

1.1.5 ADDITIONAL IN VITRO BIOCHEMICAL STUDIES OF MK-0966

1) Effects of MK-0966 in CHO Whole Cell Assays for hCOX-1 and hCOX-2 [Vol. 1.42: p. 360]

Cyclooxygenase activity was assayed in recombinant CHO cell lines in which expression of either hCOX-1 or hCOX-2 was constitutive. In the presence of arachidonic acid stimulation, these cells will produce PGE<sub>2</sub>. PGE<sub>2</sub> production in the hCOX1 and hCOX2 recombinant lines was 260-1540 and 710-1590 pg PGE<sub>2</sub>/10<sup>6</sup> cells. The IC<sub>50</sub> for inhibition of PGE<sub>2</sub> synthesis by MK-0966 in the hCOX2 and hCOX1 line was 18 ± 7 nM [SE, N=6] and >15 μM [N=3], respectively. The IC<sub>50</sub> for inhibition of PGE<sub>2</sub> synthesis by indomethacin in the hCOX2 and hCOX1 line was 27 ± 6 nM [SE, N=12] and 18 ± 2 nM [SE, N=12], respectively. It is not apparent why the Sponsor conducted more repeats with indomethacin than with MK-0966. The specificity for COX-2 in this assay [e.g. COX-1:COX-2 IC<sub>50</sub> ratio] is >800.

2) Effect of MK-0966 on Purified hCOX-2 Activity [Vol. 1.42: p. F-364]

Purified hCOX2 activity was measured in a chromogenic assay based on the enzymatic oxidation of a reporter compound following reduction of PGG<sub>2</sub> to PGH<sub>2</sub>. In this assay, COX-2 activity was inhibited by MK-0966 with an IC<sub>50</sub> value of approximately 0.4 μM. MK-0966 was approximately equipotent to indomethacin [IC<sub>50</sub> = 0.36 μM] under the conditions of this assay.

3) Effects of MK-0966 on Cyclooxygenase Activity of U-937 Cell Microsomes [Vol. 1.42: p. F-365]

This assay was designed to be a sensitive indicator of COX-1 activity in the presence of low substrate concentrations [arachidonic acid at 0.1 μM]. A microsomal preparation, which was obtained from U-937 cells that express COX-1, was incubated with arachidonic acid ± MK-0966 or indomethacin. The level of PGE<sub>2</sub> measured. The IC<sub>50</sub> for MK-0966 [N=7] and indomethacin [N=24] was 2.0 ± 0.5 μM and 21 ± 2 nM, respectively. Therefore, in this system, MK-0966 was a weak inhibitor of COX-1

4) Effect of MK-0966 on Rat COX-1 and COX-2 [Vol. 1.42: p. 368]

Cyclooxygenase activity was assayed in recombinant Sf9 insect cells in which expression of rat COX-2 was constitutive. PGE<sub>2</sub> production in these transfected cells, in the presence of arachidonic acid stimulation, was 4.0-8.1 ng PGE<sub>2</sub>/10<sup>6</sup> cells. The IC<sub>50</sub> for inhibition of PGE<sub>2</sub> synthesis by MK-0966 and indomethacin was 46 ± 9 nM [SE, N=3] and 18 ± 4 nM [N=2], respectively. [Despite the title, only COX-2 was evaluated.]

5) Determination of the Inhibition of Purified hCOX-1 by MK-0966 [Vol. 1.42: p. F-369]

The IC<sub>50</sub> for MK-0966 inhibition of PGE<sub>2</sub> production by solubilized, recombinant hCOX1 in the presence of low concentrations of radiolabeled arachidonic acid was 26.3 ± 6.4 μM [SE; N = 11]

*The following studies were submitted in a report entitled "Additional Pharmacological Studies of MK-0966" [Vol. 1.42; p. F-374] and were conducted at the Merck Frost Centre for Therapeutic Research in Quebec, Canada. With the exception of one study, these studies were described in the report entitled "Pharmacological studies of L-748,731" [Vol. 1.42; p. F-227] and will not be reviewed here. Additional data, which do not alter the previous conclusions, were provided for several studies. These studies include:*

1. *Effect of MK-0966 on Carrageenan-Induced Paw Edema in the Rat [Vol. 1.42; p. F-378]*
2. *Effect of MK-0966 on LPS-Induced PGE<sub>2</sub> Generation by Human and Rat Mononuclear Cells [Vol. 1.42; F-386]*
3. *Effects of MK-0966, Celecoxib, Meloxicam, Diclofenac and Indomethacin in LPS-Induced Human Whole Blood PGE<sub>2</sub> and Serum TXB<sub>2</sub> Assay - This assay compared MK-0966 to other COX inhibitors. The COX-2 selectivity ratios [COX-1 IC<sub>50</sub>:COX-2 IC<sub>50</sub>] for the various drugs was 35.5 for MK-0966, 6.6 for celecoxib, 2 for meloxicam, 3 for diclofenac, and 0.4 for indomethacin [Vol. 1.42; p. F-390]*
4. *Effect of MK-0966 on LPS-Induced Pyrexia in Conscious Rats [Vol. 1.42; p. 394]*

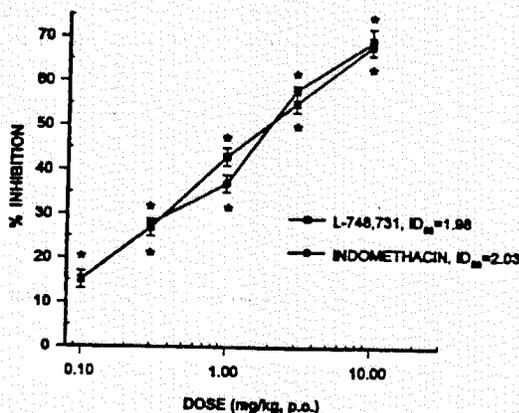
The initial Pharmacology/Toxicology Reviewer, Dr. Will Coulter, reviewed the following studies. Additional comments by the current Pharmacology/Toxicology are in italics.

### 1.2 PHARMACOLOGICAL STUDIES OF L-748,731 - *In Vivo* Studies

#### 1.2.1) Effect of L-748,731 on Carrageenan-Induced Rat Paw Edema [Vol. 1.42: p. F-315]

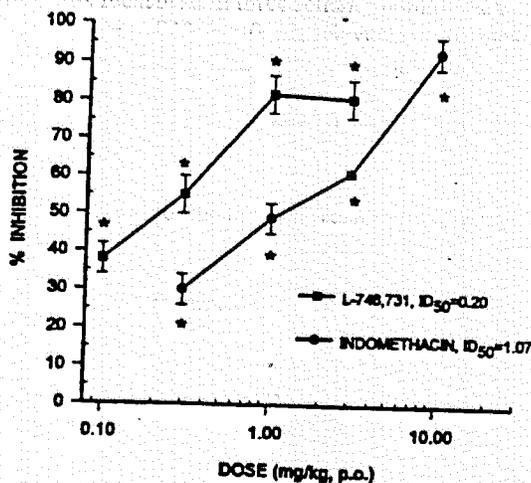
Both COX-2mRNA and protein are induced in the rat paw following injection of carrageenan. The results indicated that L-748,731 ( $ID_{50} = 1.98 \pm 0.17$  mg/Kg) was similar to indomethacin ( $ID_{50} = 2.03 \pm 0.23$  mg/Kg) potency. The following figure (p. F-317) indicated the dose-related inhibition of rat paw volume.

