Study Design – Liver S9 fractions and cytosol from human [7 and 9 pooled donors, respectively], non-induced rats, dogs, and rhesus monkeys were incubated with 100 μM of MK-0966 for 4 or 24 hours. Supernatants were analyzed for metabolites[redacted]

Results – Results indicate that the metabolism of MK-0966 *in vitro* is qualitatively similar but quantitatively dissimilar for the species evaluated. Whereas the oxidative pathway is important in the rat, the reductive pathways were predominant in humans. The table below provides a comparison of the in *vitro* metabolic profile. Values are expressed as % of initial substrate.

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

b) Non-Cytochrome P-450-Mediated Oxidation of MK-0966 to L-755,190 by Hepatic Subcellular Fractions [Vol. 1.46; p. G-870]
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. 2, 1998
QA statement signed: Yes (X)

Study Design – Liver microsomes ± cytosol from humans [pooled from 9 donors, rhesus monkeys, male Beagle dogs, and non-induced, male, Sprague-Dawley rats were incubated with 10 μM of MK-0966 in the presence of an NADPH generating system containing either NAD^+ or $NADP^+$. Supernatants were analyzed for metabolites[redacted]

Results- The data indicated that there is an NAD^+-supported oxidative pathway generating L-755,190 from MK-0966 that involves a 2-step process. This 2-step process encompasses reactions in both the cytosol and the S9 fractions. The NAD^+-supported activity was approximately 60% of the NADP-dependent oxidative metabolism. The rank order for the NAD^+-supported oxidation is rat >> human ≈ monkeys >> dogs.

c) Kinetics of the In Vitro Metabolism of MK-0966 by Human Liver S9 and Cytosolic Fractions [Vol. 1.46; p. G-881]
Site: Merck Research Laboratories; West Point, PA
Formulation and Lot No.: Test article - L-748,731-000R009 and L-755,190-000H007
Vehicle - DMSO
Certificate Analysis: No (X)
Final Report (X) Nov. 21, 1997
QA statement signed: Yes (X)

Study Design – Human liver S9 fractions [pooled from 7 donors] or cytosol [pooled from 9 donors} was incubated with redox factors plus MK-0966 or L-755,190 [5-60 μM] to determine the kinetic parameters of *in vitro* metabolism in this system.

Results – Metabolism of MK-0966 by human liver *in vitro* had a relatively high apparent K_m of ≥90 μM. Oxidative metabolism of MK-0966 to L-755,190 predominates at the early time points compared to reductive metabolism. Reductive metabolism is predominant at the later time points. The Sponsor suggests that this may be in part a function of "back-reduction" of L-755,190 to the parent compound.

3.5.1.iv Hepatocyte Preparation Studies

a) Study of the Biotransformation of L-748,731 by Rat Hepatocytes [Vol. 1.42; p. F-300] - L-748,731 was incubated with Sprague-Dawley rat hepatocytes. The hydroxylated metabolite and its two glucuronide stereoisomers were detected, increasing with time. These results would also indicate no protein binding to hepatocyte protein was occurring.

b) Effects of L-748,731 on Cytochrome P450 3A Protein Levels in Rat Hepatocytes [Vol. 1.42; p. F-303] - Sprague-Dawley hepatocyte cytochrome P450 3A was induced in a dose related manner by L-748,731, with maximum induction occurring at 50 μM. This induction was not as great as was observed with 10 μM dexamethasone, the positive control. No induction was observed with the metabolite of L-748,731, i.e., L-755,190.

3.5.2. *In Vivo* Studies

3.5.2.i. Mechanistic Studies on the Metabolism of MK-0966 in the Rat. Investigations with Oxygen-18-Labeled Probes [Vol. 1.45; G-237]
Site: Merck Research Laboratories; West Point, PA
Formulation and Lot No. : Test article [$^{18}O_2$]L-748,731-003X001 with ^{18}O at both the ring and carbonyl oxygens of the furanone ring and [$^{18}O_1$]L-755,190-002M001with ^{18}O at the 5-OH substituent.

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

Certificate Analysis: No (X)
Final Report (X) March 2, 1998
GLP and QA statements signed: No (X)
Objective: To better understand [1] the metabolism of MK-0966 *in vivo* and [1] the reversible metabolism of L-755, 190 in following iv administration of MK-0966 in rats.

Study Design – Fasted male Sprague-Dawley rats were administered either [$^{18}O_2$]L-748,731or [$^{18}O_1$]L-755,190 either 4 mg/kg iv [N=1 or 3 for MK-0966 or L-755,190, respectively] or 10 mg/kg po [N=2 or 3 for MK-0966 or L-755,190, respectively. The metabolism of these radiolabeled compounds in the plasma [e.g. fate of the labeled oxygen] was determined as a function of time by analysis. Blood was collected for 24-48 hours. Total concentrations of MK-0966 and L-755, 190 was determined by

Results – The Sponsor states that the data from these analyses indicated the following. [1] MK-0966 undergoes reversible metabolism to L-755, 190 by a pathway that involves furanone ring opening of the lactone moiety by direct hydrolysis. [2] L-755, 190 appears to reversion of MK-0966 by "reductive metabolism of the acyclic aldehyde tuatomer of L-755,190 followed by dehydration and ring closure".

