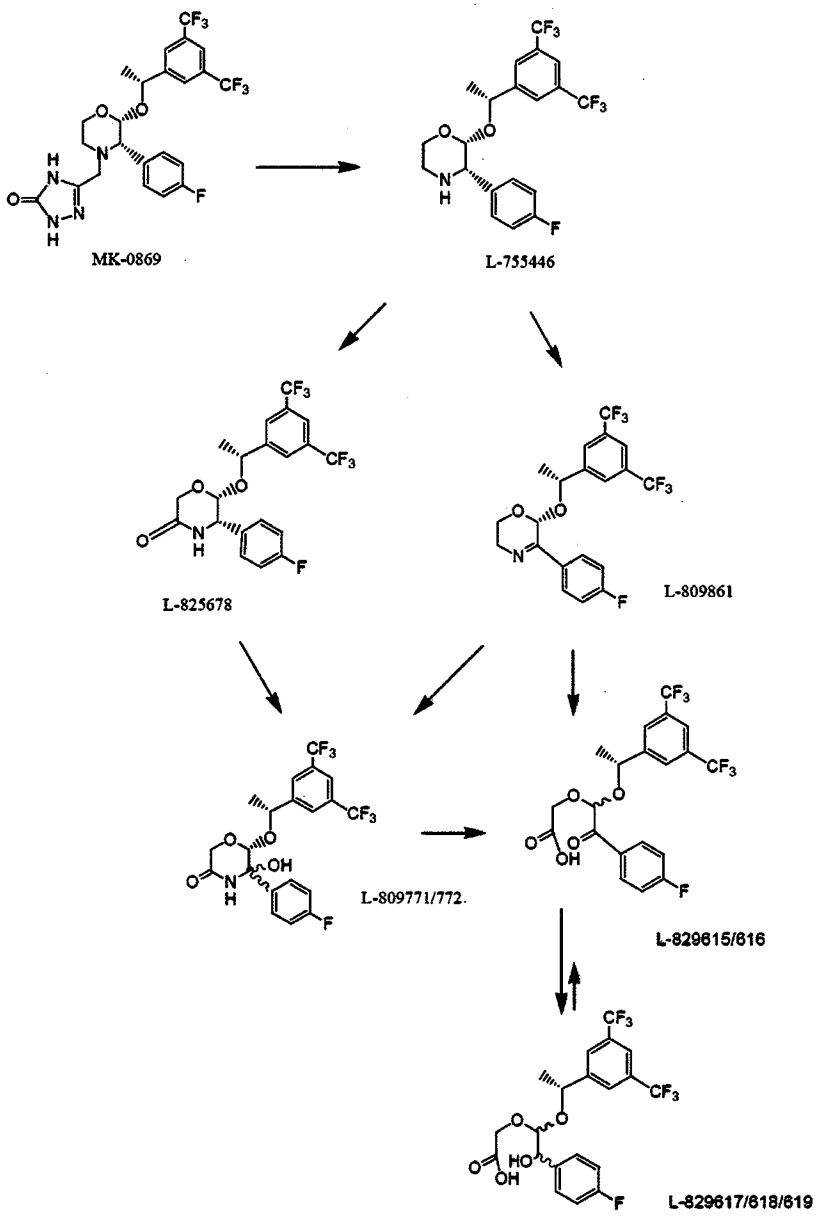
Systemic Exposure of Nonpolar and Polar Metabolites in Rats and Mice Following Single or Multiple Oral Doses of MK-0869.

Methods: For the single dose studies, male and female rats and male mice were dosed orally with [^{14}C]MK-0869 (specific activity 1 μCi/mg) by oral gavage at a dose of 10 mg/kg. Following dosing, groups of 3-5 rats and 6 mice were euthanized and blood samples were collected by cardiac puncture at 2, 4, 6, 8, 10 (males only), 18 and 24 hours for rats, and 2, 5, 7, 18 and 24 hours for mice.
For the multiple dose study, male rats were administered [^{14}C]MK-0869 at a dose of 100 mg/kg twice daily for 14 days, and on Day 15, a last dose was administered in the morning. Blood samples were collected from 3 animals at each time point at 2, 4, 6, 8, 18 and 24 hr following the last dose and the animals were sacrificed. The plasma samples were analyzed by HPLC and/or LC-MS/MS methods.

Results: The metabolic pathways of MK-0869 in rats are shown in the Figure below. These metabolites are formed by at least 6 metabolic events: N-dealkylation, (L-755446), oxidation at the benzylic carbon on the morpholine ring (L-809861), oxidation alpha to the morpholine nitrogen to form a lactam (L-825678), oxidation of the lactam to form a hydroxylated lactam (L-809771), morpholine ring-opening followed by hydrolysis to the corresponding keto acid (L-829615) and reduction of the keto acid to hydroxyl acid (L-829617).

Metabolic Pathways of MK-0869 in Rats



Single dose studies: In male rats, MK-0869 accounted for at least 50% of the total plasma radioactivity between 2 to 8 hours following administration of a 100 mg/kg oral dose. The polar and nonpolar metabolites accounted for 12% and 21% of the total radioactivity during the same time period. In female rats, 86% to 93% of the total radioactivity accounted for the parent compound, only less than 10% of the plasma radioactivity accounted for metabolites. This

suggests that the metabolism and the clearance of MK-0869 in female rats are slower than that in male rats. The distribution of radioactivity among parent compound and metabolites in male mice were similar to that in male rats. By 24 hr, the parent compound accounted for 18% to 33% of total plasma radioactivity, indicating that it was still a major component in plasma. At least 6 metabolites were identifies in the plasma of rats and mice. In male rats, up to 10 hr after dosing, concentrations of the parent compound were 4 to 10 time higher than the combined concentrations of the 6 metabolites. The concentrations of MK-0869 and its metabolites in the plasma of male rats, female rats and male mice are summarized in the Tables below.

Summary of the Concentrations of MK-0869 and its Metabolites Quantified by LC-MS/MS in Plasma of Male Rats Receiving a Single Dose of [^{14}C]MK-0869 at 100 mg/kg[a]

	Mean Concentration (ng/mL)[b]						
Time Postdose (hr)	2	4	6	8	10	18	24
Compound							
L-829617	3.7	10.5	25.0	32.9	33.4	41.4	29.5
L-829615	16.7	33.3	49.7	69.5	89.0	64.2	49.2
MK-0869	937.1	1530.7	1697.9	1386.4	1208.8	98.3	63.6
L-809771	9.1	19.9	41.0	44.0	54.4	82.6	73.5
L-825678	29.5	58.4	78.6	83.4	77.9	12.2	6.3
L-755446	12.6	21.3	21.5	23.5	22.1	2.6	1.4
L-809861	18.5	16.8	27.7	23.0	23.9	10.6	8.2
Ratio of MK-0869 to Metabolites	10.4	9.6	7.0	5.0	4.0	0.5	0.4

Summary of the Concentrations of MK-0869 and its Metabolites Quantified by LC-MS/MS in Plasma of Female Rats Receiving a Single Dose of [^{14}C]MK-0869 at 100 mg/kg[a]

	Mean Concentration (ng/mL)[b]					
Time Postdose (hr)	2	4	6	8	18	24
Compound						
L-829617	BLQ[c]	BLQ	BLQ	7.7	42.5	105.4
L-829615	BLQ	BLQ	BLQ	BLQ	85.0	161.3
MK-0869	1348.0	2118.1	1604.9	2941.3	995.4	310.4
L-809771	BLQ	3.6	7.3	16.8	135.4	167.1
L-825678	16.9	42.9	51.8	96.0	79.3	28.3
L-755446	BLQ	BLQ	BLQ	BLQ	8.2	7.6
L-809861	2.8	5.4	7.5	15.8	62.9	37.6
Ratio of MK-0869 to Metabolites	68.5	40.8	24.1	21.6	2.4	0.6

Summary of the Concentrations of MK-0869 and its Metabolites Quantified by LC-MS/MS in Plasma of Male Mice Receiving a Single Dose of [^{14}C]MK-0869 at 100 mg/kg[a]

	Mean Concentration (ng/mL)[b]				
Time Postdose (hr)	2	5	7	18	24
Compound					
L-829617	16.2	21.1	28.5	14.4	BLQ
L-829615	BLQ[c]	BLQ	BLQ	BLQ	BLQ
MK-0869	5638.1	5627.1	5580.4	114.8	BLQ
L-809771	28.1	52.6	105	615.0	248.1
L-825678	193.1	259.5	386.9	186.7	15.4
L-755446	44.1	42.8	57.7	11.9	BLQ
L-809861	BLQ	14.0	25.6	BLQ	BLQ
Ratio of MK-0869 to Metabolites	20.0	14.4	9.2	0.1	0.0

Multiple dose study: After multiple dosing in male rats, the distribution of total radioactivity in plasma was somewhat different from that of rats receiving the single doses. The polar metabolites, eluting in the same regions as those observed on rats given a single dose, were the major plasma component accounting for 48 to 56% of the total radioactivity in the plasma between 2 to 8 hr post-dose. Following multiple dosing in rats, the concentrations of MK-0869 were only 1 to 2 times higher than the combined concentrations of the 6 metabolites. Among the 6 metabolites, L-829617 and L-829615, the two most polar metabolites, were the major metabolites. The plasma concentrations of the metabolites in male rats following oral administration of multiple doses are summarized in the Table below.

Summary of the Concentrations of MK-0869 and its Metabolites Quantified by LC-MS/MS in Plasma of Male Rats Receiving Multiple Doses of MK-0869 and a Last Dose of [^{14}C]MK-0869 at 100 mg/kg[a]

	Mean Concentration (ng/mL)[b]					
Time Postdose (hr)	2	4	6	8	18	24
Compound						
L-829617	13.1	35.5	51.8	42.7	7.1	10.4
L-829615	56.6	115.3	117.4	105.2	30.0	28.3
MK-0869	218.2	315.3	257.5	220.6	18.3	25.8
L-809771	13.9	27.7	34.7	30.9	10.9	5.3
L-825678	9.6	19.9	20.0	15.9	BLQ[c]	BLQ
L-755446	4.6	6.7	5.8	4.3	BLQ	BLQ
L-809861	6.5	8.3	6.9	7.1	BLQ	BLQ
Ratio of MK-0869 to Metabolites	2.1	1.5	1.1	1.1	0.4	0.6

Conversion of MK-0517 to Aprepitant *In Vitro* in Liver Microsomal Incubations from the Sprague-Dawley Rat, Beagle Dog and Human.