Figure 1: Rat Paw Edema Assay



#### 1.2.2) Effect of L-748,731 on LPS Induced Pyrexia in Conscious rats [Vol. 1.42: p. F-318]

L-748,731 was compared to indomethacin in reducing the rectal temperature of rats treated with an ip injection of lipopolysaccharide. The ID values were: L-748,731  $ID_{50} = 0.20 \pm 0.05$  mg/Kg, indomethacin  $ID_{50} = 1.07 \pm 0.16$  mg/Kg. L-748,731 was 5 times more effective than indomethacin as an antipyretic in the conscious rat. The percent inhibition of pyrexia is indicated in the following figure (p. F-320)

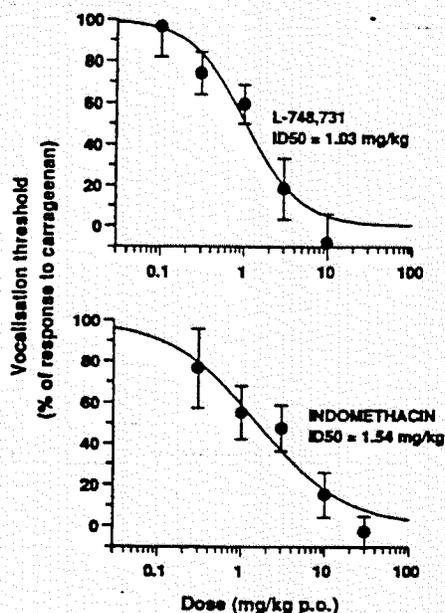


1.2.3) Effect of L-748,731 on LPS Induced Pyrexia in Conscious Squirrel Monkeys [Vol. 1.42: p. F-321]

The anti-pyretic activity of L-748,731 was measured in squirrel monkeys by inducing pyresis with an iv administration of 3-6  $\mu\text{g}/\text{Kg}$  of LPS. L-748,731 was compared to the anti-pyretic activity of diclofenac. Each drug at 3 mg/Kg po reduced rectal temperatures; however, diclofenac appeared to produce a more rapid response than L-748,731. These doses were said to be effective at inhibiting LPS-induced generation of  $\text{PGE}_2$  in squirrel monkey blood *ex vivo*.

1.2.4) Effect of L-748,731 on Acute Inflammatory Hyperalgesia Induced by Carrageenan [Vol. 1.42: p. 324]

This study compared the antinociceptive effect of L-748,731 to that of indomethacin against carrageenan induced hyperalgesia in male Sprague-Dawley rats. *Control* animals received saline (0.15 mL intraplantar). L-748,731 (0.1-10 mg/Kg po), indomethacin (0.3-30 mg/Kg po), or vehicle (5% Tween 80 po) were administered at 4 mL/Kg 2 hr after carrageenan (4.5 mg into one hind paw). The  $\text{ID}_{50}$  values (the dose that produces 50% of the maximum observed response) were: L-748,731 -  $\text{ID}_{50} = 1.03 \text{ mg/kg}$  and indomethacin -  $\text{ID}_{50} = 1.54 \text{ mg/Kg}$ . The analgesic effects of these drugs are indicated in the following figure (p. F-327).



1.2.5) Effect of L-748,731 on Chronic Inflammatory Hyperalgesia Induced by Injection of Freund's Complete Adjuvant into the Ankle Joint [Vol. 1.42: p. F-328]:

The intra-articular injection of Freund's complete adjuvant containing *Mycobacterium butyricum* into the left ankle joint of male Sprague-Dawley rats induces inflammation and hyperalgesia. The hyperalgesic effect was evaluated 7-8 days later by comparing the number of vocalizations emitted following gentle flexion and extension of the ankle joint. Indomethacin (10-30 mg/Kg po) and L-748,731 (3-30 mg/Kg po) significantly reduced the number of vocalizations at 1 and 3 hours compared to pretreatment baseline values; however, L-748,731 was not as effective as indomethacin.

*The following study was submitted in a report entitled "Additional Pharmacological Studies of MK-0966" [Vol. 1.42; p. F-374] as well as a separate reference. It was conducted at the Merck Frost Centre for Therapeutic Research in Quebec, Canada.*

1.2.6) Effects of L-748,731 on Adjuvant Induced Arthritis in Lewis Rats [Vol. 1.43: p. F-480]

Female Lewis rats (150-170 g body wt), 10 per group, were evaluated in an adjuvant-induced arthritis study consisting of a negative control group, a vehicle control group (5% Tween 80), four groups of L-748,731 (0.1, 0.3, 1.0, 3.0 mg/Kg/day po), and an indomethacin (1.0 mg/kg/day po) group. Body wt, thymus wt, spleen wt, contralateral paw volumes, and lateral radiographs were measured Days 0, 14, and 21 following sub plantar adjuvant injection of 0.5 mg *Mycobacterium butyricum*.

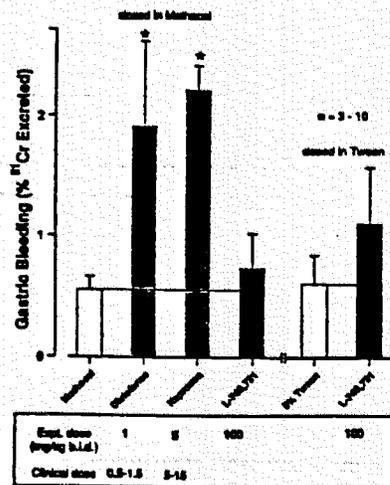
Body weight gain of the control group was significantly greater than the vehicle, L-748,731, or indomethacin groups at Day 14 and Day 21. Foot swelling was significantly reduced Days 14 and 21 at 1 and 3 mg/Kg L-748,731 and 1.0 mg/Kg indomethacin. The ID<sub>50</sub> value [50% inhibition of paw swelling] was 0.84 mg/Kg on Day 14 and 0.74 mg/Kg on Day 21 for L-748,731. Radiographic scores were also significantly reduced compared to the vehicle group on Days 14 and 21 by indomethacin (1 mg/Kg/day) and L-748,731 (1 and 3 mg/Kg/day). Thymic wt loss was significantly less with L-748,731 (1 and 3 mg/Kg) and indomethacin (1 mg/Kg) when compared to the vehicle control. No significant changes were seen with the splenic weights. L-748,731 appeared to be comparable to indomethacin in the parameters measured in this study, particularly at 3.0 mg/Kg.

1.2.7) Effects of L-748,731 on Fecal <sup>51</sup>Cr Excretion in Rats [Vol. 1.42: p. F-331]

Male Sprague-Dawley rats were dosed once with L-748,731 (100 mg/Kg), indomethacin (10 mg/Kg), or diclofenac (10 mg/Kg) followed immediately by an injection of <sup>51</sup>Cr-labeled red blood cells, and the increase in fecal <sup>51</sup>Cr excretion was evaluated over a 48 hour period. In a 5 day chronic study, the effects of 3 mg/Kg bid indomethacin or diclofenac, 100 mg/Kg bid L-748,731 on fecal <sup>51</sup>Cr excretion was also studied. In the acute study, diclofenac produced a 12% increase and indomethacin a 4% increase in fecal <sup>51</sup>Cr excretion of the administered <sup>51</sup>Cr dose. Both were said to be significant from the control (0.35%), methocel. The <sup>51</sup>Cr excretion for the L-748,731 dosed animals was no different than the methocel control. In the chronic study, the indomethacin dose was toxic to the animals and no results could be obtained. Diclofenac produced a significant increase in percent excretion of the injected <sup>51</sup>Cr dose compared to the control. Again, no change was seen with L-748,731.

1.2.8) Effects of L-748,731 of Fecal <sup>51</sup>Cr Excretion in Squirrel Monkeys [Vol. 1.42: p. F-335]

L-748,731 (5, 10, 25, 100 mg/Kg bid in 5% Tween 80, and 100 mg/Kg bid in 1% methocel), diclofenac (1 mg/Kg bid in 1% methocel), naproxen (5 mg/Kg bid in 1% methocel), 5% Tween 80 (3 mL/Kg bid) in water, or 1% methocel (3 mL/Kg bid) were administered to squirrel monkeys for 4-5 days. <sup>51</sup>Cr (5 μCi/Kg in 1 mL/Kg PBS) was administered iv 1 hr after the last drug/vehicle dose. Feces were collected for 24 hr and the <sup>51</sup>Cr content determined. The results are indicated below (p. F-338).