3.5.2.ii. Exploratory Enzyme Induction Study in Rats of L-748,731, a COX-2 Inhibitor submitted by D. Patrick [Vol. 1.21; B-4936 and Vol. 1.49; p. Q-63]
Study Identification: TT #93-281-0,-2; Gene-Tox TT #93-8980
Site: Not provided
Study Dates [In-Life]: Not provided
Formulation and Lot No.: Test article - L-748,731-000R
Vehicle - 0.5% aqueous methylcellulose
Certificate Analysis: No (X)
Memo (X) Dec. 9, 1993

GLP and QA statements signed: No (X)
Objective: "To determine the P-450 mediated 7-ethoxy-4-trifluoromethylcoumarin O-demethylase [EFCOD] and peroxisomal FACO activities in rats administered L-748,731 by oral gavage"

Test Material/ Group Designation	Dose*				Sex	N#	Species/Strain
	mg/kg	ml/kg	Route	# days dosed			
Control 1 - neg.	-	not provided	oral, gavage	4 SID	M/ F	4	Sprague Dawley Rats 100-200 g
Control 2 - positive phenobarbital	50						
bezafibrate	50						
Group 3 - L-748,731	400						

Livers were harvested and weighed approximately 24 hours after the last dose on Day 5. Microsomal preparations were made and EFCOD activity was determined fluorescently and FACO activity determined spectrophotometrically.

Liver weights – There was a 52% and 18% increase in liver weights in males and females, respectively, following administration of the positive control. Liver weights in L-748,731 treated rats were not significantly affected.

EFCOD activity – EFCOD activity was increased by 692% and 364% in males and females, respectively following administration of the positive control. EFCOD activity was decreased by 27% and 57% in males and females, respectively, following administration of L-748,731.

FACO activity – FACO was increased by 605% and 16% in males and females, respectively, following administration of the positive control. FACO activity was not significantly altered in males and females following administration of L-748,731.

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

1. Neither EFCOD nor FACO were induced in male and female Sprague-Dawley rats following administration of L-748,731 "under this dosing regimen". It was suggested that the decrease in EFCOD was secondary to "other factors". Based on Western blot data not shown, the Sponsor states that there was a mild induction in CYP2B and CYP3a. **Reviewer's Comment** - Based on the data provided, the Reviewer concurs.

3.5.2.iii. Exploratory Enzyme Induction Studies in Mice of L-748,731, a COX 2 Inhibitor: Memo Submitted by D. Patrick. [Vol. 1.49; p. Q-57]
Study Identification: TT #93-283-0,-5; Gene-Tox TT #93-8988
Site: Not provided
Study Dates [In-Life]: Not provided
Formulation and Lot No.: Not Provided
Certificate Analysis: No (X)
Memo (X) Dec. 10, 1993
GLP and QA statements signed: No (X)
Objective: "To determine the P-450 mediated 7-ethoxy-4-trifluoromethylcoumarin O-demethylase [EFCOD] and peroxisomal FACO activities in mice administered L-748,731 by oral gavage"

Test Material/ Group Designation	Dose*				Sex	N#	Species/Strain
	mg/kg	ml/kg	Route	# days dosed			
Control 1 - neg.	-	10	oral, gavage	4 SID	M/F	4	CD-1 mice 20-30 g
Control 2 - positive phenobarbital	75						
bezafibrate	75						
Group 3 - L-748,731	400						

Livers were harvested and weighed approximately 24 hours after the last dose on Day 5. Microsomal preparations were made and EFCOD activity was determined fluorescently and FACO activity determined

Liver weights – The positive controls increased liver weights by approximately 45% in both males and females. There was no treatment-related effect associated L-748,731 treatment [e.g. weights were increased by approximately 4%].

EFCOD activity – EFCOD activity was increased by 287% and 235% in males and females, respectively following administration of the positive control. There was no treatment-related effect with L-748,731.

FACO activity – FACO was increased by 209% and 226% in males and females, respectively, following administration of the positive control. There was no treatment-related effect with L-748,731.

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

1. Neither EFCOD nor FACO were induced in male and female CD-1 mice following administration of L-748,731 "under this dosing regimen". Based on Western blot data not shown, the Sponsor states that there was a moderate induction in CYP2B and CYP3A. **Reviewer's Comment** - Based on the data provided, the Reviewer concurs.

3.5.2.iv. Exploratory Enzyme Induction Study in Rats of L-748,731 a Cox 2 Inhibitor; Memo submitted by M. Kloss [Vol. 1.49; p. Q-70]
Study Identification: TT #94-253-0,-2; Gene-Tox TT #94-8905
Site: Not provided
Study Dates [In-Life]: Not provided
Formulation and Lot No.: Not Provided
Certificate Analysis: No (X)
Memo (X) Nov. 14, 1994
GLP and QA statements signed: No (X)
Objective: "To determine the P-450 mediated 7-ethoxy-4-trifluoromethylcoumarin O-demethylase [EFCOD] activity in rats administered L-748,731 by oral gavage"

Test Material/ Group Designation	Dose*				Sex	N#	Species/Strain
	mg/kg	ml/kg	Route	# days dosed			
Control 1 - neg.	-	5	oral, gavage	4 SID	M/F	4	Sprague-Dawley rats 100-200 g
Control 2 - positive phenobarbital	50						
Group 3 - L-748,731	3						
Group 4 - L-748,731	10						

Livers were harvested and weighed approximately 24 hours after the last dose on Day 5. Microsomal preparations were made and EFCOD activity was determined fluorescently.