Methods: The study was conducted to examine the conversion of MK-0517 (pro-drug) to the pharmacologically active entity, aprepitant (MK-0517) *in vitro* in liver microsomal incubations from rats, dogs and humans. [^{14}C]MK-0517 (10 μM) was incubated with the liver microsomes (500 μg protein; pH 7.4) at 37^{0}C, and duplicate samples were collected at 0, 5, 15, 30 or 60 min for analysis by HPLC. The percent of aprepitant formed from MK-0517 was calculated from the aprepitant peak area.

Results: Hydrolysis of the phosphate moiety to generate aprepitant was the major metabolic pathway for MK-0517 in rat, dog and human liver microsomes. There was a similar turnover rate in all species examined. The conversion of MK-0517 to aprepitant by liver microsomes from these species are summarized in the Table below.

Conversion of [^{14}C]MK-0517 to [^{14}C]Aprepitant in Rat, Dog and Human Liver Microsomes[a]

	Percent of aprepitant formed		
Time, min	Rat	Dog	Human
0	0	1	0.4
5	23	24	33
15	66	58	72
30	86	79	88
60	93	87	89

Thus, in incubates of misrosomes from rat, dog and human, MK-0517 underwent a rapid and nearly complete hydrolysis to generate aprepitant.

Dose Proportionality Studies of L-758,298 in Rats.

Methods: Dose proportionality studies of L-758298 were conducted in male rats following intravenous administration of 1, 8 and 25 mg/kg single doses. The doses were prepared by dissolving L-758,298 (bis-N-methyl-D-glucamine salt) in a solution of lactose (50 mg/ml), potassium carbonate (1.38 mg/ml), citric acid monohydrate (0.85 mg/ml) and sodium chloride (4 mg/ml). Blood samples were collected from the femoral vein at 2-3, 5, 15, 30 min, and 1, 2, 4, 6, 8, 10, 24, 30, 48 and 72 hr after dosing. An LC/MS/MS method was used to determine the plasma levels of L-758,298 and L-754,030 simultaneously.

Results: Intact L-758,298 concentrations were quantifiable only at two early time points of determination (22.8 to 515 ng/ml) for 1 and 8 mg/kg doses, and up to 1.0 hr for the 25 mg/kg dose (1117 to 75 ng/ml). Plasma concentrations of L-758, 298 diminished rapidly from 1117 to75.3 ng/ml from 3 to 60 min following dosing at 25 mg/kg. Plasma concentrations of L-754,030 reached the highest level by the first time point of sampling, and declined thereafter. At 10 hr post-dosing, the plasma concentrations of L-754, 030 were approximately 13.5, 140 and 620 ng/ml

at 1, 8 and 25 mg/kg doses, respectively. At 24 hr post-dosing, L-754,030 was detectable only in one rat dosed at 25 mg/kg. The plasma exposure levels (AUC) of L-754, 030 increased with increasing dose of L-758, 298 in an apparent dose-proportional manner. The mean plasma concentrations of L-758, 298 and L-754, 030 in the rat plasma at different times of dosing are shown in the Table below.

Table 7

Concentrations of L-758,298 and L-754,030 in Plasma of Rats Dosed Intravenously with L-758,298 at 1, 8, or 25 mg/kg[a]

Dose	1 mg/kg						8 mg/kg						25 mg/kg					
	Concentrations (ng/ml)[b]						Concentrations (ng/ml)[b]						Concentrations (ng/ml)[b]					
Time	L-758,298			L-754,030			L-758,298			L-754,030			L-758,298			L-754,030		
(hr)	mean	±	S.D.	mean	±	S.D.	mean	±	S.D.	mean	±	S.D.	mean	±	S.D.	mean	±	S.D.
0.05[c]	22.8	±	9.7	275.8	±	16.0	515.0	±	398.0	3130	±	331	1117.0	±	501.6	9070	±	256
0.08	NQ[d]			211.8	±	15.6	80.7	±	69.3	2552	±	351	404.3	±	88.0	6293	±	800
0.25	NQ			184.1	±	23.4	NQ			1954	±	242	186.1	±	65.5	5731	±	620
0.5	NQ			144.5	±	18.9	NQ			1555	±	119	112.0	±	34.9	4775	±	560
1	NQ			110.6	±	18.5	NQ			1177	±	76	75.3	±	29.2	3830	±	139
2	NQ			69.8	±	10.8	NQ			747.0	±	157.2	NQ			2409	±	214
4	NQ			41.6	±	9.7	NQ			526.9	±	122.5	NQ			1982	±	97
6	NQ			28.1	±	5.7	NQ			370.3	±	108.5	NQ			1602	±	108
8	NQ			19.0	±	4.9	NQ			238.2	±	88.7	NQ			1027.5	±	99.6
10	NQ			13.5	±	4.3	NQ			139.9	±	70.4	NQ			620.2	±	69.1
24	NQ				±		NQ			NQ			NQ			3.9	±	7.7
48	NQ			NQ			NQ			NQ			NQ			NQ		
72	NQ			NQ			NQ			NQ			NQ			NQ		
AUC[0-∞] (ng·hr/ml)				588	±	121				6249	±	1375				22392	±	1158

a Four male Sprague Dawley rats were dosed intravenously with L-758,298 (bis-N-methyl-D-glucamine salt) prepared in a solution of lactose (50 mg/ml), potassium carbonate (1.38 mg/ml), citric acid monohydrate (0.85 mg/ml) and sodium chloride (4 mg/ml) (pH 7.0). The vehicle was provided by ———— b(4)

b Plasma samples (0.2 ml) were extracted with ———— cartridges and eluted with methanol prior to LC/MS/MS analysis. The concentrations of L-758,298 and L-754,030 were determined simultaneously with L-757,678 and L-752,611 as the respective internal standards. The limits of quantitation for L-758,298 were 6.25, 62.5 and 62.5 ng/ml, for L-754,030 were 6.25, 12.5 and 12.5 ng/ml at 1, 8 and 25 mg/kg dosing of L-758,298, respectively.

c Following dosing at 1 mg/kg, blood samples were taken at 2 min in rats; blood samples were taken at 3 min in rats after dosing at 8 or 25 mg/kg.

d NQ: not quantifiable.

Dose Proportionality Studies of L-758,298 in Dogs.

Methods: Dose-proportionality studies of L-758,298 were conducted in male beagle dogs following single intravenous administration of 0.5, 2.0 and 32 mg/kg doses. The doses were prepared by dissolving L-758,298 (bis-N-methyl-D-glucamine salt) in a solution of lactose (50 mg/ml), potassium carbonate (1.38 mg/ml), citric acid monohydrate (0.85 mg/ml) and sodium chloride (4 mg/ml). Blood samples were collected from the femoral vein at 2.5-3, 5-6, 15, 30 min, and 1, 2, 4, 6, 8, 10, 24, 30 (only at 0.5 mg/kg), 48 and 72 hr after dosing. An LC/MS/MS method was used to determine the plasma levels of L-758,298 and L-754,030 simultaneously.

Results: Following i.v. administration of L-758,298 to male dogs, it was rapidly converted to its active metabolite, L-754,030. Intact L-758,298 concentrations were quantifiable only at 2.5 min (0.1 to 0.2 µg/ml) for 0.5 and 2.0 mg/kg doses, and at 3 and 6 min at the 32 mg/kg dose. Plasma concentrations of L-758,298 decreased rapidly from 57 to 1.3 µg/ml from 3 to 6 minutes following administration of the 32 mg/kg dose. Plasma concentrations of L-754,030 reached the highest level by the first time point of sampling and declined thereafter. The plasma levels of L-754,030 were still quantifiable at 72 hours after dosing. A non-dose-proportional increase in the AUC of L-754,030 with dose was observed following i.v. administration of 0.5, 2.0 and 32 mg/kg doses of L-758,298. There were 8- and 40-fold increases in AUC of L-754,030 as the doses increased from 0.5 to 2.0 mg/kg and 2.0 to 32 mg/kg, respectively. The plasma concentrations of L-758,298 and L-754,030, and the AUC values of L-754,030 following i.v. administration of L-758,298 are shown in the Table below.

Concentrations of L-758,298 and L-754,030 in Plasma of Dogs Dosed Intravenously with L-758,298 at 0.5, 2, or 32 mg/kg (Periods 1-3) [a]

Dose	0.5 mg/kg (period 3)				2 mg/kg (period 1)				32 mg/kg (period 2)			
	Concentrations (ng/ml)[b]				Concentrations (ng/ml)[b]				Concentrations (ng/ml)[b]			
Time (hr)	L-758,298 mean ± S.D.		L-754,030 mean ± S.D.		L-758,298 mean ± S.D.		L-754,030 mean ± S.D.		L-758,298 mean ± S.D.		L-754,030 mean ± S.D.	
0.04[c]	109	± 59	333	± 84	232	± 73	1529	± 61	57132	± 22730	19530	± 1635
0.08[c]	NQ[d]		234	± 33	NQ		992	± 77	1274	± 908	16830	± 2217
0.25	NQ		190	± 21	NQ		991	± 207	NQ		13377	± 1792
0.5	NQ		169	± 15	NQ		728	± 56	NQ		10943	± 405
1	NQ		142	± 17	NQ		717	± 59	NQ		9623	± 623
2	NQ		119	± 21	NQ		652	± 61	NQ		8907	± 691
4	NQ		92	± 14	NQ		491	± 45	NQ		8963	± 496
6	NQ		74	± 11	NQ		408	± 56	NQ		8037	± 97
8	NQ		39	± 6	NQ		344	± 42	NQ		7923	± 358
10	NQ		28	± 10	NQ		284	± 52	NQ		8100	± 763
24	NQ		6	± 2	NQ		120	± 48	NQ		5770	± 542
48	NQ		NQ		NQ		22	± 13	NQ		3860	± 1040
72	NQ		NQ		NQ		NQ		NQ		2560	± 800
AUC[e] (ng-hr/ml)			1095	± 281			8926	± 1504			375363	± 55169

a Three male beagle dogs (ords 0836, 1808 and 2847) were dosed intravenously with L-758,298 (bis-N-methyl-D-glucamine salt) chloride (4 mg/ml) (pH 7.0). The vehicle was provided by [redacted] b(4)

b Plasma samples (0.5 ml) were extracted with [redacted] cartridges and eluted with methanol prior to LC/MS/MS analysis. The concentrations of L-758,298 and L-754,030 were determined simultaneously with L-757,678 and L-752,611 as the respective internal standards. The limits of quantitation for L-758,298 was 25, 50, 100 ng/ml; for L-754,030 were 5, 5 and 20 ng/ml at 0.5, 2 and 32 mg/kg dosing of L-758,298, respectively.

c In period 2, blood samples were taken at 3 min for all dogs and 6 min 15 sec, 5 min 15 sec and 5 min 45 sec for dog 0836, 1808 and 2847, respectively.

d NQ: not quantifiable.

e AUC was estimated from 0-∞ at the 0.5 mg/kg dose level; due to non-linear kinetics, AUC was estimated from 0-48 and 0-72 hr at the 2 and 32 mg/kg dose levels, respectively.