Fecal <sup>51</sup>Cr (as a % of a 5μCi/Kg i.v. dose) was measured in groups of 3-10 squirrel monkeys after 4-5 days b.i.d. treatment with 1% methocel or 5% Tween vehicles, diclofenac, naproxen or L-748,731. The latter compound showed negligible excretion of the isotope compared with the standard NSAIDs when given at doses approximating clinical usage.  
\* p < 0.05 compared with methocel controls, Mann-Whitney U Test.

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L-748,731 in methocel or Tween-80 did not result in a statistically significant increase in fecal <sup>51</sup>Cr compared to the concurrent control animals. The mean was increased, however, for animals administered L-748,731 in methocel or Tween-80 and the variability was greater [based on S.E.M.] compared to the concurrent control animals. Individual animal data were not provided. The data suggest that individual animals may have demonstrated variable sensitivity to L-748,731-induced GI toxicity.

The current Pharmacology/Toxicology Reviewer reviewed the following study.

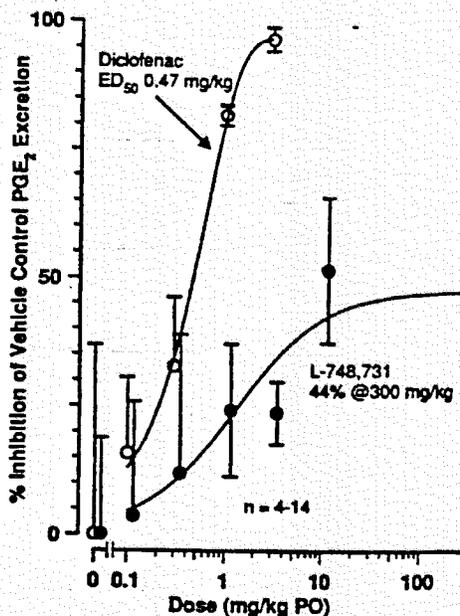
1.2.9) Effect of MK-0966 on Urinary <sup>51</sup>Cr Excretion in Rats [Vol. 1.42: p. F-381]

<sup>51</sup>Cr-EDTA is normally restricted to the GI tract unless there is GI mucosal disruption that allows absorption of the complex. Once absorbed, the kidney excretes 95% of the complex. Therefore, measurement of urinary excretion of <sup>51</sup>Cr allows assessment of GI mucosal integrity. The Sponsor states that this assay is 3-10X more sensitive than the fecal <sup>51</sup>Cr method described above. Administration of 3 mg/kg BID X 3 days of either indomethacin or diclofenac to male and female Sprague-Dawley rats resulted in a significant increase in urinary <sup>51</sup>Cr excretion during the 24 hour period following dosing. There was a significant increase in urinary <sup>51</sup>Cr excretion in male rats only that were administered MK-0966 at 200 mg/kg/day X 5 days. The increase in urinary <sup>51</sup>Cr excretion in females following administration of MK-0966 was not statistically significant. GI mucosal disruption was not demonstrated for MK-0966 using the fecal <sup>51</sup>Cr excretion method.

The initial Pharmacology/Toxicology Reviewer, Dr. Will Coulter, reviewed the following studies.

1.2.10) Effects of L-748,731 on Rat Urinary PGE<sub>2</sub> Excretion [Vol. 1.42: F-339]

This study looked at the PGE<sub>2</sub> excretion from renal origin (not metabolized) as distinguished from PGE<sub>2</sub> from systemic origin, which is metabolized. Rats were dosed po with 5% Tween vehicle (1 mL/Kg), diclofenac (0.1-3 mg/Kg), or L-748,731 (0.1-300 mg/Kg), followed by 2.5 mL/Kg H<sub>2</sub>O. After 1 hr the urine was collected for 3 hr and spiked with <sup>3</sup>H-PGE<sub>2</sub>. L-748,731 at 300 mg/Kg produced 44% inhibition of vehicle control PGE<sub>2</sub> excretion. Diclofenac produced almost 100% inhibition, with an ED<sub>50</sub> = 0.47 mg/Kg. This study was intended to be an index of the nephrotoxicity of NSAIDs. Graphic results from p. F-341 are indicated below.



Urinary PGE<sub>2</sub> excretion could be inhibited in groups of 4-5 rats by the standard NSAID diclofenac (>95% inhibition at 3 mg/kg). In contrast, L-748,731 showed greatly reduced activity in this assay.

Values Represent Mean ± S.E.M.

*These results suggest that, based on PGE<sub>2</sub> production inhibition, L-748,731 is potentially less nephrotoxic than diclofenac.*

**1.2.11) Effects of L-748,731 on Urinary PGE<sub>2</sub> Excretion in Anesthetized Dogs and on AA-Induced PGE<sub>2</sub> Formation in LPS Stimulated Dog Whole Blood *Ex Vivo* [Vol. 1.42; p. F-342]**

According to the sponsor, PGE<sub>2</sub> of renal origin is excreted in the urine unmetabolized, while systemic derived PGE<sub>2</sub> is metabolized prior to urinary excretion. L-748,731 produced a dose related inhibition (75%) of *ex vivo* LPS stimulated dog blood prostanoid generation and approximately a 64% reduction in urinary PGE<sub>2</sub>. Diclofenac (2.5 µg/Kg/min continuous infusion) produced a dose related inhibition (>85%) of urinary PGE<sub>2</sub> and almost 100% of *ex vivo* LPS stimulated dog whole blood. The Sponsor concludes that "L-748,731 has some *in vivo* selectivity for COX-2 in anaesthetised male dogs".

**1.2.12) Effects of L-748,731 on Urinary Eicosanoid and Sodium Excretion in Conscious Male Dogs [Vol. 1.42; p. F-346].**

L-748,731 (25 mg/Kg) produced a dose related inhibition (75%) of *ex vivo* LPS stimulated dog whole blood PGE<sub>2</sub> at 8 µg/Kg/min. The urinary PGE<sub>2</sub> excretion was decreased 35-40% by 3-4 hr. Diclofenac (2.5 µg/Kg/min infusion) also reduced the *ex vivo* biosynthesis of LPS stimulated dog whole blood to 12% of the predose value and urinary PGE<sub>2</sub> excretion to 22% of the baseline value by 3-4 hr. Both drugs resulted in a decrease in urinary sodium excretion. These results indicate that L-748,731 partially inhibits renal eicosanoid activity which may be involved in the control of Na excretion.

**1.3 SUMMARY OF PHARMACOLOGY** – Studies were conducted both *in vitro* and *in vivo* to delineate [1] the selectivity of L-748,731 for inhibition of COX-2 compared to COX-1 and [2] efficacy of L-748,731 in inflammatory, pain, pyrexia, and arthritis animal models. Generally, for these studies, indomethacin or diclofenac was used as a comparator. Table 1 outlines the studies conducted *in vitro*.

TABLE 1

Study	IC <sub>50</sub>			
	COX-2*		COX-1*	
	L-748,731	Indometh.**	L-748,731	Indometh.**
1. Whole Cell Cyclooxygenase [CO] Assay – PGE <sub>2</sub> synthesis by COX-1 [U-937] or COX-2 [osteosarcoma] expressing cells	26 ± 10 nM	20 nM	>50,000 nM	10 nM
2. Human, dog, and rat kidney microsomal CO assays	-	-	-	-
Human			14 µM	0.3 µM
Dog			>30 µM	0.25 µM
Rat			>30 µM	0.32 µM
3. CO Activity of U-937 Cell Microsomes	-	-	≈ 7 µM	≈ 30 nM
4. LPS-Induced Human Whole Blood PGE <sub>2</sub> [COX-2] and TXB <sub>2</sub> [COX-1]	0.59 µM	0.50 µM	13.12 µM	0.16 µM
5. LPS-Induced PGE <sub>2</sub> Generation by Mononuclear Cells				
Human	29 µM	26 µM	-	-
Rat	23 nM	87 nM		
6. Arachidonic Induce PGE <sub>2</sub> Production LPS Stimulated Dog Blood	10.8 µM	[diclofenac] 0.14 µM	-	-
7. CHO Whole Cell Assays for hCOX-1 or hCOX-2	18 ± 7 nM	27 ± 6 nM	>15 µM	18 ± 2 nM
8. Purified hCOX-2 Activity	0.4 ± 0.05 µM	0.36 ± 0.03 µM	-	-
9. CO Activity of U-937 Cell Microsomes	-	-	2.0 ± 0.5 µM	21 ± 2 nM
10. CO Activity in Sf9 Insect Cells Expressing COX-2	46 ± 9 nM	18 ± 4 nM	-	-
11. Inhibition of Purified hCOX-1	-	-	26 ± 6 µM	