Liver weights – The positive control increased liver weights by approximately 15-20% in both males and females. There was no treatment-related effect associated L-748,731 treatment [e.g. weights were increased by approximately 4%].

EFCOD activity – EFCOD activity was increased by 618% and 552% in males and females, respectively following administration of the positive control. EFCOD was decreased in a dose-dependent fashion at both doses of L-748,731. The maximum decrease at 10 mg/kg/day was 45-50% in males and females.

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

1. EFCOD was decreased in male and female Sprague-Dawley rats following administration of L-748,731. A-NOEL was not determined. Western blot analysis [data not shown] indicated, according to the Sponsor, that there was a slight induction in CYP3A in females at 10 mg/kg/day. **Reviewer's Comment** - Based on the data provided, the Reviewer concurs.

3.5.2.v. Western and Northern Blots from Exploratory Enzyme Induction Study in Mice of L-748,731 [Vol. 1.51; p. Q-1009]
Study Identification: TT #93-283-0, -5
Site: Not provided
Study Dates [In-Life]: Not provided
Formulation and Lot No.: Not Provided
Certificate Analysis: No (X)
Memo (X) Jan. 30, 1995
GLP and QA statements signed: No (X)
Objective: To determine levels of P450 2B and P450 3A by Western and Northern blots in CD-1 mice administered L-748,731.

Male and female CD-1 mice were administered L-748,731 at 400 mg/kg/day X 4 days. Liver microsomes were prepared. Phenobarbital and bezafibrate served as positive controls. P450 3A mRNA was not assessed due to lack of reactivity with the oligonucleotide probe.

Results – P450 3A protein was increased by 1.8X in males but not in females. P450 2B protein was increased 10X in males and marginally in females. P450 2B RNA was increased by 2.5X in males but not in females.

Sponsor's Conclusions

1. "The quantitative difference in induction between males and females is derived from the lower level of basal P450 3A and 2B proteins in male mice since the P450 3A and 2B proteins in the microsomes from the positive control groups and the L-748,731 group reached the same levels in both males and females".

2. The biological significance of the minor effects in males is not known.

3.5.2.vi. Western and Northern Blots from Exploratory Enzyme Induction Study in Rats of L-748,731 [Vol. 1.51; p. Q-1020]
Study Identification: TT #93-281-0, -2
Site: Not provided
Study Dates [In-Life]: Not provided
Formulation and Lot No.: Not Provided
Certificate Analysis: No (X)
Memo (X) Jan. 30, 1995

GLP and QA statements signed: No (X)
Objective: To determine levels of P450 2B and P450 3A by Western and Northern blots in Sprague-Dawley rats administered L-748,731.

Male and female Sprague-Dawley rats mice were administered L-748,731 at 400 mg/kg/day X 4 days. Liver microsomes were prepared. Phenobarbital and bezafibrate served as positive controls. Western blots were conducted in females only since they were considered more sensitive to P450 changes.

Results – P450 3A protein was increased marginally in males and 19X in females. P450 2B protein was increased 7.5X in males and 14X in females. P450 2B RNA was increased by 3.5X in females and the P450 3A was increase by 3X in 1/2 females.

Sponsor's Conclusions

1. The biological significance of the minor effects in males is not known.

3.5.2.vii. In Situ Metabolism of [^{14}C]L-755,190, a Metabolite of MK-0966, in the Rat Isolated Intestinal Segment Model [Vol. 1.46; p. G-773]
Site: Merck Research Laboratories; West Point, PA
Formulation and Lot No.: Test article - [^{14}C]L-755,190-001K001 with ^{14}C in the 4-C position of the furanone ring
Certificate Analysis: No (X)
Final Report (X) Oct. 12, 1997
QA statement signed: Yes (X)

Study Design – A dose of 200 µg of radiolabeled L-755,190 was injected into the lumen of a perfused, isolated jejunal segment in anesthetized, male Sprague-Dawley rats [N=2]. Mesenteric blood was collected every 5 minutes for 1 hour. The jejunal segment was excised 1 hour after dosing and the contents and wash collected. Total radioactivity was determined in all samples by [redacted] Analysis for the presence of L-755,190 and MK-0966 in the plasma was conducted by [redacted] Metabolite profile in the intestinal segment and contents was evaluated by [redacted]

Results- A mean of 86.5% of total radioactive dose was recovered in the blood, GI segment, and lumenal contents. Radiolabeled L-755,190 was absorbed rapidly [within 5 minutes] into the mesenteric blood. There was rapid formation of MK-0966 that reached a plateau of 1% of the dose. It should be noted that analysis of the L-755,190 preparation revealed an impurity [app. 0.3%] which may have been trace amounts of radiolabeled MK-0966. Only unchanged L-755,190 was detected in the jejunal homogenate and segment contents.