Characterization of the Human Liver Microsomal Cytochrome P450 Isozymes Involved in the In Vitro Metabolism of MK-0869.

Methods: The relative contribution of different human Cytochrome P_{450} (CYP) isozymes to the metabolism of MK-0869 and its nonpolar metabolites (L-755446 and L-809861) was examined *in vitro* using CYP isozyme-selective inhibitors and microsomes expressing individual recombinant human CYP isozymes. The following agents were used as inhibitors of specific CYP isozymes: ketoconazole (1-2 μM) for CYP3A4, quinidine (1 μM) for CYP2D6, sulfaphenazole (1 μM) for CYP2C9, furafylline (25 μM) for CYP1A2 and 4-methylpyrazole (100 μM) for CYP2E1. Microsomes prepared from baculovirus-infected Sf21 cells expressing recombinant human CYP isozymes, CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 or CYP3A4 were used to identify the oxidative metabolism of MK-0869, L-755446 and L-809861. L-825678 was incubated with only with microsomes expressing CYP3A4. Inhibition of the following metabolism processes by MK-0869, L-755446, L-829617 or L-829615 were studied in pooled human liver microsomes: CYP1A2-mediated phenacetin O-deethylation, CYP2C9-mediated tolbutamide 3-hydroxylation, CYP2D6-mediated bufuralol 1'-hydroxylation, CYP2E1-mediated chlorzoxazone 6-hydroxylation, CYP3A4-mediated testerone 6β-hydroxylation, CYP3A4-mediated midazolam 1'- and 4-hydroxylation, CYP3A4-mediated diltiazem N-demethylation, CYP3A4-mediated terfenadine metabolism, CYP2C9-mediated R/S-warfarin 7-hydroxylation and CYP2C19-mediated mephenytion 4-hydroxylation. The metabolites were analyzed by LC-MS/MS and spectroscopic methods.

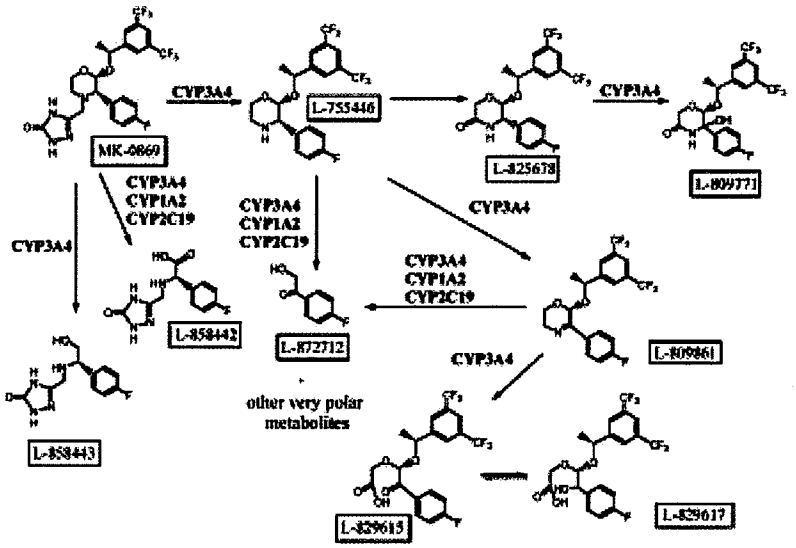
Results: The metabolism of MK-0869 in human liver microsomes was completely inhibited by the CYP3A4-specific inhibitor, ketoconazole (1 μM). No inhibition was observed with CYP2D6, CYP2C9, CYP1A2 or CYP2E1 isozyme-specific inhibitors, quinidine, sulfaphenazole, furaphylline or 4-methylpyrazole, respectively. CYP3A4 was also primarily responsible for the metabolism of two nonpolar metabolites, L-755446 and 809861, formed from N-dealkylation of MK-0869. In addition, the lactum L-825678, generated by incubation of L-755446 with human hepatocytes, was metabolized by microsomes expressing CYP3A4 to the hydroxylactum L-809771.

[^{14}C]MK-0869 was also metabolized by Sf21 cells expressing recombinant human CYP1A2, CYP2C19 or CYP3A4 isozyles to two very polar metabolites, L-858442 and L-858443. CYP3A4 generated both L-858442 and L-858443, while CYP1A2 and CYP2C19 generated only L-858442. This suggests that MK-0869, in addition to the N-dealkylation to L-755446, underwent O-dealkylation leading to the formation of the metabolites with intact triazolone groups. Similarly, the nonpolar metabolites, L-755446 and L-809861 underwent O-dealkylation by recombinant CYP1A2, CYP2C19 or CYP3A4 to generate a very polar metabolite, L-872712. In addition to L-872712, recombinant CYP3A4 catalyzed also the formation of a nonpolar metabolite, L-809861.

In pooled human liver microsomes, MK-0869 was a moderate inhibitor of CYP3A4. The K_i values for the inhibition of 1'- and 4-hydroxylation of midazolam, N-demethylation of diltiazem and the metabolism of terfenadine were 10, 10, 11 and 21 μM, respectively. On the other hand, MK-0869 was a very weak inhibitor of CYP2C9 (7-hydroxylayion of R/S warfarin) and CYP2C19 (4-hydroxylation of S-mephenytion) with K_i values of 108 and 66 μM, respectively. MK-0869 was also a very weak inhibitor of CYP2D6, CYP1A2 and CYP2E1, with IC_{50} values of >100 μM. The in vitro

metabolic pathways of MK-0869 by Sf21 cells or microsomes expressing recombinant human CYP isozymes are shown in the sponsor's Figure below.

In Vitro Metabolism of MK-0869 by Sf21 Cells or Microsomes Expressing Recombinant Human CYP Isozymes



The nonpolar metabolite of MK-0869, L-755446, was a weak inhibitor of CYP1A2 (IC_{50} = 20 to 30 μM), CYP2C9 (IC_{50} = 63 μM), CYP2D6 (IC_{50} = 75 μM), CYP2E1 (IC_{50} >100 μM) and CYP3A4 (IC_{50} = 30 to 35 μM). The two polar metabolites, L-829615 and L-829617, were very weak inhibitors of CYP1A2, CYP2C9, CYP2E1, CYP2D6 and CYP3A4, with IC_{50} values of >100 μM).
The results suggest that CYP3A4 is the major enzyme involved in the metabolism of MK-0869. In addition, CYP1A2 and CYP2C9 are also involved in the metabolism of MK-0869.

Evaluation of MK-0869 as an Inhibitor of CYP2C8 and CYP2B6 Activities in Pooled Human Microsomes.

Methods: MK-0869 was evaluated as an inhibitor of human liver microsomal CYP2C8 (taxol 6α-hydroxylase) and CYP2B6 (bupropion hydroxylase) activity. Pooled human microsomes were incubated with taxol and bupropion to determine the K_m value of each marker substrate. To evaluate the IC_{50} value, a single concentration of the marker substrate (approaching K_m) and varying concentrations of MK-0869, or positive control inhibitors (quercetin and N-(α-methylbenzyl)-1-aminobenzatriazole) were incubated for 20 min at 37°C. The incubates were analyzed by an LC-MS/MS method.

Results: MK-0869 was a weak inhibitor of CYP2B6 (IC_{50} = 44.7 μM) and CYP2C8 (IC_{50} >100 μM) when compared with the positive controls (IC_{50} < 3.0 μM). CYP2C8 and CYP2B6 inhibitory activities of MK-0869 and the positive controls are shown in the Table below.

Table 2. Evaluation of MK-0869 as an inhibitor of CYP2C8 and CYP2B6 activities in pooled human liver microsomes.

Enzyme Involved	Reaction (Substrate conc.)[a]	Compound Tested	Conc. Range (μM)	IC_{50} (μM)[d]
CYP2C8	Taxol 6α-hydroxylation (15 μM)	Quercetin[b]	0.023 - 50	2.7 ± 0.5
		MK-0869	0.046 - 100	>100 ± 10.0
CYP2B6	Bupropion hydroxylation (100 μM)	MBA[b, c]	0.0046 - 10	0.03 ± 0.006
		MK-0869	0.046 - 100	44.7 ± 2.4

[a] Substrate concentration used as K_m values (see table 1).
[b] Inhibitor used as positive control.
[c] MBA = *N*-(α-methylbenzyl)-1-aminobenzotriazole.
[d] IC_{50} = Mean ± SE.

Effect of Aprepitant on CYP2B6-Catalyzed Bupropion Hydroxylation in Human Liver Microsomes.

Methods: The reversibility of the inhibitory effect of aprepitant on CYP2B6-catalyzed bupropion hydroxylation was examined in human liver microsomes. Pooled human liver microsomes (0.25 mg/ml) were incubated at 37^{0}C for 10 min in a reaction mixture containing bupropion and aprepitant (5 to 40 μM). N-(α-methylbenzyl)-1-aminobenzatriazole was used as a positive control.

Results: Aprepitant was a weak inhibitor of CYP2B6-catalyzed reaction in human liver microsomes, with a K_i value of 12±1.2 μM. The effect of aprepitant on CYP2B6-catalyzed bupropion hydroxylation is shown in the Table below.

Table 1
Effect of Aprepitant on CYP2B6-Catalyzed Bupropion Hydroxylation in Human Liver Microsomes

Compound Tested	IC_{50} or K_i values[a]	Type of Inhibition
Aprepitant (MK-0869, L-000754030)	K_i = 12 ± 1.2 μM	Full Mixed
MBA[b] (Positive Control Inhibitor)	IC_{50} = 0.084 ± 0.008 μM	ND[c]

[a] K_i and IC_{50} values represent parameter value from curve fit ± standard error.
[b] MBA: *N*-(α-methylbenzyl)-1-aminobenzotriazole.
[c] ND - Not determined.