\*Isoform reported to be the predominantly active enzyme for the listed response

\*\*Indomethacin

Based on these findings, L-748,731 is relatively specific for COX-2 vs. COX-1. *In vitro* kinetic studies indicated that L-748,731 inhibition of microsomal hCOX-2 was a two step process involving "a rapidly reversible binding of enzyme followed by a slow isomerization to a tightly bound inhibited complex". Inhibition of hCOX-1 was characterized by a weak and rapidly reversible binding [ $IC_{50} \approx 15 \mu M$ ]. L-748,731 did exhibit some COX-1 activity inhibition [e.g. human serum  $TXB_2$  assay, kidney microsomal CO assay, CO activity of U-937 cell microsomes] in the assay systems evaluated. However, the concentrations required to achieve inhibition were high [ $\mu M$ ] and significantly greater than the concentrations of indomethacin required [ $nM$ ] to demonstrate inhibition within the same assay. In general, the potency for COX-2 inhibition was similar for L-748,731 and indomethacin.

In the inhibition of  $PGE_2$  production in LPS stimulated dog blood, however, diclofenac was more potent [approximately 80X] than L-748,731. The data from the kidney microsomal cyclooxygenase [CO] assays suggests that human renal microsomal CO activity is more sensitive to the inhibitory effects of L-748,731 than the rat and dog preparations.  $PGE_2$  synthesis was inhibited by a maximum of 70-90% in human microsomal preparations with an  $IC_{50}$  of 14  $\mu M$ . The  $IC_{50}$  in rats and dogs was  $>30 \mu M$ . It should be noted, however, that indomethacin was approximately 50X more potent than L-748,731 in this system. The biological significance of this finding is not known.

These studies also demonstrated that L-748,731 exhibited little to no inhibitory activity against enzymes involved in leukotriene biosynthesis [e.g. lipooxygenase activity]. The  $IC_{50}$  in these assays was  $>20-25 \mu M$ . In addition, L-748,731 did not demonstrate activity at any other receptors or against enzymes evaluated in the [redacted]. L-748,731 did not inhibit collagen induced platelet aggregation up to 10  $\mu M$ . However, in this system, indomethacin resulted in only a 28% decrease in this response.

There was considerable variability in the pattern of COX-1 distribution in the gastrointestinal tracts of the various species evaluated. The rank order of COX-1 levels throughout the GI tract was generally humans  $>$  monkey  $>$  dog  $>$  rat. The COX-1 level in the stomachs of the squirrel monkey and the antrum of the dog, however, was greater than that observed in humans. The Sponsor suggests that there "appears to be an inverse relationship between levels of PGHS-1 protein observed and the sites within the gastrointestinal tract where ulceration is commonly observed".

L-748,731 demonstrated efficacy in the following animal models: [1] acute inflammatory model [carrageenan-induced rat paw edema]; [2] pyrexia reversal in conscious rats and squirrel monkeys; [3] antinociception in an acute inflammation [carrageenan-induced rat paw hyperalgesia] and chronic inflammation [CFA-induced hyperalgesia in rats]; and [4] adjuvant induced arthritis in rats. In general, the efficacy was comparable to indomethacin or diclofenac in the various models under the conditions tested. L-748,731 was less effective in the chronic inflammatory hyperalgesia model but more effective in the pyrexia reversal in conscious rats. Table 2 outlines the  $ID_{50}$  or  $ED_{50}$  provided by the Sponsor for selected studies.

Study	$ID_{50}$ or $ED_{50}$ [mg/kg]			
	COX-2*		COX-1*	
	L-748,731	Indometh.**	L-748,731	Indometh.**
1. Carrageenan-Induced Rat Paw Edema	1.98 $\pm$ 0.17	2.03 $\pm$ 0.23	-	-
2. LPS-Induced Pyrexia in Conscious Rats	0.20 $\pm$ 0.05	1.07 $\pm$ 0.16	-	-
3. Acute Inflammatory Hyperalgesia Induced by Carrageenan	1.03	1.54		
4. Adjuvant Induced Arthritis	0.74-0.84 mg/kg	-	-	-

\*Isoform reported to be the predominantly active enzyme for the listed response

\*\*Indomethacin

Three studies were conducted to assess the integrity of the GI tract. No statistically significant increase in fecal  $^{51}Cr$  excretion was observed in either rats or squirrel monkeys following administration of L-748,731 at doses up to 100 mg/kg/day for 4-5 days. There was a statistically significant increase in

urinary <sup>51</sup>Cr excretion in male rats and a nonstatistically significant increase in female rats administered L-748,731 at 200 mg/kg/day for 5 days. <sup>51</sup>Cr excretion [both urinary and feces] was significantly increased in these studies following administration of either indomethacin or diclofenac. The development of gastrointestinal lesions in rats following administration of L-748,731 is both dose and time-dependent. It is probable, therefore, that had these parameters been measured under different experimental conditions [e.g. following chronic dose administration], there would have been a statistically significant increase in <sup>51</sup>Cr excretion.

Three studies were conducted to assess the potential of L-748,731 to induce nephrotoxicity. The assumption is that the nephrotoxicity is associated with an inhibition of PGE<sub>2</sub> which plays a role in control of renal vasculature [e.g. vasodilatory] and in sodium excretion [e.g. increases excretion]. *In vitro* studies indicated that the predominant isoform involved in inhibition of PGE<sub>2</sub> synthesis is COX-1. In the *in vivo* studies, L-748,731 administration resulted in a decrease in urinary PGE<sub>2</sub> by approximately 40-60%. In conscious male dogs, this decrease was transitory with baseline values observed after 3 hours. In addition, urinary Na excretion was decreased. These findings indicate that L-748,731 does inhibit renal eicosanoid synthesis. However, the degree of inhibition is less than that observed with diclofenac.

**2. Safety Pharmacology:** *The initial Pharmacology/Toxicology reviewer, Dr. Will Coulter, reviewed the following studies. Additional comments by the current Pharmacology/Toxicology reviewer are indicated in italics.*

### 2.1 In Vivo Studies

#### 2.1.1 Ancillary Pharmacology of L-748,731-000R, a Selective Cyclooxygenase II (COX-2) Inhibitor [Vol. 1.43: F-398]

L-748,731-000R was evaluated for: a) cardiovascular effects and autonomic interactions in anesthetized dogs, b) respiratory function and hemostasis in anesthetized dogs, c) gastric acid secretion in conscious chronic gastric fistula dogs, and d) gastrointestinal motility in conscious mice.

Three conditioned, *vagotomized*, anesthetized mongrel dogs (8.3-10.0 Kg) were administered an intraduodenal (ID) bolus of 25 mg/Kg L-748,731-000R. The mean arterial pressure measured at 17 to 75 minutes after drug administration was increased about 10% at 30 min in one dog, the average heart rate increased 3% at 75 min. The EKG lead II showed no uniform average changes, and the arterial BP responses to (McNeil-343-A, DMPP, peripheral vagal stimulation, methacholine, epinephrine, norepinephrine, phenethylamine) autonomic *stimulus were not significantly altered*.

The respiratory function was measured in three female mongrel dogs (9.1-10.6 Kg) administered a suspension (5% Tween 80) of 25 mg/Kg L-748,731-000R. Several of the mean respiratory parameters (peak expiratory flow, intrapulmonary pressure, tidal volume, airway resistance, respiratory rate, minute volume, and end expiratory work) increased/decreased with time (0 to 90 min observation) when compared to time 0. *Blood pressure and heart rate were not altered*. Bleeding time was measured (Simplate®) by buccal bleeding time and said to be unaltered.

