treatment related or of a random nature.

4.3 REPEAT DOSE STUDIES - RATS

4.3.1. Exploratory 15-Day Oral Toxicity Study in Rats: [Vol. 1.11, B-910]

Study: TT#93-149-0,
Compound: L-748,731-000R, lot 003,
Formulation: Suspension in 5% polysorbate 80 (Tween®, L-521,660- 000C008) in deionized water each day.
Route: Oral, gavage, 5 mL/Kg body wt.
Dose Levels: 0, 30, 100, 300 mg/Kg/day x 14 days
Strain: Crl:CD®(SD) BR, 59 days old, F 173-255 g, M 248-358 g
Number: 10/sex/group
Control Treatment: 5% Tween 80® in DI water
Study Site: Merck Research Laboratories, West Point, PA
Date: November 1993 - March 1994
GLP/QAU Statements: Not present.

The study was conducted to evaluate the toxicity, determine plasma drug levels, and evaluate P450 mediated 7-ethoxy-4-trifluoromethylcoumarin O-demethylase (EFCOD) and peroxisomal FACO (peroxisomal) enzyme induction. *[No positive controls were included in the enzyme evaluation.]* The animals were examined daily. Food consumption was estimated 2x/week by observation. Body weights were determined predose, once Week 1 and twice Week 2. Hematology and serum chemistry were evaluated Week 2. Plasma drug levels were determined D14-15 at 1, 2, 4, 8, and 24 hours after drug administration from 2/sex/group. No statistical workup was done. Necropsy was limited to examination of livers, kidneys, and GI tract. Organ weights were recorded for kidneys and livers. Histopathologic examination was limited to the stomach, small intestine, large intestine, liver, kidneys from all control and high dose rats.

Results and Discussion

-signs: none
-mortality: none
-body wt: F body wt declined in all groups and M body wt increased in all groups over the study period - drug groups were not evenly balanced in body wt compared to controls-
-food consumption: no changes indicated-
-hematology: no treatment related changes *[54, 30, 80% ↑ in PMN count in G2, G3, G4]*
-blood chemistry: no treatment related changes *[23% ↑ in BUN in M all dose groups]*
-organ weights:
F: slight ↑ in kidney (11%) and liver (11%) wts -*absolute and relative*
M: slight ↑ in absolute (2.3%) and relative (8%) liver wt
-gross/histopathology: nothing remarkable
-plasma levels: from 2/sex/group non-fasted animals

Dose (mg/Kg):	30	100	300
C_{max} (h):	2	2	2
t_{max} (μM):	22.2	28.2	31.2
$AUC_{0\ 24h}$ (μM h):	224.5	312.5	376.2

-enzyme induction -

Dose (mg/kg):		0	30	100	300
EFCOD[1] (mean):	M	638.5	438.7	494.5	535.7
SD:		92.9	59.0	130.4	139.8
	F	843.0	325.6	259.2	262.0
SD:		86.9	65.7	61.1	49.9

[1] pmole/min/mg microsomal protein

FACO[1] (mean):	M	1.06	0.88	1.14	0.84
SD:		0.35	0.31	0.37	0.21
	F	1.08	1.07	1.27	1.35
SD:		0.11	0.16	0.18	

[1] nmoles/min/mg protein

Results and Discussion

The dosages used in this exploratory study showed no adverse effects. Drug plasma levels increased with the dose, but were not proportional to the dose. AUC values were also not proportional to the dose. EFCOD activity was decreased in all drug groups about 25% in males and 70% in females. It was stated that Western blot analysis (data not shown) indicated CYP2B and CYP3A proteins are induced. The FACO activity was increased in females in the 100 and 300 mg/Kg groups. This was said to be "a spurious result since female SD rats are refractory to FACO induction and no effect was seen in males."

The current Pharmacology/Toxicology Reviewer reviewed the following studies

4.3.2 L-752,860 and L-748,731: Exploratory Sixteen-Day Oral Toxicity Study in Rats [Vol. 1.17; p. B-3102]

Study Identification: TT #95-615-0
Site: Laboratoires Merck Sharp & Dohme-Chibret, Riom, France
Study Dates: May 5 - Sept 26, 1995
Formulation and Lot No.: L-748,731-000R014 and L-752, 860-000R005
Vehicle control - 0.5% aqueous methylcellulose
Certificate of Analysis Submitted: No (X)
Final Report (X) Sept. 28, 1995
GLP and QA statements signed: No (X) not GLP
Objective: "To determine the potential toxicity of L-752,860, an inhibitor of cyclooxygenase 2, on selected target organs when administered for 16 days in rats and compare with effects of L-748,731 given at the same highest dosage level."