Conversion of MK-0517 to Aprepitant In Vitro in S9 Fractions from Human Liver, Kidney, Lung and Ileum.

Methods: The conversion of MK-0517 (fosaprepitant) to the pharmacologically active entity, aprepitant, in S9 fractions from human liver, kidney, lung and ileum was examined *in vitro* following incubation at 37^{0}C and analysis of the incubates using a LC-MS/MS method.

Results: There was rapid hydrolysis of the phosphoramidate moiety of MK-0517 to generate aprepitant in S9 fractions from the human liver, kidney, lung and ileum. The results suggest that in addition to liver, the conversion of MK-0517 to aprepitant can occur in multiple human tissues. The conversion of MK-0517 to aprepitant in S9 fractions from different human tissues are summarized in the Table below.

Conversion of MK-0517 to Aprepitant in S9 Fractions From Human Liver, Kidney, Lung, and Ileum[a]

	Disappearance of MK-0517 (μM)					Formation of Aprepitant (μM)			
Incubation Time (min)	Liver	Kidney	Lung	Ileum		Liver	Kidney	Lung	Ileum
0	12.2 ± 0.6	11.1 ± 1.0	12.0 ± 0.6	11.5 ± 0.5		0	0	0	0
15	5.8 ± 0.2	4.9 ± 0.2	8.9 ± 0.5	3.7 ± 0.3		4.5 ± 0.1	4.8 ± 0.3	3.1 ± 0.1	6.1 ± 1.7
30	2.9 ± 0.1	2.0 ± 0.1	6.3 ± 0.1	1.4 ± 0.1		7.4 ± 0.4	6.8 ± 0.4	5.3 ± 0.3	8.9 ± 0.1
60	BLQ	BLQ	2.8 ± 0.1	BLQ		10.1 ± 0.3	9.4 ± 0.4	9.2 ± 0.4	10.8 ± 0.7

Thus, in humans, fosaprepitant can be converted to aprepitant by tissues other than the liver.

2.6.4.6 Excretion

1. **Excretion of [4-flurophenyl-3-^{3}H]L-754,030] after intravenous administration**

Animals: Male Sprague-Dawley rats (body weight range of 300-400 g; ages were not provided by the sponsor).

Methods: [4-Flurophenyl-3-^{3}H]L-754,030] was prepared in ethanol:propylene glycol:water [13:58:2,(v/v/v)] at a specific activity of 33.6 μCi/mg. Three rats were intravenously administered 2 mg/kg of [4-Flurophenyl-3-^{3}H]L-754,030] via the

tail vein. After dosing, rats were housed in metabolism cages, and urine and feces were collected for 4 days. Radioactivity was determined by scintillation counters.

Results: As shown in the following table, 41% of the total dose of intravenously administered [4-flurophenyl-3-^{3}H]L-754,030] was recovered from the urine over 96 hr after dosing, and 58% in the feces. These data suggest the possibility that fecal excretion, presumably via bile, is a major pathway for the elimination of L-754,030 in rats.

Percent of radioactive [4-flurophenyl-3-^{3}H]L-754,030 (2 mg/kg, i.v.) recovered from urine and feces

Time (hr)	Percent of radioactive dose	
	Urine	Feces
0-24	29.9	38.9
24-48	7.6	13.9
48-72	2.2	3.6
72-96	1.1	1.5
Total	**41**	**58**

2.6.4.7 Pharmacokinetic drug interactions

In the human liver microsomes, CYP3A4 was identified as the major cytochrome P-450 isozyme involved in the metabolism of MK-0869. Two other CYP450 isozymes, CYP1A2 and CYP2C19, were also involved in the metabolism of the compound. In addition, aprepitant has been found to cause inhibition of CYP3A4 and activation of some drug metabolizing enzymes. Thus, there is a potential for drug-drug interactions with compounds which are metabolized by these enzyme systems.

2.6.4.8 Other Pharmacokinetic Studies

Oral Toxicokinetic Study in Pregnant Rats (Report Date/Number TT #97-736-0).

Methods: Concentrations of L-754,030 were determined in maternal and fetal plasma following administration of drug to pregnant female Sprague Dawley rats. Pregnant dams received L-754,030 by oral gavage at doses of 0, 125, 250, 500, and 1000 mg/kg/day from days 6 to 20 of gestation. Maternal blood was collected at 2, 4, 6, 8, 12, and 24 hr after dosing on day 20 of gestation. There were 15 pregnant dams/group. Rats in the control group received the vehicle, 0.5% methylcellulose/0.02% sodium lauryl sulfate. Blood was collected from 4 pregnant dams/group for each time point. Immediately following collection of maternal blood at the 4-hr time point, the uterus of each female was removed and fetuses were excised. Fetal blood was collected from umbilical vessels. L-754,030 was isolated by solid phase extraction and quantified by Liquid Chromatography/Mass Spectrometry/Mass Spectrometry.

Results: Plasma C_{max} and AUC values for L-754,030 in dams did not change with ascending doses. Plasma C_{max} and AUC values plateaued over the range of 125 to 1000 mg/kg/day. This observation may indicate a saturation of absorption of the parent drug or increased conversion of the parent compound to a metabolite. At 4 hr post-dosing, the fetal to maternal plasma drug level ratio ranged from 0.104 to 0.139. Placental transfer of L-754,030 was evident and fetal plasma drug concentrations were 10-14% of maternal plasma drug concentrations.

Plasma toxicokinetics of L-754,030 in pregnant female rats on day 20 of gestation. Rats received L-754,030 by oral gavage at doses of 125, 250, 500, and 1000 mg/kg/day from days 6 to 20 of gestation.

Dose, mg/kg/day	C_{max}, µg/mL	T_{max}, hr	$AUC_{0\text{-}24hr}$, µg*hr/mL
125	2.13	6	28.9
250	1.77	8	22.6
500	2.54	6	28.5
1000	2.18	6	25.4

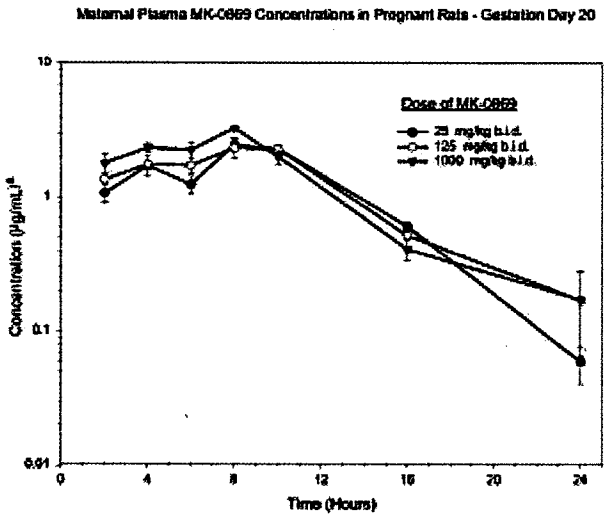
Fetal and maternal plasma concentrations of L-754,030 on day 20 of gestation at 4 hr after dosing. Dams received L-754,030 by oral gavage at doses of 125, 250, 500, and 1000 mg/ kg/day from days 6 to 20 of gestation.

Dose, mg/kg/day	125	250	500	1000
Fetal plasma, µg/mL	0.166	0.142	0.131	0.219
Maternal plasma, µg/mL	1.41	1.37	1.25	1.58
Fetal/Maternal Ratio	0.118	0.104	0.105	0.139

Oral Toxicokinetic Study in Pregnant Rats (Study # 01-732-0):

Methods: An oral toxicokinetic study was conducted with MK-0869 in pregnant Sprague-Dawley rats following administration of the drug from Gestation Day (GD) 6 through GD 20. MK-0869 colloidal dispersions (200 mg/ml) in deionized water, with approximate particle size of — m, were administered to 3 groups of rats at 15 mg/kg b.i.d., 125 mg/kg b.i.d. and 1000 mg/kg b.i.d. doses. On GD 20, blood samples were collected from 4 dams/time point/group at 2, 4, 6, 8, 10, 16 and 20 hours following the first daily dose. Plasma drug concentrations were determined by liquid chromatography – tandem mass spectrometry (lower limit of detection 0.00210 µg/ml). b(4)

Results: Following oral administration of MK-0869 to pregnant rats on GD 6 through GD 20, the mean maximal plasma drug concentration (C_{max}) was achieved 8 hours after administration of the first daily dose on GD 20. The C_{max} values at 25 mg/kg and 125 mg/kg b.i.d. doses were similar, and was slightly higher at the 1000 mg/kg b.i.d dose. The $AUC_{0\text{-}24hr}$ values increased slightly with increasing doses, and were not dose-proportional. Thus, it appears that there is a saturation of absorption at the 25 mg/kg b.i.d. dose. Plasma drug concentrations at different times after dosing are shown in the sponsor's Figure below.



The pharmacokinetic parameters of MK-0869 in pregnant rats are summarized in the sponsor's Table below.

Maternal Plasma MK-0869 Toxicokinetic Parameters
Gestation Day 20

	MK-0869 (mg/kg b.i.d.)		
	25	125	1000
Maternal Toxicokinetic Parameters			
$AUC_{0-24\ hr}$ (µg•hr/mL)[a]	26.8 ± 1.26	28.1 ± 2.29	31.3 ± 1.62
C_{max} (µg/mL)[b]	2.49	2.36	3.30
T_{max} (hr)[c]	8.0	8.0	8.0

[a] Mean ± SEM calculated using all individual plasma concentrations.
[b] Maximum mean plasma concentration.
[c] Time at which C_{max} occurred.

Oral Toxicokinetic Study in Pregnant and Lactating Rats: (Study #01-738-0)

Methods: The study was conducted to determine the concentrations of MK-0869 in maternal and fetal plasma and maternal milk following oral administration of the drug to female Sprague-Dawley rats during gestation and lactation periods. MK-0869 colloidal dispersion (---- particle size) was administered to the animals by oral gavage (1000 mg/kg b.i.d. or 2000 mg/kg/day) on GD 6 to 20 or through lactation Day 14. On GD 20, maternal blood samples were collected from 4 animals/group at 8 and 24 hours after administration of the first daily dose. Fetal blood samples (collected from the

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umbilical vessels) were also collected at the same time and pooled by litter. At 8 hours after the first daily dose on lactation Day 14, maternal plasma and milk samples were collected for determination of drug concentrations. Plasma or milk concentrations of MK-0869 were determined by liquid chromatography/tandem mass spectrometry.