The effect of 25 mg/Kg L-748,731-000R in 5% Tween 80 was measured on the gastric acid secretion in conscious gastric fistula female beagles. No radical change was seen on basal and gastrin-stimulated gastric acid secretions, and emesis was not reported. Likewise, this dosage did not enhance or inhibit GI motility in female CF<sub>1</sub> mice, as determined by the advancement of acacia/charcoal in the small bowel.

Motor signs, autonomic signs, and spinal reflexes were evaluated in mice. The animals, two per dose, were administered 1, 30, or 100 mg/Kg po in a suspension of 5% Tween 80. There were no observable signs reported over 60 minutes.

2.1.2 Effect of the Selective Cyclooxygenase II Inhibitor (COX-2), L-748,731, on Renal Function and Electrolyte Excretion in Conscious, Trained Dogs and Conscious, Volume-Depleted, Normotensive Rats [Vol. 1.43: p F-424]

The renal effects of L-748,731 (5 and 25 mg/Kg po) in conscious female dogs (22-32 Kg) maintained on a normal sodium diet were evaluated in this study. The results showed a significant decrease in urinary flow, and sodium excretion from control at 5 mg/Kg (n = 4) and 25 mg/Kg (n = 5). Indomethacin, although not evaluated in this study, was said to produce a similar results (Altsheler et al., Am J Physion 4:F338-F344, (1978). Glomerular filtration rate, renal plasma flow, potassium excretion, plasma renin activity, or antidiuretic hormone levels were not affected, but urinary PGE<sub>2</sub> levels were reduced. *The Sponsor suggests that these results indicate that L-748,731 activity is at the level of tubular function and may be secondary to a reduction in renal PGE<sub>2</sub>. The plasma level at which these effects were observed was 1.0 μM.*

In conscious volume-depleted, furosemide, normotensive male Wistar rats (200-300 g) in which a bladder catheter was attached, 10 mg/Kg po of L-748,731 decreased the glomerular filtration rate, renal blood flow, sodium excretion, and urine volume. These decreases were said not to differ significantly from the vehicle group. On the other hand, indomethacin at (5 mg/Kg iv) significantly decreased the glomerular filtration rate and urine volume. *The decrease in these parameters following administration of L-748,731 was not statistically different from the decrease observed with indomethacin.*

**2.2 Summary of Safety Pharmacology** – Cardiovascular function, autonomic responses, respiratory function and gastric secretion were not significantly altered in dogs following administration of 25 mg/kg of L-748,731. GI motility, motor function, autonomic responses, and spinal reflexes were not altered in mice administered 1, 30, or 100 mg/kg po.

In female dogs administered either 5 or 25 mg/kg of L-748,731, there was a significant decrease in urine volume, Na excretion, and urinary PGE<sub>2</sub> levels. Glomerular filtration rate, renal plasma flow, plasma renin activity, or antidiuretic hormone levels were not altered. According to the Sponsor, these results suggest that L-748,731 activity is at the level of tubular function and not at the glomerulus. The changes in urinary volume and Na excretion may be secondary to a reduction in renal PGE<sub>2</sub>. The plasma level of L-748,731 at which these effects were observed was >1.0 μM.

In volume-depleted, normotensive male Wistar rats administered 10 mg/kg of L-748,731 po, glomerular filtration rate, renal blood flow, sodium excretion, and urine volume was decreased. These renal parameters were significantly different in the indomethacin treated animals compared to the control animals. The magnitude of the decrease in the animals administered L-748,731 was not statistically significant compared to either control animals or rats administered 5 mg/kg of indomethacin iv. Therefore, the Sponsor concluded that the renal effects of L-748,731 were less than or equal to the renal effects of indomethacin.

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3. Pharmacokinetics/Toxicokinetics

3.1. Pharmacokinetic Studies - Absorption, Distribution, Metabolism, Excretion Studies

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

3.1.1 Absorption, Distribution, Metabolism, and Excretion of L-748,731 in Rats and Dogs [Vol. 1.45: p. G-60]

Compound:  $^{14}\text{C}$  L-748,731 -  $^{14}\text{C}$  at 4 position of furanone ring.

Chemical and radioactive purity

Formulation: Solution in DMSO for iv administration and suspension in 0.5% methylcellulose for po administration.

Strain: Sprague-Dawley rats and beagle dogs

Study Site: Merck Research Labs., West Point, PA

Date: October 4, 1994

GLP/QAU Statements: No

Samples were analyzed for L-748,731 by [redacted] degrade resulting from [redacted] analyze for radioactivity in plasma or urine. Dosage used in the rat and dog iv studies was 2 mg/Kg. The oral doses were 5, 10, 100, or 300 mg/Kg in the rat and 2, 5, 10, or 50 mg/Kg in the dog. Drug was administered to fasted animals. The study design for the dog was a crossover with a 1-3 week washout period between doses. The quantitation limit was 0.05  $\mu\text{g/mL}$  following iv administration and 2.5 ng/mL following oral administration.

Rat Data

The Sponsor states that "variable but relatively flat terminal phase precluded reliable estimate of total AUC, and consequently any conclusion regarding dose-proportionality".

Estimated PK Parameters in Rats Following  $^{14}\text{C}$  L-748,731 at 2 mg/Kg iv or 5 mg/Kg po

Route	AUC <sub>0-10</sub> ng hr/mL	CL <sub>p</sub> <sup>b</sup> mL/min/Kg	Vd <sup>c</sup> L/Kg	t <sub>1/2</sub> <sup>d</sup> min	C <sub>max</sub> <sup>e</sup> ng/mL	T <sub>max</sub> <sup>f</sup> min
IV	1357	<25	0.7	-	-	-
PO	441	-	-	-	205	30

<sup>a</sup> Values derived from mean plasma data.

<sup>b</sup> Based on partial AUC<sub>0-10</sub> derived from mean plasma concentration.

<sup>c</sup> Derived from dose divided by the mean extrapolated Co.

<sup>d</sup> The half-life could not be determined due to variable but flat terminal phase.

<sup>e</sup> From the mean plasma concentration values. Occurred at 30 min.

<sup>f</sup> From the mean plasma concentration values.

Excretion Route	Administration	
	IV	Oral
Biliary	74.5%	61.3%
Urinary	23.1%	14.9%
Total	97.6%	76.2% (96 hour data)

<sup>a</sup> The major portion of the administered dose was eliminated in the first 24 hrs.

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In a repeat study [A Repeat Study of the Excretion of Radioactivity in the Urine and Feces of Rats Following Oral Administration of [<sup>14</sup>C]MK-0966 (5mg/kg) [Vol. 1.45; p. G-322], 97.9 ± 6.9% of radioactivity was recovered over a 96-hour interval with 25.6 ± 2.0% in the urine and 72.3 ± 7.0% in the feces.

Dose dependence of L-748,731 (10, 100, 300 mg/Kg In Rats (n = 4) Following Oral Administration

Dose	AUC <sup>a</sup>		C <sub>max</sub>	T <sub>max</sub>		
	µg hr/mL	mg/Kg	µg/mL	min		
	Mean (SD)		Mean (SD)	Mean (SD)		
10	1.36	(0.675)	0.324	(0.09)	38	(15)
100	23.5	(13.8)	3.44	(0.58)	180	(0)
300	76.5	(31.4)	5.41	(1.20)	135	(30)

<sup>a</sup> Mean partial AUC values.

Four major metabolites (M-1, M-2, M-3, and M-4) were observed 24 hr urine samples from iv or po drug administration. These four metabolites represented 80-93% of the total <sup>14</sup>C recovered. No unchanged drug was observed in the samples. Following β-glucuronidase treatment of the urine, completely hydrolyzed the glucuronide of M-2 to M-3. M-3 is believed to be the furanone 5-hydroxyl derivative of the parent drug.

Dog Data

Mean PK Data in Dogs Administered 2 mg/Kg, 10 min IV Infusion or 5 mg/Kg PO

"The intra- and inter-animal variability in rats was not evident in dogs."