Test Material/ Group Designation	Dose*				Sex	N	Species/Strain
	mg/kg	ml/kg	Route	# days dosed			
Group 1 - vehicle	-	5	oral gavage	treated = 15 control = 16	M	9	Crl:CD®(SD)BR strain - Sprague Dawley
Group 2 - L-752,860	30				F	9	
Group 3 - L-752,860	100						app. 8 wks at study start
Group 4 - L-752,860	300						M - 245-285 g
Group 5 - L-748,731	300						F - 151-199 g

*fed approximately 15.5 or 24 gm for females and males, respectively

Parameter Evaluated	Time Point(s)
Physical examination/mortality	Daily
Body weight	pretest, 1X/ Drug Week 1, 2X/Drug Week 2
Food consumption	estimation
Hematology - RBC count, Hb, Hct, MCV, MCH, MCHC	Drug Week 2
Serum Biochemistry - BUN, creatinine, AST, ALT, AP	Drug Week 2
Necropsy - stomach, small and large intestine, liver, kidneys, and cervical and mesenteric lymph nodes	Day 15 or 16 2 hours after the last dose
Organ weights - brain, liver, kidneys	
Histopathology -stomach, small and large intestine, liver, kidneys, and cervical and mesenteric lymph nodes - control and 300 mg/kg/day groups	Day 15 or 16
Plasma Drug Levels - ether anesthesia - retroorbital bleed - HPLC/UV detection system	Day 14 - 2 and 6 hours post dosing

Results -

Clinical signs - No treatment related effects

Mortality - There were no deaths during the study

Body weight - No treatment related effects

Food consumption - No treatment related effects

Hematology/Serum Biochemistry - There was approximately a 37% increase in BUN in males administered L-748,731 as compared to the control values.

Organ weights - There was an 11-16% increase in liver weight [absolute and relative] in females administered L-748,731 at 300 mg/kg/day. This increase was observed in both sexes administered L-752,860 at ≥30 mg/kg/day.

Necropsy - No treatment related effects

Histopathology - No treatment related effects

Plasma drug levels - The plasma drug concentrations of L-748,731 at 2 and 10 hours were 23.9[44.2] and 12.1[29.2] μM in males[females]. Drug levels of L-748,731 were approximately 3 and 15X higher at 2 and 10 hours post dosing than L-752,860

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

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

1. The increase in BUN following administration of L-748,731 was considered incidental due to a lack of histopathological correlate and the small number of animals in the group. **Reviewer's Comment** - Based on the findings in the other repeat dose toxicity studies in rats, it is felt that the increase in BUN was treatment-related. *The fact that there was no concomitant increase in creatinine suggests that this increase in BUN was not related to renal changes.*

2. There was an increase in liver weights in females administered L-748,731 at 300 mg/kg/day.

3. Female rats had higher plasma concentrations of L-748,731 than males.

Reviewer's Comment – Both an increase in liver weights and higher plasma concentrations in females have been observed in other studies.

4.3.3. L748,731, Ibuprofen: Exploratory Fifteen-Day Cyclooxygenase Inhibition Study in Female Rats [Vol. 1.17; p. B-3018]
Study Identification: TT #95-055-0
Site: Merck Research Laboratories; West Point, PA
In-life Phase Study Dates: Sept. 13-27, 1995
Formulation: L-748,731-000R009; 99.9% purity
Vehicle control - 0.5% aqueous methylcellulose
Comparative control - Ibuprofen
Certificate Analysis:No (X)
Final Report (X) Jan. 18, 1996
GLP and QA statements signed: No (X) not GLP
Objective: "To determine the effects of a selective cylclooxygenase II [COXII] inhibitor [L-748,731] and a non-selective cyclooxygenase inhibitor [ibuprofen] on gastrointestinal cyclooxygenase activity."

Test Material/ Group Designation	Dose*				Sex	N	Species/Strain
	mg/kg	ml/kg	Route	# days dosed			
Group 1 - vehicle	-	5	oral gavage SID	15	F	16	Crl:CD®(SD)BR - Sprague-Dawley rats 78 days at study start, 248-316 g individually housed
Group 2 - ibuprofen	100						
Group 3 - L-748,731	100						

*fed *ad libitum*