Results: Following oral administration of a 1000 mg/kg b.i.d. dose of MK-0869 to pregnant rats, drug concentrations were detected in the maternal and fetal plasma at both 8 and 24 hours after the first b.i.d. dose on GD 20. The mean placental transfer of the drug was approximately 9% (range 6%-12%) and 26.5% (range 14% to 44%) at 8 and 24 hours, respectively. The transfer of the drug to milk was assessed by the ratio of milk concentration to maternal plasma concentration. On lactation Day 14, the milk transfer of the drug at 8 hours after the first b.i.d. dose was 90% (range 85% to 98%). Maternal and fetal plasma drug concentrations on GD 20 and milk transfer of the drug on lactation Day 14 are summarized in the sponsor's Table below.

Maternal Plasma MK-0869 Toxicokinetic Parameters
Placental Transfer - Gestation Day 20
Milk Transfer - Lactation Day 14
(Values are the Mean ± SEM)

	MK-0869 (mg/kg b.i.d.)
	1000
Gestation Day 20	
Maternal Plasma Conc. (µg/mL)[a]	
8 hours	2.59 ± 0.428
24 hours	0.104 ± 0.0348
Fetal Plasma Conc. (µg/mL)[a]	
8 hours	0.228 ± 0.0348
24 hours	0.0223 ± 0.00409
Fetal/Maternal Plasma Ratio[b]	
8 hours	0.0910 ± 0.0124
24 hours	0.265 ± 0.0727
Lactation Day 14	
Maternal Plasma Conc. (µg/mL)[a]	
8 hours	5.42 ± 0.702
Milk Conc. (µg/mL)[a]	
8 hours	4.84 ± 0.582
Milk/Maternal Plasma Ratio[c]	0.898 ± 0.0295

[a] Values are the mean ± SEM.
[b] Values are the mean ± SEM of the individual fetal plasma concentration divided by the corresponding maternal plasma concentration.
[c] Values are the mean ± SEM of the individual milk concentration divided by the corresponding maternal plasma concentration.

Intravenous Toxicokinetic Study with L-758298 in Pregnant and Lactating Rats: (Study #97-741-0)

Methods: The study was conducted to determine the concentrations of L-754, 030 in maternal and fetal plasma and maternal milk following i.v. administration of L-758, 298 (a pro-drug of L-754, 030; 4 mg/kg/day) to female Sprague-Dawley rats during gestation and lactation periods. L-758, 298 (in normal saline) was administered to pregnant animals by infusion into a tail vein on GD 6 to 20 or through lactation Day 14. On GD 20, maternal blood samples were collected from 5 animals/group at 4 and 8 minutes and 1, 5, 10 and 24 hours after administration of the dose. Fetal blood samples (collected from the umbilical vessels) were collected at 1 hr timepoint and pooled by litter. On lactation Day 14, at 8 hours after dosing, maternal plasma and milk samples were collected for determination of drug concentrations. Plasma or milk concentrations of L-754, 030 were determined by liquid chromatography/tandem mass spectrometry.

Results: Following i.v. administration of a 4 mg/kg/day dose of L-758, 298 (a pro-drug of L-754, 030) to pregnant rats, L-754, 030 concentrations were observed in maternal and fetal plasma at 1 hour after dosing on GD 20. The mean fetal to maternal plasma drug concentration ratio was 0.0992. The transfer of the drug to milk was assessed by the ratio of milk concentration to maternal plasma concentration. On lactation Day 14, the milk transfer of drug at 1 hour after dosing was 111.5% (range 101% to 121%). Maternal and fetal plasma L-754, 030 concentrations on GD 20 and milk transfer of the drug on lactation Day 14 are summarized in the Table below.

Maternal and fetal plasma L-754, 030 concentrations and transfer of the drug to milk.

Maternal plasma concentration	0.905 ± 0.0649 µg/ml
Fetal plasma concentration	0.0898 ± 0.00715 µg/ml
Fetal/maternal plasma concentration ratio	0.0992 (0.0947 – 0.105
Plasma concentration	0.733 ± 0.0250 µg/ml
Milk concentration	0.814 ± 0.0138 µg/ml
Milk/Plasma concentration ratio	1.11 (1.01 – 1.21)

Following i.v. administration of L-758, 298 (a pro-drug of L-754, 030) to pregnant and lactating rats, L-754, 030 concentrations were detected in fetal plasma and in the milk of lactating mothers. Fetal plasma concentration was about $1/10^{th}$ of that of maternal plasma concentration at 1 hour after dosing on GD 20. The concentration in the milk was slightly higher than the plasma concentrations of lactating mothers.

Intravenous Toxicokinetic Study with L-758, 298 in Pregnant Rats: (Study #98-708-0)

Methods: The study was conducted to determine the toxicokinetic profiles of L-758, 298 and its *in vivo* hydrolysis product, L-754, 030 in maternal plasma following i.v. administration of L-758, 298 (4 mg/kg/day) to pregnant Sprague-Dawley rats. L-758, 298 (in normal saline) was administered to the animals by infusion into a tail vein on GD 6 through 20. On GD 20, maternal blood samples were collected from 6 animals/group at 4 and 8 minutes and 1, 5, 10 and 24 hours after administration of the dose. Plasma concentrations of L-758, 298 and L-754, 030 were determined by liquid chromatography/tandem mass spectrometry. The $AUC_{0\text{-}24h}$, C_{max} and T_{max} values were calculated from the plasma concentrations.

Results: Following i.v. administration of a 4 mg/kg/day dose of L-758, 298 to pregnant rats, the drug was rapidly hydrolyzed to L-754, 030. The plasma concentration of L-758, 298 at 4 minutes was 0.0155 μg/ml, and declined to 0.00715 μg/ml at 8 minutes. At 1 hour, the plasma concentrations of L-758, 298 were below the detection limit of 0.00126 μg/ml. The AUC value for L-758, 298 was 0.00437 μg.hr/ml. The maximum plasma concentrations (C_{max}) for L-754, 030 were observed at 8 minutes after dosing. The C_{max} for L-754, 030 was about 125-fold higher than that of L-758, 298 (pro-drug), suggesting a very rapid hydrolysis of L-758, 298. In 24 hours after dosing >98% of L-754, 030 was cleared from the plasma. The plasma exposure level ($AUC_{0\text{-}24hr}$) for L-754, 030 was 10.3 μg.hr/ml, which is approximately 2400-fold higher than that of L-758, 298. The Toxicokinetic parameters for L-758, 298 and L-754, 030 in pregnant rats on Gestation Day 20 are summarized in the Table below.

Parameters	L-758, 298	L-754, 030
$AUC_{0\text{-}24hr}$ (μg.hr/ml)	0.00437	10.3
C_{max} (μg/ml)	0.0155	1.93
T_{max} (hr)	0.0667	0.133

Following i.v. administration of L-758, 298 to pregnant rats, it was rapidly hydrolyzed to the active drug, L-754, 030. The C_{max} and AUC values for L-754, 030 were 125- and 2400- fold higher than that of L-758, 298, respectively.

Oral Toxicokinetic Study in Pregnant Rabbits (Report Date/Number TT #97-736-0).

Methods: Concentrations of L-754,030 in maternal and fetal plasma were determined following oral administration of l-754,030 to New Zealand White pregnant female rabbits. Pregnant dams received L-754,030 by oral intubation at doses of 5 and 25 mg/kg/day from days 7 to 21 of gestation. There were 6 pregnant dams/dose. On day 20 of gestation, blood for determination of maternal plasma drug concentrations was collected at 2, 4, 8, 12, and 24 hr after dosing. Blood was collected from 4 pregnant dams per time point. Following the collection of the 24-hr time point on day 21, pregnant dams were treated with L-754,030. At 4 hr after dosing on day 21, females were anesthetized, blood was collected from the vena cava, and the uterus from each selected female was removed. Fetuses were excised and blood was collected from the great vessels. L-754,030 was isolated by solid phase extraction and quantified by Liquid Chromatography/Mass Spectrometry/Mass Spectrometry.

Results: Plasma C_{max} and AUC values for L-754,030 in pregnant dams were approximately proportional to dose. At 4 hr post-dosing, the fetal to maternal plasma drug level ratios were 0.345 and 0.564 with maternal doses of 5 and 25 mg/kg/day, respectively. Placental transfer of L-754,030 was evident and fetal plasma drug concentrations were 34.5 and 56.4% of maternal plasma drug concentrations at doses of 5 and 25 mg/kg/day, respectively.

Plasma toxicokinetics of L-754,030 in pregnant female rabbits on day 20/21 of gestation. Rabbits received L-754,030 by oral intubation at doses of 5 and 25 mg/kg/day from days 7 to 21 of gestation.

Dose, mg/kg/day	C_{max}, μg/mL	T_{max}, hr	$AUC_{0\text{-}24hr}$, μg*hr/mL
5	0.390	5.00	4.73
25	1.55	16.0	26.9

Fetal and maternal plasma concentrations of L-754,030 on day 21 of gestation at 4 hr after dosing. Dams received L-754,030 by oral gavage at doses of 5 and 25 mg/kg/day from days 7 to 21 of gestation.

Dose, mg/kg/day	**5**	**25**
Fetal plasma, µg/mL	0.0820	0.790
Maternal plasma, µg/mL	0.238	1.40
Fetal/Maternal Ratio	0.345	0.564

2.6.4.9 Discussion and Conclusions

Fosaprepitant (L-758,298; pro-drug) was rapidly converted to aprepitant in *in vitro* liver preparations from rat, dog and humans. In addition to liver, the conversion of the pro-drug to active drug can take place in extrahepatic tissues. Following i.v. administration to rats and dogs and humans, L-758,298 was rapidly converted to its active metabolite. After i.v. administration, the half-life of aprepitant was shorter in rats (3 hr) when compared to that of ferrets (10 hr) and humans (9-13 hours). MK-0869 was a weak substrate and inhibitor of P-glycoprotein. It had high binding affinities for plasma proteins from rats, dogs and humans (>98%). It penetrated the blood brain barrier in rats and ferrets following i.v. and oral administration, and was excreted in the milk of lactating rats following oral administration. Thus, fosaprepitant should be administered to lactating mothers with caution. CYP3A4 was the major CYP enzyme involved in the metabolism of MK-0869. Two other CYP enzymes, CYP1A2 and CYP2C19 were also involved in the metabolism of the compound. Aprepitant is also an activator of several drug metabolizing enzymes, an inhibitor of CYP3A4. Thus, there is a potential for drug-drug interaction of fosaprepitant with drugs metabolized by these enzymes.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

N/A

2.6.5 TOXICOLOGY

Study title: L-758298: Acute Oral and IntravenousToxicity Studies in Mice and Rats

Sponsor's ID # TT #95-2584, TT #95-2585, TT #95-2586, TT #95-2587

Conducting laboratory (and location if not Sponsor): Merck Research Laboratories, West Point, PA.