Dose	AUC <sub>0</sub>	Cl <sub>p</sub>	Vd <sub>ss</sub>	t <sub>1/2</sub>	C <sub>max</sub>	T <sub>max</sub>	F
mg/Kg	ng hr/mL	mL/min/Kg	L/Kg	min	ng/mL	min	%
2 [iv]	9225(406)	3.6(0.2)	1.0(0.1)	154(18)	-	-	-
5 [po]	5997(1194)			198(49)	1114(156)	90(35)	26.1(6.1)

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Excretion	Administration	
	IV	Oral
Biliary	34.8%	76.0%
Urinary	49.2%	17.5%
Total	84.0%	93.5% (96 hr data)

<sup>a</sup> The major portion of the administered dose was eliminated in the first 24 hrs.

Dose Dependence of L-748,731 (2, 10, 50 mg/Kg) in Dogs (n = 4) Following Oral Administration

Increases were less than dose proportional.

Dose	AUC <sup>a</sup>		C <sub>max</sub>	T <sub>max</sub>		
	µg hr/mL	mg/Kg	µg/mL	min		
	Mean (SD)		Mean (SD)	Mean (SD)		
2	2.02	(0.52)	0.635	(0.099)	53	(15)
10	5.14	(1.41)	1.35	(0.42)	75	(30)
50	16.3	(4.77)	3.71	(1.07)	105	(30)

<sup>a</sup> Mean partial AUC values.

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Metabolism in the dog was more extensive than in the rat, with more than 14 metabolites observed, and none of the metabolites appeared to be identical to what was seen in the rat. No parent drug was detected in the urine from either iv or po drug administration. The major metabolites were identified as M-5, M-6, M-7, and M-15, and were observed in most of the samples. Treatment with  $\beta$ -glucuronidase indicated at least 4 new phase I metabolites in the urine of iv dosed dogs. The urine metabolite pattern from a dog dosed orally did not change after  $\beta$ -glucuronidase treatment.

When  $^{14}\text{C}$  L-748,731 was incubated with induced or uninduced microsomal preparation of liver, very little metabolite formation was seen with dog or human microsomes. The putative hydroxylated metabolite was the only metabolite identified. With phenobarbital induced liver microsomes from rats, the parent drug was metabolized to the putative hydroxylated metabolite at approximately 29% of the total radioactivity.

#### Plasma protein binding

The high degree of plasma protein binding was determined

The results are indicated below.

#### Plasma Protein Binding of $^{14}\text{C}$ L-748,731

Concentration $\mu\text{g/mL}$	Percent $^{14}\text{C}$ L-748,731 Bound <sup>a</sup>		
	Rat	Dog	Human
0.05	91.7	84.2	85.9
0.10	92.9	82.2	86.7
0.50	93.0 <sup>b</sup>	82.1	86.6
1.00	93.1	81.4	86.7
5.00	92.8	81.6	86.6

<sup>a</sup> Values represent the mean of n = 2

<sup>b</sup> Value represents n = 1

#### Erythrocyte Partitioning in rat, dog, and human whole blood

The blood:plasma ratio of radiolabeled L-748,731 was 0.76. This indicates that clearance from the blood will be somewhat greater than from the plasma.

The current Pharmacology/Toxicology Reviewer reviewed the following studies.

#### 3.1.2. L-748,731: Dose-Dependence of MK-0966 Pharmacokinetics in Rats and Dogs [Vol. 1,45: p. G-152]

Site: Merck Research Laboratories; West Point, PA

Formulation and Lot No. : Test article L-748,731-000R009

Vehicle - DMSO for iv administration

- 0.5% aqueous methylcellulose for po administration

Certificate Analysis: No (X)

Final Report (X) April 2, 1996

GLP and QA statements signed: No (X)

Objective: To determine the effect of dose on the systemic exposure of MK-0966

Study Design - Fasted male Sprague-Dawley rats [N=4] were administered MK-0966 as an iv bolus [0.5 ml/kg] at 1, 2, or 4 mg/kg or orally [5 ml/kg] at 2, 5, and 10 mg/kg. Fasted male Beagle dogs [N=4] were administered L-748,731 as an iv infusion [0.2 ml/kg over 10 minutes] at 1, 2, or 4 mg/kg or orally [5 ml/kg] at 2, 5, or 10 mg/kg. A crossover study was conducted in dogs with a 1-2 week washout period between doses. Four dogs were administered 5 mg/kg of radiolabeled MK-0966 as part of a separate study. Blood samples were collected over 48 hours. The following parameters were measured: partial AUC,  $C_{\text{max}}$ , and apparent  $t_{1/2}$ . MK-0966 was measured in plasma detection.

Results – The partial AUC values for 24 hours for the rat and dog following oral administration are provided below.

Dose [mg/kg]	AUC <sup>a</sup> [ng•hr/ml]	
	Rat	Dog
2	179 ± 53	4822 ± 2103
5	3001 ± 1078	7375 ± 1878
10	2963 ± 1540	11853 ± 4088

<sup>a</sup>Partial AUC value from 0-24 hours

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

1. Exposure increased as a function of dose following iv administration in both dogs and rats and following po administration in dogs.
2. Exposure increased as a function of dose following po administration between 2-5 mg/kg but not between 5-10 mg/kg in rats.

**Reviewer Comments:** The Reviewer concurs. There were differences in the AUCs seen in rats in these earlier pharmacokinetic studies compared to the toxicokinetic studies. This difference reflects a difference in the method of AUC determination. In the initial studies, partial AUCs<sub>0-24 hr</sub> were calculated. In the toxicokinetic studies, trapezoidal rule was used to calculate AUCs<sub>0-24 hr</sub>. Therefore, the latter values are higher.

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

**3.1.3. Study of the Non-Covalent Binding of L-748,731 with Plasma Proteins [Vol. 1.42; p. 309].**

The plasma protein binding values determined by equilibrium dialysis for 80 µM L-748,731 (25 µg/mL) are indicated in the following table (p. F-310).

Species	Percent Bound ± S.D.
Rat	85.3 ± 1.9
Rhesus	87.3 ± 1.6
Human	89.1 ± 1.3

**3.1.4. Study of the Potential for Covalent Binding between L-748,731 and Proteins [Vol. 1.42; p. F-297]** Incubations with <sup>14</sup>C-L-748,731 and microsomal proteins of the rat and dog did not indicate covalent binding due to reactive oxidative metabolites.

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

**3.2. Pharmacokinetic Studies - Absorption**

**3.2.1. The Effect of Food on the Absorption Kinetics of MK-0966 in Dogs Following Oral Administration [Vol. 1.45; p. G-377]**

Site: Merck Research Laboratories; West Point, PA  
Formulation and Lot No.: Test article - L-748,731-000R009  
Vehicle - 0.5% methylcellulose

Certificate of Analysis Submitted: No (X)  
Final Report (X) Oct. 26, 1997  
QA statements signed: Yes (X)

Study Design – Male Beagle dogs [N=4] were administered 5 mg/kg of L-748,731 po after fasting or 1 hour post feeding. The study was conducted in a crossover design with a 1-week washout period.

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**3.3.2 Distribution of MK-0966 and Its Metabolite L-755,190 in the Gastrointestinal Tract of the Rat One Hour After Oral Doses of [<sup>14</sup>C]MK-0966 or [<sup>14</sup>C]L-755,190 [Vol. 1,46: p. G-570]**  
 Site: Merck Research Laboratories; West Point, PA  
 Formulation and Lot No.: Test article [<sup>14</sup>C]L-748,731-002V002 and [<sup>14</sup>C]L-755,190-001K001 with <sup>14</sup>C in the 4-C position of the furanone ring [L-755,190 is the major metabolite of L-748,731 in the rat].

Vehicle – not identified

Certificate of Analysis Submitted: No (X)

Final Report (X) Oct. 15, 1997

QA statement signed: Yes (X)

**Study Design** – One hour after fasted male Sprague Dawley rats [N=1] were administered 5 mg/kg po of either MK-0966 or L-755,190, segments from the duodenum, ileum, jejunum, cecum and large intestine were collected. Total radioactivity in tissue and contents was determined by liquid scintillation counting.