3.5.2.viii. Isolation and Identification of L-755,190 and Its Glucuronide Conjugate in the Urine of Rats Dosed Intravenously with [^{14}C]MK-0966 at 2 mg/kg [Vol. 1.46; G-622]
Site: Merck Research Laboratories; West Point, PA
Formulation and Lot No.: Test article with [^{14}C]L-748,731-002V002 ^{14}C in the 4-C position of the furanone ring
Vehicle – DMSO
Certificate Analysis: No (X)
Final Report (X) Nov. 5, 1997
QA statement signed: Yes (X)

Study Design – Fasted, male, Sprague-Dawley rats [N=3] were dosed with 2 mg/kg L-748,731 and urine collected over 24 hours. Total radioactivity in the urine was determined by [redacted] Urine was pooled and metabolite identification was attempted following [redacted]

Results – Total radioactivity recovered in the urine ranged from 17.9 – 22.5%. One minor and 3 major metabolites were identified. Of the major metabolites 1 was not identified but the other 2 were L-755,190 and its glucuronide. The minor metabolite was not identified.

3.5.2.ix. Metabolite Profiles of Urine From Mice and Rats Following Oral Administration of [^{14}C]MK-0966 at 100 mg/kg [Vol. 1.46; p. G-638]
Site: Merck Research Laboratories; West Point, PA
Formulation and Lot No.: Test article with [^{14}C]L-748,731-002V007 ^{14}C in the 4-C position of the furanone ring

Vehicle – 0.5% methylcellulose
Certificate Analysis: No (X)
Final Report (X) Dec. 2, 1997
QA statement signed: Yes (X)

Study Design – Nonfasted, female, CRL:CD-1 mice [N=4 or 5/group] and nonfasted, male, Sprague-Dawley rats [N=4] were administered 100 mg/kg of radiolabeled MK-0966 and urine was collected over 24 hours. Samples were pooled for mice. Total radioactivity in the urine was determined Rat urine was evaluated

Results – Total radioactivity recovered in the urine of mice and rats was 9% and 3.6-8.3% of the dose, respectively. The following metabolites were identified: [1] glucuronide of L-755,190 - 80% and 34% of total urine radioactivity in mice and rats; [2] dihydro metabolite - <1% of the *trans* and 3% and 4% of the *cis* and *trans* in mice and rats; [3] L-755,190 – 3-4% in both species; [4] MK-0966 – ≤3-4% in the rat; and [5] 2 unidentified 3-4% in the rat.

3.5.2.x. Identification of the 3',4'-Dihydrodiol and 4'-Phenol Sulfate Metabolites of MK-0966 in Urine of Rats Dosed Orally with MK-0966 (100 mg/kg) [Vol. 1.46; p. G-652]
Site: Merck Research Laboratories; West Point, PA
Formulation and Lot No.: Test article - L-748,731-000R009

Vehicle – 0.5% methylcellulose
Certificate Analysis: No (X)
Final Report (X) Dec. 6, 1997
QA statement signed: Yes (X)

Study Design – Male Sprague-Dawley rats [N=3] were administered MK-0966 at 100 mg/kg po and urine was collected over 24 hours. The metabolic profile was analyzed Two unidentified were collected and pooled, further purified, and analyzed

Results – The initial were considered to represent MK-0966, L-755,190 and its glucuronide. The two analyzed by were identified as 3', 4'-*trans* dihydrodiol and the 4' phenol glucuronide. The was not characterized since the Sponsor considered it to be unrelated to the drug.

3.5.2.xi. Metabolite Profiles of Plasma and Urine From Mice Following Oral Administration of [^{14}C]MK-0966 at 5 mg/kg/ and of Plasma from Rats After Intravenous (2mg/kg/) or Oral (5 mg/kg) Administration of [^{14}C]MK-0966 [Vol. 1.46; p. G-761]
Site: Merck Research Laboratories; West Point, PA
Formulation and Lot No.: Test article - [^{14}C]L-748,731-002V002 ^{14}C in the 4-C position of the furanone ring

Vehicle – DMSO for iv administration
- 0.5% methylcellulose for oral administration
Certificate Analysis: No (X)
Final Report (X) Dec. 2, 1997
QA statement signed: Yes (X)

Study Design – Nonfasted, male, Crl:CD (ICR)BR mice [N=5] were administered by oral gavage 5 mg/kg of radiolabeled MK-0966. Urine samples were collected from 1 group over a 24-hour period. Blood samples were collected at 30 and 60 minutes in 2 and 1 group[s], respectively. Urine and plasma samples were pooled. Nonfasted, male, Sprague-Dawley rats [N=3] were administered radiolabeled MK-0966 at 2 or 5 mg/kg iv or po, respectively. Blood samples were collected after 1 hour. Total radioactivity of the samples was determined Metabolite profiles were analyzed by

Results - Total radioactivity in the urine sample was 12.6% of the radioactive dose. The major metabolite [49% of the total radioactivity] was identified as L-755,190. Four unidentified constituted ≤14% of the total radioactivity. L-755,190 was also the major metabolite [60-70%] in both the mouse and the rat. There were 2-3 that were not characterized.

3.5.2.xii. Isolation and Identification of *trans*-Dihydro-MK-0966 Lactone, a Metabolite of MK-0966, From Urine of Dogs Following Intravenous Administration of [^{14}C]MK-0966 at 2 mg/kg.