Dates of study initiation & completion: April 05, 1995 & July 17, 1995

GLP compliance: Yes

QA Report Yes (X) **No** ( )

Methods:

Species/strain: Mouse: (———) BR mice, 6 to 7 weeks old. b(4)

Rat: ——— BR rats, 6 to 7 weeks old.

Single doses of L-758298 was intravenously or orally administered to groups of mice and rats (3 animals/group) at the following doses: Mouse, oral – **500 mg/kg; mouse, intravenous – 200 mg/kg and 500 mg/kg; rat, oral – 500 mg/kg; rat, intravenous – 200 mg/kg and 500 mg/kg. The drug was dissolved** in normal saline for both intravenous and oral administration. The animals were observed daily for 14 days for clinical signs and mortality. Body weights of the animals were measured before initiation of dosing and on Days 7 and 14.

Results:

Mice: Oral administration of a single 500 mg/kg dose to mice was not associated with any treatment-related clinical signs or mortality. Intravenous administration of the 500 mg/kg dose produced death in 1 of 3 mice within 12 minutes of dosing. There were no deaths at the 200 mg/kg dose. Animals receiving the i.v. doses exhibited gasping, kicking convulsions, and bradypnea during dosing. Immediately after dosing of the 500 mg/kg dose, decreased activity and loss of righting reflex were observed while gasping and kicking convulsions ceased. Thus, the i.v. minimal lethal dose (MLD) in mice was 500 mg/kg, and the oral MLD is not known.

Rats: In rats, oral administration of L-758298 at a single dose of 500 mg/kg was not associated with any clinical signs or mortality. Intravenous administration of the 500 mg/kg dose caused death within 8 minutes preceded by gasping and bradypnea. Because of the rapid death of the animal, no additional animals were administered the 500 mg/kg dose. Thus, the i.v. MLD in rats was <500 mg/kg, and the oral MLD is not known.

The mortalities for different groups of mice and rats following i.v. or oral administration of L-758298 are summarized in the Table below.

TABLE 1. L-758,298: Acute Oral and Intravenous Toxicity Studies in Mice and Rats.
TT #95-2584, TT #95-2585, TT #95-2586, TT #95-2587

Mortality

Route of Administration	Species and Sex	Vehicle* % Conc.	Dose mg/kg	No. of Animals	No. of Deaths	ALD_{50} mg/kg	Time of Deaths
Oral TT #95-2584	Mouse Female	* 1%	500	3	0	> 500	No deaths occurred
Intravenous TT #95-2585	Mouse Female	* 1%	500	3	1	> 500	12 minutes
			200	3	0		
Oral TT #95-2586	Rat Female	* 1%	500	3	0	> 500	No deaths occurred
Intravenous TT #95-2587	Rat Female	* 1%	500	1	1		8 minutes
			200	3	0	> 200	

* = Vehicle used was 0.9% saline.

Study title: L-754030: Acute Oral and Intraperitoneal Toxicity Studies in Mice and Rats

Sponsor's ID # TT #95-2686, TT #95-2687, TT #95-2688, TT #95-2689

Conducting laboratory (and location if not Sponsor): Merck Research Laboratories, West Point, PA.

Dates of study initiation & completion: August 25, 1995 & October 19, 1995

GLP compliance: Yes

QA Report Yes (X) **No** ( )

Methods:

Species/strain: Mouse: [redacted] BR mice, 6 to 7 weeks old. b(4)

Rat: [redacted] BR rats, 6 to 9 weeks old.

Single doses of L-754030 (lot # 8, purity 99.4%) was intraperitoneally or orally administered to groups of mice and rats (3 female animals/group) at the following doses: **Mouse, oral – 2000 mg/kg; mouse, intraperitoneal – 2000 mg/kg and 500 mg/kg; rat, oral – 2000 mg/kg; rat, intraperitoneal – 800**

mg/kg and 2000 mg/kg. The drug was suspended in 0.02% sodium lauryl sulfate/0.5% aqueous methylcellulose for intraperitoneal or oral administration in both mice and rats. The animals were observed daily for 14 days for clinical signs and mortality. Body weights of the animals were measured before initiation of dosing and on Days 7 and 14.

Results:

Mice: Oral or intraperitoneal administration of a single 2000 mg/kg dose to mice was not associated with any treatment-related clinical signs or mortality. Thus, the intraperitoneal or oral minimal lethal dose (MLD) of L-754030 in mice was not known.

Rats: In rats, oral administration of L-754030 at a single dose of 2000 mg/kg was not associated with any clinical signs or mortality. One of 3 rats administered the intraperitoneal dose of the 2000 mg/kg exhibited decreased activity on Day 2, and labored breathing and urine staining on Day 3. The signs lasted until death of the animal on Day 9. No clinical signs or mortality was observed in animals receiving the 800 mg/kg intraperitoneal dose.

Thus, the intraperitoneal MLD was 2000 mg/kg in rats, and the oral MLD was not known. However, as only female animals were used in the study, the MLD in males is not known.

Study title: L-754030: Exploratory Acute Oral Toxicity Study in Mice

Sponsor's ID # TT #96-2583

Conducting laboratory (and location if not Sponsor): Merck Research Laboratories, West Point, PA.

Dates of study initiation & completion: April 02, 1996 & June 03, 1996

GLP compliance: Yes

QA Report Yes ( ) No (X)

Methods:

Species/strain: (———) BR mice, approximately 23 weeks old. b(4)

L-754030 (lot # 1, purity 99.7%) was administered to three female mice at a single dose of 500 mg/kg. The drug was suspended (2%) in 0.5% aqueous methylcellulose. The animals were observed daily for 7 days for clinical signs and mortality. The study was terminated on Day 7 without any further investigation.

Results: Oral administration of a single 500 mg/kg dose of L-754030 to mice was not associated with any treatment-related clinical signs or mortality. Thus, the minimal lethal dose (MLD) of L-754030 was not known. The animals were observed for only 7 days, instead of 14 days, following administration of the dose.

Study title: L-758298: Acute Oral Toxicity Study in Mice

Sponsor's ID # TT #96-2585

Conducting laboratory (and location if not Sponsor): Merck Research Laboratories, West Point, PA.

Dates of study initiation & completion: April 02, 1996 & April 09, 1996

GLP compliance: Yes

QA Report Yes ( ) No (X)

Methods:

Species/strain: ———— BR mice, approximately 126 days old. b(4)

L-758298 (lot # L-758298-003C009, purity 96.3%) was administered to three female mice at a single dose of 500 mg/kg (25 ml/kg). The drug was suspended (2%) in 0.5% aqueous methylcellulose. The animals were observed daily for 7 days for clinical signs and mortality. The study was terminated on Day 7 without any further investigation.

Results: Oral administration of a single 500 mg/kg dose of L-758298 to mice was not associated with any treatment-related clinical signs or mortality. Thus, the minimal lethal dose (MLD) of L-758298 was not known. The animals were observed for only 7 days, instead of 14 days, following administration of the dose.

2.6.6.3 Repeat-dose toxicity

Study Title: L-000758298: Seventeen (17)-Day Intravenous Toxicity Study in Rats

Study no. TT#04-6005

Conducting laboratory (and location if not Sponsor): Merck Research Laboratories, Route de Marsat, Riom, 63963 Clermont-Ferrand Cedex 9, France.

Dates of study initiation & completion: February 26, 2004 and March 15, 2004.

GLP compliance: Yes

QA Report Yes (X) **No** ( )

Drug, Lot #, radiolabel (if applicable), and % purity: L-000758298; Lot # L-000758298-003C016; purity 99.6%.

Formulation/vehicle: L-000758298 lyophilized formulation was reconstituted in sterile 0.9% NaCl solution for injection.

Methods: L-000758298 was administered intravenously to Sprague Dawley rats at dose levels of 2.5, 5.0 or 7.5 mg/kg/day for 2 weeks. The males received 14 doses, and the females received 14 or 15 doses depending on the schedule of sacrifice. The 7.5 mg/kg/day surviving animals were terminated on Day 9 and received only 8 doses.

Dosing:

Species/strain: Sprague Dawley rats

#/sex/group or time point (main study): 15 animals/sex/group were used in the study.

Satellite groups used for toxicokinetics or recovery: N/A

Weight: males – 262 to 313 g; females – 167 to 203 g.

Age: 55 to 56 days

Doses in administered units: L-000758298 was administered at i.v. doses of 2.5, 5 and 7.5 mg/kg/day.

Route, form, volume and infusion rate: The doses were administered intravenously at dosing volumes of 0.1, 0.2 and 0.3 ml/kg for low, mid and high doses, respectively.

Times at which Observations were made:

Clinical signs- The animals were observed daily for clinical signs and mortality. In addition, each animal was given a detailed physical examination at the time of dosing.

Body weights- Body weights were measured once in Week 1 and twice in Week 2 of the dosing period.

Food and water consumption- Male and female animals received 22 and 16 g/day of [redacted] dies, respectively. The cages were watched twice weekly before dosing to determine whether any food was remaining. b(4)

Ophthalmoscopy: Ophthalmologic examinations (indirect ophthalmoscopy and slit lamp bimicroscopy) were performed on all control and 5.0 mg/kg/day group animals in week 2 of the dosing period.

Hematology: Blood samples for hematology were collected in week 2 of the dosing period.

Clinical chemistry- Blood samples for clinical chemistry analyses were collected from all animals in week 2 of the dosing period.

Urinalysis- Urine samples were collected from 10 animals/sex/group in week 2 of the dosing period.

Gross pathology- At the end of the dosing period or early termination, the animals were sacrificed and a complete necropsy examination was performed on all animals.

Organs weighed- The weights of the following organs were recorded.

Adrenals, brain, heart, kidneys, liver, ovary, pituitary, prostate, spleen, testes and thyroid.

Histopathology- Following organs from the control and high dose animals were fixed and examined microscopically. In addition, organs with gross changes from the 2.5 mg/kg/day group were also examined histologically.