**Results** – Approximately 43% [5% in tissue and 38% in contents] and 51% [11% in tissue and 40% in contents] of the total radioactivity of a dose of MK-0966 and L-755,190 were found in the gastrointestinal tract. The majority of the radioactivity was present in the jejunum and ileum. The levels of MK-0966, L-755,190, and the glucuronide of L-755,190 in the tissues were fairly comparable. MK-0966 was the predominant form in the intestinal contents.

**3.3.3. Distribution of MK-0966 and Its Metabolite L-755,190 in the Gastrointestinal Tract of the Rat 1, 4, and 6 Hours After Oral Doses of [<sup>14</sup>C]MK-0966 or [<sup>14</sup>C]L-755,190 at 5 mg/kg [Vol. 1,46: p. G-582]**

Site: Merck Research Laboratories; West Point, PA

Formulation and Lot No.: Test article [<sup>14</sup>C]L-748,731-002V007 and [<sup>14</sup>C]L-755,190-001K002 with <sup>14</sup>C in the 4-C position of the furanone ring

Vehicle – not identified

Certificate of Analysis Submitted: No (X)

Final Report (X) Oct. 19, 1997

QA statement signed: Yes (X)

**Study Design** – One, 4, and 6 hours after fasted male Sprague Dawley rats [N=2] were administered 5 mg/kg po of either MK-0966 or L-755,190, segments from the duodenum, ileum, jejunum, cecum and large intestine were collected. Distribution of radioactivity and drug [metabolites, parent] in tissue and contents was determined. Jejunum tissue homogenates and luminal contents from the 6-hour time point were incubated with  $\beta$ -glucuronidase.

**Results** - Following administration of radiolabeled MK-0966 and L-755,190, the percentage of the radioactivity administered remained fairly constant [approximately 60%] in the GI tract. At 1 hour, >95% of the radioactivity was associated with the jejunum and ileum and at 6 hours was distributed evenly throughout the ileum, jejunum, and cecum. Following administration of MK-0966, the parent was the predominant form in the jejunum and ileum, but decreased to barely perceptible levels by 6 hours. After 4 hours, the predominant component of the radioactivity was the glucuronide of L-755,190. The predominant component in the cecum, however, was parent compound. After administration of radiolabeled L-755,190, only L-755,190 and its glucuronide were detected in the ileum and jejunum at 1 and 4 hours. At 6 hours after dosing, MK-0966 was found in trace levels in the ileum but was the predominant form in the cecum. The Sponsor states that these data support the hypothesis that metabolic conversion of L-755,190 and/or its glucuronide to MK-0966 occurs in the lower GI tract beginning at the level of the cecum.

**3.3.4. CNS penetration of MK-966 [L-748,731] in the rat [Vol. 1,3: Reference C-139] – The results of this study were submitted as a Memo. It is identified as DMPK Report No. 97015**

laboratory was not GLP certified.

The

**Study Design** - Fasted male Sprague-Dawley [N=3/timepoint] were administered L-748,731-000R002 in 0.5% methylcellulose po at 5 and 50 mg/kg. At 2, 5, 10, 15, 30, and 120 minutes post dosing [5 mg/kg only], rats were anesthetized and a blood sample and the brain were collected. At 2, 4, 12, and 24 hours post dosing [5 and 50 mg/kg], rats were anesthetized and a blood and CSF sample, and the brain were collected.

**Results** - Equilibrium between plasma and blood was reached within 5 minutes with a brain:plasma ratio of approximately 0.4. The  $T_{max}$  in the plasma, brain, and CSF was 4 hours.  $C_{max}$  was  $454 \pm 85$  ng/ml,  $160 \pm 30$  ng/g, and  $35 \pm 7$  ng/ml in the plasma, brain, and CSF, respectively. Elimination profile in the brain and CSF was similar to plasma elimination. By 24 hours, the brain concentration of L-748,731 was  $9.6 \pm 1.9$  ng/g.

### 3. 4. Pharmacokinetic Studies - Excretion

#### 3.4.1. Biliary Excretion of MK-0966 in Rats Following Administration of [ $^{14}$ C]MK-0966 at 2 mg/kg/ I.V. or 5 mg/kg/P.O. and Identification of Two Biliary Metabolites [Vol. 1.45; p. G-330]

Site: Merck Research Laboratories; West Point, PA

Formulation and Lot No.: Test article - [ $^{14}$ C]L-748,731-002V001 with  $^{14}$ C incorporated at the C-4 position of the furanone ring

Vehicle - DMSO for iv administration  
- PEG-400 for po administration

Certificate of Analysis Submitted: No (X)

Final Report (X) Nov. 1, 1997

QA statements signed: Yes (X)

**Study Design** - Bile-cannulated, fasted, male Sprague-Dawley rats [N=3] were administered radiolabeled MK-0966 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. [redacted] conducted on bile samples. Metabolites were identified by [redacted] analysis in 1 rat. Samples were evaluated [redacted] and NMR both before and after  $\beta$ -glucuronidase treatment.

**Results** - Radioactivity recovery in the bile ranged from 37-94% and 58-76% following the iv and po doses, respectively. Radioactivity recovery in the urine ranged from 2.8-4.3% and 2.6-7.3% 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  $\beta$ -glucuronidase resulted in hydrolysis of the major metabolite [ $\beta$ -O-glucuronide conjugate of L-755,190] to the form the minor metabolite [L-755,190, the 5-OH furanone metabolite of MK-0966].

#### 3.4.2. 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]MK-0966 [Vol. 1.45; p. G-388]

Site: Merck Research Laboratories; West Point, PA

Formulation and Lot No.: Test article - [ $^{14}$ C]L-748,731-002V008 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 of Analysis Submitted: No (X)

Final Report (X) March 12, 1998

QA statements signed: Yes (X)

**Study Design** - Fasted, male Sprague Dawley rats, with or without bile cannulation, [N=4] were administered 2 or 5 mg/kg of L-748,731 via the iv or po routes, respectively. Bile and blood were collected

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at 0, 0.083 [blood only], 0.25, 0.5, 1, 2, 4, 6, 8, 10, and 24 hours. MK-0966 and L-755,190 concentrations were analyzed

**Results** – Following iv administration in intact rats, the level of MK-0966 declined following  $T_{max}$  and then reached a second peak at 10 hours. Following iv administration in intact rats, the level of MK-0966 decreased following  $T_{max}$  to a plateau 6-10 hours post dosing. Following iv and oral administration of drug in cannulated rats, parent and metabolite underwent a steady decrease over 6 and 10 hours, respectively. Regardless of dosing regimen, there was a rapid formation of L-755,190. The kinetics of this metabolite were similar to the parent compound. The interruption of enterohepatic recirculation following bile cannulation resulted in AUC levels that were less than in the intact animals for both parent [approximately 20% either route] and metabolite [approximately 70-80%]. The decrease in exposure to parent compound was not statistically significant. The table below compares pharmacokinetic parameters in the cannulated vs. intact rats.

	Intravenous Route <sup>a</sup>		Oral Route <sup>a</sup>	
	Intact	Cannulated	Intact	Cannulated
MK-0966				
Partial AUC - ng•hr/ml <sup>b</sup>	2367 ± 351	1966 ± 173	7965 ± 1901	6385 ± 2749
L-755,190				
Partial AUC - ng•hr/ml	675 ± 95.8	120 ± 9.61 <sup>c</sup>	905 ± 69.2	314 ± 51.6

<sup>a</sup>2 mg/kg for iv, 5 mg/kg for po

<sup>b</sup>0-24 hour interval for intact, 0-10 hour interval for cannulated

<sup>c</sup>value represents 0-6 hour interval

**Note:** The Sponsor did not provide individual animal data. There appears to be considerable variability in the MK-0966 concentrations in the rats, either intact or cannulated, following oral administration.

**Conclusion:** Enterohepatic recirculation plays a role in the pharmacokinetics of L-748,731. The systemic exposure to L-755,190 was significantly decreased in the cannulated animals.