Site: Merck Research Laboratories; West Point, PA

Formulation and Lot No.: Test article - [^{14}C]L-748,731-002V008 with 002V002 ^{14}C in the 4-C position of the furanone ring

Vehicle – DMSO

Certificate Analysis: No (X)
Final Report (X) Nov. 12, 1997
QA statement signed: Yes (X)

Study Design – Fasted, male, Beagle dogs [N=2] were administered radiolabeled MK-0966 iv at 2 mg/kg. Urine was collected at 0-8 and 8-24 intervals. Total radioactivity in urine was determined and metabolites evaluated A new identified in acid treated urine was isolated and characterized

Results – The mean total radioactivity in urine collected over 24 hours was 44.8% of the dose. The peak that was observed in 3/4 urine samples following treatment was the *trans*-dihydro-MK-0966. This metabolite constituted 14-22% of the total radioactivity.

3.6 Pharmacokinetic Studies - Studies to Assess Potential Drug-Drug Interaction

3.6.1. An In Vitro Plasma Protein Binding Displacement Interaction Study With [^{14}C]MK-0966 and [^{14}C]Salicylic Acid [Vol. 1.46; G-604]

Site: Merck Research Laboratories; West Point, PA

Formulation and Lot No.: Test article with [^{14}C]L-748,731-002V008 ^{14}C in the 4-C position of the furanone ring

Vehicle – DMSO

Certificate Analysis: No (X)
Final Report (X) Dec. 15, 1997
QA statement signed: Yes (X)

Study Design – Plasma protein binding of MK-0966 and salicylic acid was determined by an Human plasma was incubated with 0.5, 0.1, and 2 µg/ml of radiolabeled MK-0966 or 13.8, 27.6, and 69.1 µg/ml of radiolabeled salicylic acid. To determine displacement potential unlabeled salicylic acid [27.6 µg/ml] or unlabeled MK-0966 [1 µg/ml] was added to the tubes of plasma plus labeled MK-0966 or salicylic acid, respectively.

Results – Protein binding was comparable for both compounds [approximately 90%] and was independent of concentration within the range used. There was no displacement of labeled drug under the conditions of this experiment. The Sponsor does not provide a rationale for the selected concentrations.

3.6.2. Effects of Cimetidine and Ketoconazole on the In Vitro Metabolism of MK-0966 by Human Liver Microsomes, S9 Fractions and Cytosol [Vol. 1.46; G-901]
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. 13, 1997
QA statement signed: Yes (X)

Study Design – Various combinations of human liver subcellular fractions [microsomes, S9, and cytosol] were incubated with 0.1 mM of MK-0966, various redox cofactors ± inhibitors [ketoconazole or cimetidine at 0.1 – 100 μM]. Supernatants were analyzed [redacted]

Results – Total metabolism, including both oxidative and reductive pathways, were unaffected by incubation with either inhibitor. Ketoconazole, however, inhibited oxidative metabolism by up to 80% with an IC_{50} <0.1 μM. Approximately 20% of the NAD^+-supported oxidation was resistant to ketoconazole inhibition. Ketoconazole increased the net accumulation of reductive metabolites by approximately 34%. Cimetidine was a weak inhibitor of oxidative metabolism with an IC_{50} >100 μM.

3.6.3. Evaluation of MK-0966 as an Inhibitor of Human Liver Microsomal Cytochrome P-450 Activity [Vol. 1.46; p. G-913]
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) Sept. 22, 1997
QA statement signed: Yes (X)

Study Design – Human liver microsomes [pooled] or cDNA expressed CYP3A4 were incubated with marker substrate and either MK-0966 [0.1, 1, 10, and 100 μM], a positive control, or a VH control. The marker substrates included phenacetin *O*-demethylase [CYP1A2], diclofenac 4'-dehydroxylase [CYP2C9], (S)-(+)-mephenytoin 4'-hydroxylase [CYP2C19], bufuralol 1'-hydroxylase [CYP2D6], and testosterone 6β-hydroxylase or midazolam 1'-hydroxylase [CYP3A4/5]. [redacted] were analyzed by [redacted]

Results – MK-0966 was a weak inhibitor of the CYP activity evaluated with an IC_{50} >100 μM. Inhibition was ≤40%.

3.6.4. Characterization of P-450 Activities in Microsomal Samples Using Testosterone Hydroxylation Assay; Memo from S.J. Grossman [Vol. 1. 51; p. Q-1014]
Study Identification: TT #93-281-0,-2
Site: Not provided
Formulation and Lot No.: Not provided
Final Report (X) Nov. 22, 1994
Objective: "To characterize the P-450 enzymes that were present in liver microsomal samples from rats treated with L-748,731."

Study Design – "The pattern of testosterone metabolism formed after microsomal incubations serves as a 'fingerprint of the distribution of P-450 activities present in a microsomal sample." Liver microsomes from rats [species not indicated] administered L-748,731 were incubated with testosterone.

Results – The rates of formation for 15-BHT, 2-BHT, 6-BHT, and 16-BHT were increased

Sponsor's Conclusion – This pattern suggests a small increase in CYP 3A and CYP 2B.