Salivary gland, esophagus, stomach, small intestine (duodenum, jejunum, ileum), large intestine (colon), liver, pancreas, adrenals, pituitary, thyroid, parathyroid, kidneys, urinary bladder, ovaries, uterus, testes and epididymides, prostate, skin, mammary gland, lung, heart, spleen lymph nodes, thymus, bone marrow, bone, skeletal muscle, brain, spinal cord, peripheral nerve, eye, Harder's **gland and injection sites (tail** veins).

Toxicokinetics- Blood samples were collected from 3 or 4 animals/sex/time point at approximately 2, 5, 15 and 30 minutes and 1, 4, 8 and 24 hours post-dose in week 2 of the dosing period.

Results:

Mortality: From Day 4 to Day 7, 3 males from the mid dose, and 3 females and 8 males from the high dose group were sacrificed because dosing was not feasible due to treatment-related changes at the injection sites of the tail. The high dose group was terminated early on Day 9 of the treatment period.

Clinical signs: Treatment-related changes were observed at the injection sites of the tails at all doses. There was a treatment-related increased incidence of swelling and/or cutaneous discoloration and desquamation at low (up to 33% of the animals), mid (up to 100% males and 73% females) and high (up to 100% males and females) doses. Loss of 2.5 cm to 4 cm tip of the tail was observed in 2 males from the mid dose group, and 3 males and 1 female from the high dose group.

Body weights: The mean body weights of the control male and female animals before initiation of dosing were 280±12 and 188±9 g and on day 11 were 294±12 and 196±6 g, respectively. Compared to controls, decreased mean body weight gain was observed in females (up to 30%) at all doses, following 1 week of dosing. The body weight gains of females receiving 2.5, 5 and 7.5 mg/kg/day doses were suppressed by 20%, 30% and 30%, respectively, during the dosing period.

Food consumption: No treatment-related changes in the food consumption were observed in any group.

Ophthalmoscopy: No treatment-related ophthalmologic abnormalities were observed in any group.

Hematology: The high dose animals had slightly decreased erythrocytes, hemoglobin and hematocrit levels, and increases in reticulocytes, absolute neutrophil, monocyte and platelet counts. Hematological changes in high dose male and female animals are shown in the Table below.

Treatment-Related Hematological Changes – Drug Week 2
(Percent Differences in Mean Values
From Concurrent Controls)

Parameter	L-000758298 (mg/kg/day)					
	Females			Males		
	2.5	5.0	7.5	2.5	5.0	7.5
Erythrocyte count	-	-	-5	-	-	-14
Hemoglobin concentration	-	-	-5	-	-	-15
Hematocrit	-	-	-4	-	-	-13
Reticulocytes	-	-	+90	-	-	+117
Absolute neutrophil count	-	-	+81	-	-	+252
Absolute monocyte count	-	-	+86	-	-	+68
Platelet count	-	-	I	-	-	+43
- = No treatment-related change. I = Increased based on individual animal values.						

Clinical chemistry: High dose male and female animals had a decrease in mean albumin levels and the albumin/globulin ratio in week 2 of dosing. The changes in mean albumin levels and albumin/globulin ratios are shown in the Table below.

Treatment-Related Serum Biochemical Changes – Drug Week 2
(Percent Differences in Mean Values
From Concurrent Controls)

Parameter	L-000758298 (mg/kg/day)					
	Females			Males		
	2.5	5.0	7.5	2.5	5.0	7.5
Albumin	-	-	-9	-	-	-12
Albumin/globulin ratio	-	-	-17	-	-	-18
- = No treatment-related change.						

Urinalysis: No treatment-related changes in urinalysis were observed in any group.

Gross Pathology: Treatment related gross pathological changes were observed at the injection sites of the tail from animals from all groups. These included blackish discoloration of the tail injection site and the presence of obstructive thrombosis.

Organ weight: No treatment related changes in any organ weight were observed.

Histopathology: Histopathological examinations of all organs were conducted only of the control and the high dose animals, except injection sites and gross changes from the low dose group. Treatment related histopathological changes were limited to the injection site of all groups, and consisted of cellular proliferation of venous intima, venous necrosis or thrombosis, skin necrosis, subcutaneous edema, cellular infiltration or fibroplasia and degeneration of muscle fibers. Histopathological changes observed at the injection sites of male and female rats are shown in the Table below.

Histomorphologic Changes
(Incidence, n = 15, except for tissues
examined at the discretion of the pathologist)

	L-000758298 (mg/kg/day)							
	Females				Males			
	Control	2.5	5.0	7.5	Control	2.5	5.0	7.5
Injection Site								
Vein								
Intima, cellular proliferation	0	6[a]	8[a]	4[a]	0	2[a]	3[a]	0
Necrosis	1	3	8[a]	7[a]	3	3	8[a]	10[a]
Thrombosis	1	3[a]	7[a]	6[a]	0	4[a]	11[a]	12[a]
Skin								
Necrosis	0	1[a]	4[a]	11[a]	0	2[a]	8[a]	12[a]
Subcutis								
Edema	0	2[a]	7[a]	14[a]	0	5[a]	11[a]	14[a]
Cellular infiltration	4	1	5	8[a]	3	1	9[a]	10[a]
Fibroplasia	2	4	11[a]	13[a]	1	5[a]	11[a]	11[a]
Muscle Fiber								
Degeneration	0	2[a]	3[a]	1[a]	0	2[a]	5[a]	0
Tail		NE	NE		NE			
Necrosis	1[b]			4[b,c]		2[b]	9[b, c]	8[b, c]
Iliac Lymph node	NE	NE		NE	NE	NE	NE	NE
Reactive hyperplasia			3[b, c]					

[a] Treatment-related changes based on incidence and/or severity.
[b] Tissue examined at the discretion of the pathologist.
[c] Change secondary to treatment.
NE = Not examined.

Toxicokinetics: Following i.v. administration of L-000758298 to male and female rats, the C_{max} and AUC values for the parent compound increased with increasing doses, but were more than dose-proportional. The C_{max} and AUC values for the parent compound were slightly higher in the males as compared with the females, while the plasma concentrations and exposure levels for the active metabolite (L-000754030) were higher in females than in males. This indicates that the conversion of L-000758298 to L-000754030 is faster in females than in males, or there may be faster clearance in males than in females. The toxicokinetic parameters in male and female rats for L-000758298 (pro-drug) and L-000754030 (active compound) are shown in the Tables below.

Mean Plasma L-000758298 Toxicokinetic Parameters – Drug Week 2

	L-000758298 (mg/kg/day)	
	Females	
	2.5	5
$AUC_{0\text{-}24\ hr}$ (µg•hr/mL)[a]	0.00404 ± 0.00138	0.0224 ± 0.0145
C_{max} (µg/mL)[a]	0.0970 ± 0.0331	0.487 ± 0.347
T_{max} (hr)	0.0333	0.0333
	Males	
	2.5	5
$AUC_{0\text{-}24\ hr}$ (µg•hr/mL)[a]	0.00603 ± 0.000887	0.0312 ± 0.0130
C_{max} (µg/mL)[a]	0.145 ± 0.0213	0.630 ± 0.305
T_{max} (hr)	0.0333	0.0333
	Sexes Combined	
	2.5	5
$AUC_{0\text{-}24\ hr}$ (µg•hr/mL)[a]	0.00503 ± 0.000846	0.0262 ± 0.00927
C_{max} (µg/mL)[a]	0.121 ± 0.0203	0.548 ± 0.220
T_{max} (hr)	0.0333	0.0333
[a] Values are the mean ± SEM.		

Mean Plasma L-000754030 Toxicokinetic Parameters – Drug Week 2

	L-000754030 (mg/kg/day)	
	Females	
	2.5	5
$AUC_{0\text{-}24\ hr}$ (µg•hr/mL) [a]	2.62 ± 0.431	5.53 ± 0.499
C_{max} (µg/mL) [a]	0.767 ± 0.0525	1.54 ± 0.571
T_{max} (hr)	0.0333	0.0333
	Males	
	2.5	5
$AUC_{0\text{-}24\ hr}$ (µg•hr/mL)[a]	1.26 ± 0.0961	2.39 ± 0.251
C_{max} (µg/mL) [a]	1.06 ± 0.121	1.97 ± 0.359
T_{max} (hr)	0.0333	0.0333
[a] Values are the mean ± SEM.		

Summary: In the 17–day intravenous toxicity study with L-000758298 in rats, the drug was administered at doses of 0, 2.5, 5.0 and 7.5 mg/kg/day. The high dose (7.5 mg/kg/day) group was terminated early on Day 9, because dosing was not feasible due to treatment-related changes at the injection sites of the tail. Treatment group females had decreased body weight gains when compared with the controls. High dose males and females had decreased erythrocytes, hemoglobin and hematocrit levels, and increased reticulocytes, neutrophil and monocyte counts. Changes at the injection site were observed in all groups, and consisted of cellular proliferation of venous intima, venous necrosis or thrombosis, skin necrosis, subcutaneous edema, cellular infiltration or fibroplasia and degeneration of muscle fibers. Thus, the injection site was the target organ of toxicity, the NOAEL was not established.

MK-0869: 16-Day Intravenous Toxicity Study in Rats (TT #99-004-0).

Testing Laboratory: Merck Institute for Therapeutic Research
Merck Research Laboratories
Merck & Co., Inc.
West Point, PA 19486

Date Started: January 11, 1999

Date Completed: June 16, 1999

GLP Compliance: Statements of compliance with GLP regulations and the Quality Assurance Unit were included.