### 3.4.3. Biliary Excretion in Dogs Following Administration of [<sup>14</sup>C]L-748,731 at 2mg/kg I.V. or 5 mg/kg P.O. [Vol. 1.45; p. G-362]

Site: Merck Research Laboratories; West Point, PA

Formulation and Lot No.: Test article - [<sup>14</sup>C]L-748,731-002V001, 002, and 008 and 3 with <sup>14</sup>C incorporated at the C-4 position of the furanone ring

Vehicle – DMSO for iv administration

- 0.5% methylcellulose for po administration

Certificate of Analysis Submitted: No (X)

Final Report (X) Nov. 11, 1997

QA statements signed: Yes (X)

**Study Design** – Bile-cannulated, fasted, male Beagle dogs [N=1] were administered radiolabeled L-748,731 at 2 mg/kg iv or 5 mg/kg po. Bile was collected every hour for the first 8 hours and then for the 8-24 hour time period. Urine was collected for 0-8 and 8-24-hour intervals. Pooled samples from an earlier study [N=4] were also analyzed to better define the metabolic profile. Total radioactivity in the plasma, bile, and urine was determined by [redacted] was conducted on bile and urine samples. Pooled samples were evaluated by [redacted]

**Results** – Radioactivity recovery in the bile was 28.2% and 26.5% following the iv and po doses, respectively. Radioactivity recovery in the urine was 32.0% and 16.8% following the iv and po doses, respectively. The major metabolite in bile following either iv or oral administration was L-755,190 which constituted approximately 29% and 36-50%, respectively. In the bile following iv administration, there were also 5 more polar, less abundant [redacted] including the glucuronide of L-755,190. There were a total of [redacted] in the urine sample following iv administration, but the [redacted]

Both the glucuronide of L-755,190 and the [redacted]

*trans*-dihydro MK-0966 [L-781,449] were identified in urine following oral administration of MK-0966. Parent compound was not demonstrated. Due to incomplete recovery of radioactivity, the pooled sample was not fully analyzed.

### 3.5. Pharmacokinetics - Metabolism

#### 3.5.1 *In Vitro* Studies

##### 3.5.1.i Microsomal Studies

a) Study of the Oxidative (Phase I) Biotransformations [Vol. 1.42; p. F-293]. - The following table shows the amount of L-748,731 metabolism observed in a one hour incubation with liver microsomal protein. Incubations were in duplicate [Table 1, p. F-295]

Species	Hydroxylation (%)	nmol/mg Protein/hr	Recovery (%)
Rat	5.9	11.8	96.5
Rhesus	3.2	6.4	98.0
Dog	< 0.5	< 1.0	98.0
Human	1.2	2.4	97.0
Human	0.6	1.2	101.0

Metabolite L-755,190 was the only oxidative metabolite detected. The OH group was on the lactone ring.

##### b) Identification of L-755,190 as the Major Metabolite of MK-0966 in Incubations with Liver Microsomal Preparations From Phenobarbital-Pretreated Rats [Vol. 1.46; p. G-806]

Site: Merck Research Laboratories; West Point, PA

Formulation and Lot No.: Test article - [<sup>14</sup>C]L-748,731-002V001 with <sup>14</sup>C in the 4-C position of the furanone ring

Vehicle - DMSO

Certificate Analysis: No (X)

Final Report (X) Nov. 4, 1997

QA statement signed: Yes (X)

Study Design - Microsomal preparations from phenobarbital-pretreated rats were incubated with 100 μM of radiolabeled MK-0966. Metabolite profile was evaluated [redacted] were isolated and collected by [redacted] methods and the structure characterized [redacted]

Results - Two [redacted] and identified as MK-0966 and L-755,190.

##### c) The Cytochrome P-450 Mediated Oxidation of MK-0966 to L-755,190 by Human Liver Microsomes [Vol. 1.46; p. G-851]

Site: Merck Research Laboratories; West Point, PA

Formulation and Lot No.: Test article - L-748,731-000R009

Vehicle - DMSO

Certificate Analysis: No (X)

Final Report (X) Oct. 10, 1997

QA statement signed: Yes (X)

Study Design - Either pooled individual human liver microsomal preparations or microsomes containing cDNA expressed CYP proteins [CYP1A2, 3A4, 2C19, D6, C9, and EI] were incubated with MK-0966 [10-80 μM] ± inhibitors. The metabolite profile was determined [redacted]

Results - NADPH-dependent oxidation by human liver microsomes has a high K<sub>m</sub> approximated at >>80 μM. At 10 μM, CYP1A2 and CYP3A4 appear to contribute equally to metabolism but at 60 μM,

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metabolism by CYP3A4 predominates. The rank order for metabolism, based on cDNA expressed CYP proteins is: CYP1A2  $\geq$  3A4 > 2C19  $\geq$  D6 > 2C9  $\approx$  2E1. MK-0966 had a weak ability to inhibit CYP isozyme specific inhibitors.

d) Study of L-748,731 on Human Cytochrome P450 3A4 Metabolizing Enzyme [Vol. 1.42: p. F-306]. - Human liver microsome preparation containing a high level of P450 3A4 was incubated with L-748,731 under oxidative conditions to determine if inhibition of 6 $\beta$ -hydroxylation of testosterone occurred. CYP 3A enzymes are, in general, responsible for the 6 $\beta$ -hydroxylation of steroids. The results of the experiment indicated L-748,731 did not show any significant inhibition at concentrations up to about 100  $\mu$ M. At 200  $\mu$ M a 15% to 20% inhibition was seen. Troleandomycin [ $\approx$ 0.4 – 8  $\mu$ M] and erythromycin [ $\approx$  20-1000  $\mu$ M] on the other hand, produced dose-related inhibitions of CYP 3A4, with troleandomycin being the more potent inhibitor.

e) Study of the Phase II Hepatic Microsome Metabolism of the Hydroxylated Metabolite of L-748,731 - Conjugation of Glucuronic Acid [Vol. 1.42: p. F-299] - Incubation of the metabolite, L-755,190, with rat hepatic microsomes under glucuronidation conditions, i.e., in the presence of UDPGA and the appropriate cofactors, resulted in the formation of two glucuronic acid adducts. These were believed to be the two possible enantiomers.

### 3.5.1.ii Cytosol Studies

#### a) Reductive Metabolism of MK-0966 by Human Liver Cytosol [Vol. 1.46: p. G-827]

Site: Merck Research Laboratories; West Point, PA

Formulation and Lot No.: Test article - L-748,731-000R009

Vehicle - DMSO

Certificate Analysis: No (X)

Final Report (X) Nov. 4, 1997

QA statement signed: Yes (X)

Study Design- Pooled cytosolic preparation from 9 human donors was incubated with 100  $\mu$ M of MK-0966  $\pm$  NADPH. The intermediate generated by incubation in the absence of redox cofactors was incubated  $\pm$  NADPH. The effects of dicoumarol [NADPH:quinone reductase inhibitor] and quercetin [carbonyl reductase inhibitor] on the metabolism of the intermediate were evaluated. The supernatant was analyzed by

Results - Cytosolic metabolism of MK-0966 was NADPH-dependent. In the presence of NADPH, the *cis* and *trans*-dihydrohydroxyacid [DHHA] was formed. In the absence of NADP, a dihydro analog of L755,190 was generated. The dihydrolactones appear to be artifacts of acidification of the sample. Total reduction of the dihydro-analog was largely unaffected in the presence of dicoumarol. However, the relative distribution of *cis* vs. *trans* DHHA was shifted. Quercetin had no effect.

### 3.5.1.iii. Subcellular Fraction Studies

#### a) The Metabolism of MK-0966 by Liver Subcellular Fractions From Human, Monkey, Dog and Rat [Vol. 1.46: p. G-816]

Site: Merck Research Laboratories; West Point, PA

Formulation and Lot No.: Test article - L-748,731-000R009

Vehicle - DMSO

Certificate Analysis: No (X)

Final Report (X) Feb. 4, 1998

QA statement signed: Yes (X)