3.7 TOXICOKINETIC STUDIES – ACUTE STUDIES IN MICE

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

3.7.1. SINGLE DOSE ORAL TOXICITY STUDY IN MICE:

Study: TT#95-617-0
Compound: L-748,731-000R, batch 014, 99.8% purity
Formulation: Suspension in 0.5% methylcellulose.
Route: Oral gavage.
Dose volume: 10 mL/Kg body weight
Dose Levels: Group: 1 2 3 4 5
mg/Kg: 30 100 300 600 1000
Strain: Crl:CD-1®(ICR)BR albino mice, 10 week old.
Body weight: 31.2-40.1 g, 24.6-32.1 g
Number: 28/sex/group
Control Treatment: No control used.
Study Site: Laboratoires MS&D-Chibret, Riom, France
Date: 13 June 1995 to 16 October 1995
GLP/QAU: Both present and signed.

The study was done to determine plasma levels of L-748,731 in mice after a single oral administration.

All mice were weighed pretest. Blood was taken from 4 animals/sex from each group at 0.5, 1, 2, 4, 6, 10, and 24 hours postdosing for plasma drug level determination.

RESULTS AND DISCUSSION

- mortality: none-

PK data:

Mean Plasma Parameters Following a Single Dose

	30 mg/Kg M F	100 mg/Kg M F	300 mg/Kg M F	600 mg/Kg M F	1000 mg/Kg M F
C_{max} (µg/mL)	2.7 1.4	6.5 4.1	9.2 6.2	10.2 6.3	11.9 10.5
T_{max} (hr)	0.5 0.5	0.5 0.5	1.0 0.5	2.0 0.5	1.0 1.0
AUC $_{(0\text{-}24hr)}$ (µg hr/mL	15.4 5.6	36.2 14.6	95.9 50.3	122.6 71.9	113.3 87.7

C_{max} and AUC values did not show a dose proportional increase. These parameters were also higher in males at all dose levels. AUC values approached a plateau in males at 600 mg/Kg, but were still increasing at 1000 mg/Kg in females.

Although $AUC_{0\text{-}24}$ did not exhibit dose proportionality, the increase was "approximately linear" in the range of 30-300 mg/kg.

3.8 TOXICOKINETIC STUDIES – ACUTE RAT STUDIES

3.8.1. Single Dose Oral Toxicokinetic Study in Rats:

Study: TT#95-027-0
Compound: L-748,731-000R, batch number 009, 98.8% purity (weight%)
Formulation: Suspension in aqueous 0.5% methylcellulose
Route: Orally by gavage at 5 mL/Kg.
Dose Levels: Group: 1 2 3 4 5
mg/Kg: 125 250 500 1000 2000
Strain: Sprague-Dawley [Crl:CD®(SD)BR], about 10 weeks old
body weight- 278 to 346 g, 207 to 228 g
Number: 9/sex/group
Control Treatment: No control group in this study.
Study Site: Merck Research Laboratories, West Point, PA 19486
Date of Study: 02May95 to 31Aug95
GLP/QAU Statements: Both present with signatures.

The objective of the study was to determine the toxicokinetic profile in rats after a single oral administration of L-748,731.

All animals were observed for mortality. Body weight was determined pre-test only. The rats were divided into three subgroups to be bled at 0.5, 6, and 24 hours or at 1 and 4 hours or at 2 and 10 hours. Each animal was bled two to three times during the 24-hour period from the orbital sinus. Heparinized blood was collected from 3/sex/group at 0.5, 1, 2, 4, 6, 10, and 24 hours after dosing. All animals were sacrificed on D2.

RESULTS AND DISCUSSION

mortality: none
toxicokinetics:

Mean Plasma Toxicokinetic Parameters at 125 and 250 mg/Kg

	125 mg/Kg		250 mg/Kg	
	Male	Female	Male	Female
Cmax (μg/mL)	9.41	12.57	8.56	11.67
Tmax (hr)	6	6	6	6
AUC (μg hr/mL)	95.56	147.36	113.84	184.61

The long time to attain Cmax was suggested related to poor aqueous solubility. A nonlinear relationship between the low and high dose AUC values is evident (19% increase in males and 25% in females). Females show a higher systemic exposure (Cmax and AUC values).

[The Sponsor states that only the 125 and 250 mg/kg dose levels were evaluated because "the toxicokinetic data [was] not [to] be used for carcinogenesis dose selection". These results suggest a "pattern of non-linear saturable toxicokinetics".]

The current Pharmacology/Toxicology Reviewer reviewed the following study

3.8.2. L-748,731: Single Dose Oral Toxicokinetic Study in Rats [Vol. 1.7; p. A-132]

Study Identification: TT #96-025-0
Site: Merck Research Laboratories; West Point, PA

Study Dates [In-life]: April 10-11, 1996
Formulation and Lot No.: Test article L-748,731-000R27 99.9% purity by weight
Vehicle - 0.5% aqueous methylcellulose,
Certificate Analysis: No (X) Sponsor states that concentration and uniformity assays were within acceptable limits
Final Report (X) Nov. 26, 1997
GLP and QA statements signed: Yes (X)
Objective: "To determine the plasma concentrations and the toxicokinetic profile after a single-dose oral administration to rats of L-748,731, a cyclooxygenase-2 inhibitor".

Test Material/ Group Designation	Dose and Regimen#				N*	Sex	Species/ Strain
	mg/kg	ml/kg	Route	# of doses			
Group 1 - L-748-731	2	5	oral, gavage	one	10	M F	Sprague-Dawley - [Crl:CD®(SD)BR] Charles River Labs; Raleigh, NC app. 73 days at start date Males - 264 to 376 gm Females - 185 to 231 gm
Group 2 - L-748-731	4						
Group 3 - L-748-731	8						
Group 4 - L-748-731	10						
Group 5 - L-748-731	15						
Group 6 - L-748-731	30						

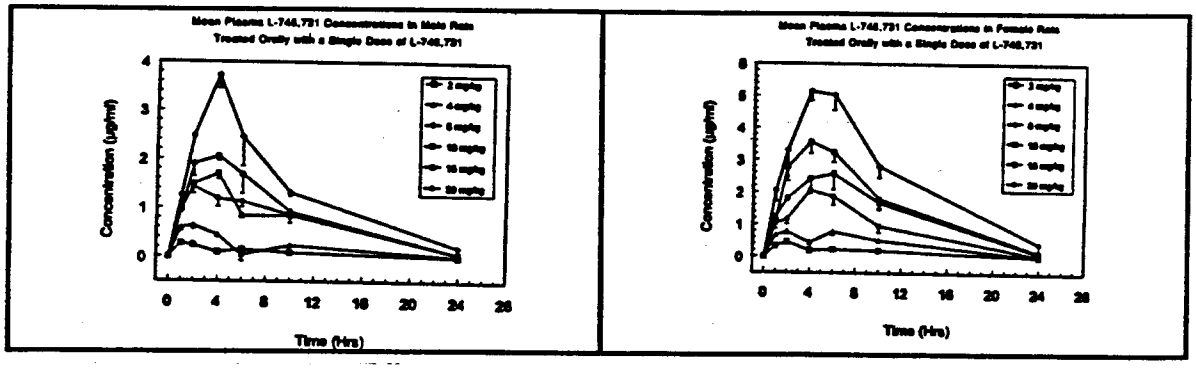
#Diet - 24 and 17 grams daily for males and females, respectively
*3 animals bled at each time point with each animal being bled twice [1 and 6; 2 and 10; and 4 and 24 hours post dosing]

Parameter Evaluated*	Time Point(s)
Mortality check	daily
Body Weight	Pretest only to determine dose
Plasma kinetic profile [C_{max}, t_{max}, AUC_{0-24}] - anesthetized, retro-orbital bleed - minor modifications to a validated method -	1, 2, 4, 6, 10, and 24

Results

Mortality – There were no unscheduled deaths or sacrifices.

Toxicokinetic Parameters - The graphs below represent mean plasma drug concentration vs. time.



The table below delineates the C_{max}, T_{max}, and AUC for male and female rats.

Dose [mg/kg]	C_{max} [µg/ml]		T_{max} [hours]		AUC [µg•hr/ml]	
	Males	Females	Males	Females	Males	Females
2	0.274	0.456	1	2	2.10	3.95
4	0.639	0.782	2	6	5.88	9.77
8	1.43	2.06	2	4	17.1	21.6
10	1.69	2.55	4	6	17.1	32.2
15	2.06	3.56	4	4	21.8	39.4
30	3.73	5.15	4	4	33.1	60.7

Reviewer's Comment (Study Design and Data Presentation) - These were adequate.

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

1. In males and females at doses of 2-8 mg/kg, increases in AUC and C_{max} were fairly dose proportional. At ≥10 mg/kg the increases in AUC and C_{max} were less than dose proportional.
2. Absorption in males tended to be rapid at doses from 2-8 mg/kg but was slower at ≥10 mg/kg. In females, absorption appeared to be prolonged at all doses except 2 mg/kg.
3. Exposure tended to be greater in females than in males.

Reviewer's Comment - The Reviewer concurs.

3.9 TOXICOKINETIC STUDIES – REPEAT DOSE MOUSE STUDIES

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

3.9.1. FIVE-WEEK ORAL TOXICOKINETIC STUDY IN MICE:

Study: TT#95-611-0
Compound: L-748,731-000R, batch #014
Formulation: Suspension prepared daily in aqueous
0.5% methylcellulose.
Route: Oral, gavage at 10 mL/Kg
Dose Levels: Group: 1 2 3 4 5
mg/Kg/day: 30 100 300 600 1000
Strain: Crl:CD-1®(ICR)BR albino mice, 36 days old
Weight: M 23.0-32.3 g, F 18.5-26.3 g
Number: 30/sex/group
Control Treatment: No vehicle control used.
Study Site: Laboratoires Merck Sharp & Dohme-Chibret
Centre de Recherché, Riom, France
Date: 25 April 1995 to 27 October 1995
GLP/QAU: Both present and signed.

The objective of this study was to determine plasma drug levels in mice after repeated oral administration.

All animals were observed daily for mortality and clinical signs of toxicity. Body weight was determined pretest, during Drug Week 1 and twice/week thereafter. Blood was collected from 4 non-fasted anesthetized mice/sex/group at 0.5, 1, 2, 4, 6, 10, and 24 hours postdosing for determining PK data.

RESULTS AND DISCUSSION

- mortality: none-
- signs: no treatment-related signs
- body weight: no body weight decrement > 4.5% in any group- *Although not significant, there was a dose dependent decrease in overall body weight gain in females as follows: 4.9, 4.3, 3.9, 3.8, and 3.4 grams at 30, 100, 300, 600, and 1000 mg/kg/day. There was also a decrease in overall body weight gain for males at 100 and 1000 mg/kg/day vs. 30 mg/kg/day at 5.7 and 5.0 grams vs. 6.6 grams, respectively.]*
- PK data:

Known amounts (10-2000 ng) of L-748,731 were added to 200 µL of plasma from untreated animals and carried through the analytical procedure to validate the assay. The absolute recovery of L-748,731 from mouse plasma was 99.3±1.4% (mean ± S.E.M., n=32).

Mean Plasma Toxicokinetic Parameters For L-748,731

Dose (mg/Kg):	30 mg/Kg		100 mg/Kg		300 mg/Kg		600 mg/Kg		1000 mg/Kg	
	M	F	M	F	M	F	M	F	M	F
C_{max} (µg/mL)	4.0	2.3	6.5	4.4	8.2	5.6	12.7	8.2	11.6	9.1
T_{max} (hr)	0.5	0.5	0.5	1.0	1.0	0.5	1.0	1.0	1.0	1.0
AUC $_{(0\text{-}24hr)}$ (µg hr/mL)	20.4	9.3	40.4	25.8	63.1	49.6	105.2	94.2	97.4	92.4

As in the single dose study, the males had higher plasma drug concentrations than females. AUC and C_{max} values appeared to plateau between 600 mg/Kg and 1000 mg/Kg.

Additional Reviewer's Comment: *The Reviewer concurs. Although the C_{max} and $AUC_{0\text{-}24}$ did not exhibit dose proportionality, the increase was "approximately linear" in the range of 30-600 mg/kg. In addition, the values tended to be comparable to those observed in the single dose study which would indicate that there was no accumulation of drug in the plasma following repeat dosing.*

The current Pharmacology/Toxicology Reviewer reviewed the following studies.

3.9.2. L748,731: Twenty-Seven-Week Oral Toxicokinetic Study in Mice [Vol 1.38; p. E-1381]

Study Identification: TT# 96-604-0
Site: Laboratoires Merck Sharp & Dohme-Chibret, Centre de Recherché, Riom, France
Study Dates: Feb. 14, - Aug 14, 1996
Formulation and Lot No.: L748,731 [Batch #027] in 0.5% aqueous methylcellulose

Certificate of Analysis Submitted: No (X) analyzed and found to be within acceptable limits, according to the Sponsor in Drug weeks 16 and 26, drug concentration was analyzed Drug Weeks 1, 7, 19, and 26
Final Report (X) Jan. 9, 1997
GLP and QA Assurance Statements Signed: Yes (X)
Objective: "To monitor plasma level of L-748,731 when given orally to mice after repeated administration under the condition of the concomitant mouse carcinogenicity study."

Test Material/ Group Designation	Dose*				Sex	N	Species/Strain
	mg/kg	ml/kg	Route	# days dosed			
Group 1 - L-748,731	5	10	oral, gavage	F and M = 30 or 182-183	M	30	-Crl:CD-1® [IBR] BR albino mice - -37 day at start of study, F - 18.3 to 25.8 g; M - 23.1 to 31.2 g
Group 2 - L-748,731	10						
Group 3 - L-748,731	20				F	30	
Group 4 - L-748,731	30						

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

Results - The following animals were found dead and were discarded without necropsy:[1] 3 females and 1 male at 5 mg/kg/day (3, 5, 104,and 5 dose); [1] 1 male at 20 mg/kg/day (127 doses); and [3] 1 female at 30 mg/kg/day (6 doses).

Body Weight - No drug-related changes in body weight or body weight gain were observed.

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

Dose [mg/kg/day]	Plasma Concentration [µg/ml ± SEM]							
	Week 5				Week 27			
	0.5 hours		10 hours		0.5 hours		10 hours	
	F	M	F	M	F	M	F	M
5	0.31 ± 0.05	0.75 ± 0.12	0.06 ± 0.01	0.18 ± 0.02	0.33 ± 0.03	0.86 ±0.09	0.06 ± 0.01	0.24 ± 0.03
10	0.54 ±0.07	1.30 ± 0.04	0.08 ± 0.02	0.45 ± 0.07	0.59 ± 0.01	1.34 ± 0.11	0.14 ± 0.03	0.47 ± 0.05
20	1.04 ± 0.06	2.08 ± 0.09	0.23 ± 0.04	0.79 ± 0.12	1.25 ± 0.09	2.03 ± 0.16	0.36 ± 0.11	0.93 ± 0.13
30	1.46 ± 0.14	2.59 ± 0.22	0.26 ± 0.05	1.27 ± 0.19	1.32 ± 0.23	2.41 ± 0.13	0.33 ± 0.10	1.22 ± 0.18

The plasma concentrations in males tended to be higher than in females by approximately 2.1 and 3.4-4.2X at 0.5 and 10 hours respectively during Drug Week 5 and 27.

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

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

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

2. The increase in plasma drug concentration at 0.5 hours was slightly less than dose proportional for both sexes at 5 and 27 weeks.

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