Animals: Sprague-Dawley rats [BR] were used in this study. At the start of treatment, animals were 57 days of age and had body weight ranges of 225-276 g for male rats and 159-210 g for female rats. b(4)

Drug Batch: MK-0869, Lot Number L-754,030-004H032 (rug particle size)

Methods: In a 16-day intravenous toxicity study, rats received MK-0869 (m drug particle size) at doses of 0, 80, 160, and 240 µg/kg/day. There were 15 rats/sex/group. Sterile dosing solutions were supplied pre-formulated in vehicle ((anhydrous), ng/mL polysorbate 80, mg/mL NaOH, pH of and an osmolality of mOsm) at a MK-0869 concentration of 20 µg/mL. MK-0869 at 20 µg/mL was the maximum feasible concentration in this vehicle solution. The intravenous dosing volumes for the 0, 80, 160, and 240 µg/kg/day groups were 12, 4, 8, and 12 mL/kg, respectively. Control animals received the vehicle, designated as L-931,175, which consisted of 2.50 mg/mL polysorbate 80, 8.20 mg/mL with a pH of and an osmolality of mOsm. Male and female rats received in quantities of 22 and 16 g/animal/day, respectively. Animals were observed for clinical signs of toxicity and mortality on a daily basis. Body weights were measured prior to the start of treatment, once in week 1, and twice in week 2. For assessment of food consumption, cages were examined twice weekly during weeks 1 and 2 at approximately 24 hr after dosing to determine whether any food was remaining. Ophthalmic examinations were performed for all animals in the control and 240 µg/kg/day groups in week 2. Blood for determination of hematology and serum biochemical parameters was collected in week 2. Urine samples for urinalysis were collected overnight from 10 rats/sex/group in drug week 2. All rats surviving to scheduled termination were sacrificed and subjected to a complete necropsy. Absolute and relative organ weights were determined for the adrenal glands, brain, heart, ovaries, kidneys, liver, pituitary, prostate, spleen, testes, and thyroid gland. The testes and epididymides from all male rats were fixed in Bouin's solution. Remaining tissues from all animals were fixed in 10% neutral buffered formalin. Tissues from the control and 240 µg/kg/day groups as well as animals that died during the treatment period were prepared by routine methods, stained with hematoxylin and eosin, and submitted to microscopic examination as follows: salivary gland (submandibular/sublingual), esophagus, stomach (glandular and nonglandular portions), small intestine (duodenum, jejunum, ileum), large intestine (colon), liver, pancreas, adrenal glands, parathyroid gland (when present in b(4)

thyroid tissue section), pituitary gland, skin (from mammary region), mammary gland (when present in skin section), lung, heart, spleen, lymph nodes (cervical, pancreatic, and mesenteric), thymus, bone marrow (in bone section), bone (femur, including femorotibial joint), skeletal muscle, thyroid gland, kidneys, urinary bladder, ovaries, uterus, testes and epididymides, prostate, brain (including cerebral cortex, and subcortical white matter, basal ganglia, cerebellum, and pons), spinal cord (cervical), peripheral nerve (sciatic), eye (with optic nerve), Harder's gland, and injection site. Gross abnormalities from all animals were processed in a similar manner and submitted to microscopic examination.

Results:

1. **Observed Effects:** There were no treatment-related observed effects.

2. **Mortality:** There was no treatment-related mortality. One control female rat was found dead on day 7. The cause of death was unknown.

3. **Body Weight and Food Consumption:** Body weight gains of female rats at 160 and 240 µg/kg/day were impaired by >10%. Body weights of male controls on days -1 and 14 were 247 and 282 g, respectively. Body weight gains of male rats at 80, 160, and 240 µg/kg/day were 114.8, 98, and 91.7% of the control, respectively. Body weights of female controls on days -1 and 14 were 177 and 190 g, respectively. Body weight gains of female rats at 80, 160, and 240 µg/kg/day were 112.9, 83.7, and 81.4 % of the control, respectively.

4. **Hematology:** There were no treatment-related changes of hematological parameters.

5. **Serum Biochemistry and Urinalysis:** There were no treatment-related changes of serum biochemical of urinalysis parameters.

6. **Ophthalmic Examination:** There were no treatment-related ophthalmic effects.

7. **Organ Weights:** Absolute and relative thyroid gland weights for female treatment groups were increased to 106-117% and 105-112% of control values (0.0108 g and 0.0060% B.W.), respectively; however, there were no corresponding histopathological changes.

8. **Gross Pathology:** There were no reported treatment-related gross pathological changes.

9. **Histopathology:** There were no treatment-related histopathological changes.

In a 16-day intravenous toxicity study, rats received MK-0869 —— drug particle size) at doses of 0, 80, 160, and 240 µg/kg/day. The no effect dose appeared to be 240 µg/kg/day. Solubility of MK-0869 in the vehicle limited the amount of drug that could be administered by the intravenous route. Therefore, doses used appeared to be inadequate to assess the toxicity of MK-0869 when administered by the intravenous route. Body weight gains of female rats at 160 and 240 µg/kg/day were impaired by >10%; however, there were no effects on corresponding male treatment groups. A target organ of toxicity was not identified. b(4)

1. 4-Week Intravenous Toxicity Study of L-758,298 (Study TT #95-607-0)

Testing Laboratory: Laboratory Merck Sharp & Dohme-Chibret
Centre de Recherche
Riom, France

Compliance with Good Laboratory Practice and Quality Assurance Requirements: Sponsor provided statements of compliance.

Date Study Started: March 14, 1995

Date Study Completed: August 7, 1995

Animals: Male (body weight range of 215 to 276 g; 7 weeks of age) and female (body weight range of 163 to 222 g; 7 weeks of age) Sprague-Dawley rats.

Methods: Four groups of 30 rats each (15 males and 15 females) were intravenously administered 0, 0.25, 1 and 4 mg/kg/day of L-758,298, respectively, for 4 weeks via the caudal vein. The basis for dose selection was not provided by the sponsor. Vehicle was 0.9% Sodium Chloride Injection, USP. Dosing volume was 10 ml/kg, not exceeding an injection rate of 6 ml/min.

Mortality and clinical signs of toxicity were observed daily. Body weight was measured once during pretest, once during Week 1 and twice a week thereafter. Food consumption was measured twice a week.

Blood samples for hematology and blood chemistry examination were withdrawn via the orbital sinus under ether anesthesia at 24 hrs post-dosing from all rats on Day 7 or 8 and on Day 23, 24 or 25. Urine samples for urinalysis were collected overnight from 10 rats/sex/group during Week 4.

Ophthalmic examinations were performed on rats receiving 0 and 4 mg/kg of L-758,298 on Day 22.

Blood samples for future determination of L-758,298 plasma levels were collected via the orbital sinus on Day 28 at 0.067, 1.33, 1, 2, 5 and 7 hrs post dose from 4 rats/sex/timepoint. Blood samples were collected at 10 and 24 hrs postdose from 3 rats/sex/timepoint.

All rats underwent complete necropsies at scheduled termination. (Details were not provided by the sponsor.) Organ weights of brain, pituitary, spleen, heart, kidney, liver, adrenal, thyroid, testis/ovary and prostates were determined for all animals.

Sections of the following tissues from all control and high-dose rats were prepared by routine methods, stained with hematoxylin and eosin, and examined microscopically: injection site, salivary gland, stomach, small intestine, large intestine, liver, pancreas, adrenal, thyroid, parathyroid, pituitary, kidney, urinary bladder, ovary/testis, uterus/prostate, skin, mammary gland, lung, heart, spleen, lymph node, thymus, skeletal muscle, bone, bone marrow, brain, cervical spinal cord, sciatic nerve, eye and Harder's gland. Tissues with gross pathology were microscopically examined for all animals.

Results:

1. Observed Effects: There were no treatment-related clinical signs of toxicity.

2. Mortality: There were no deaths in this study.

3. Body Weight: Mean body weights of control males were 244 and 303 g during Weeks 1 and 4, respectively. Mean body weights of control females were 181 and 199 g during Weeks 1 and 4, respectively. There were no treatment-related effects on mean body weights.

4. Food Consumption: Although the sponsor stated that there were no treatment-related effects on food consumption, individual data for food consumption could not be located in the submission.

5. Hematology: There were no treatment-related effects on hematology parameters.

6. Blood Chemistry: There were no treatment-related effects on blood chemistry parameters.

7. Urinalysis: There were no treatment-related effects on urinalysis parameters.

8. Ophthalmic Examination: There were no treatment-related ophthalmic effects.

9. Organ Weights: There were no treatment-related effects on organ weights.

10. Gross Pathology: There were no treatment-related gross pathological lesions.

11. Histopathology: There were no treatment-related histopathological lesions.

12. Plasma Levels of Drug: Sponsor stated that data for plasma levels of L-758,298 will be provided at a future date.

In summary, the no effect i.v. dose of L-758,298 was 4 mg/kg/day in the rat. Organs of toxicity were not identified.

Toxicokinetics: In this study, animals were treated with L-758, 298 at 0.25, 1, and 4 mg/kg i.v. doses. On Day 28, animals were bled at 4 min, 8 min, 1, 2, 5, and 7 hours postdose (4 rats/sex/timepoint) and 10 and 24 hours postdose (3 rats/sex/timepoint) for determination of L-758, 298 (pro-drug) and L-754, 030 (active drug) by liquid chromatography/tandem mass spectrometry (LC/MS/MS) method. Maximum mean plasma concentrations of the active drug, L-754, 030, were attained very rapidly (within 4 minutes postdose). The mean plasma Cmax increased in a less than dose-proportional manner. This could be due to saturation of the dephosphorylation pathway. The systemic exposure or $AUC_{0\text{-}24h}$ also increased in a less than dose-proportional manner. Apparently, the females showed higher (2 to 4-fold) exposure to active drug than males. This could be attributed to either sex-related difference in dephosphorylation of the pro-drug or sex-related difference in the metabolism of the active drug or combination of both. The following table (from vol. 1, pg. 7 of sponsor's submission) summarizes the mean toxicokinetic parameters of L-754, 030 after i.v. administration of L-758, 298 in rats.

SUMMARY OF DRUG WEEK 4 MEAN TOXICOKINETIC PARAMETERS OF L-754,030 IN MALE AND FEMALE RATS FOLLOWING INTRAVENOUS DOSING WITH L-758,298						
	Dose of L-758,298 (mg/kg/day)					
	Males			Females		
Toxicokinetic Parameters	0.25	1	4	0.25	1	4
L-754,030						
C_{max} (µg/ml)	0.100	0.356	1.069	0.127	0.377	1.212
T_{max} (hr)	0.07	0.07	0.07	0.07	0.07	0.07
AUC of the Means (µg•hr/ml)	0.232	0.895	2.737	0.937	2.361	5.621

Study title: 5-Week intravenous toxicity study in rats **(TT#97-002-0)**

Study no: TT#97-002-0

Conducting laboratory and location: Merck Research Laboratories, West Point, PA.

Date of study initiation: January 9, 1997

Date of study report: July 29, 1997

GLP compliance: A statement of compliance is included.

QA report: yes (X) no ( )

Drug, lot #, and % purity: L-758, 298. Lot No. 003C009. 98.6% pure.

Formulation/vehicle: Tween-sodium citrate diluent (TSCD), pH 7.5. The composition (per ml) of the vehicle is as follows: Polysorbate 80 ——— mg; D-lactose- ——— ng; meglumine- ———) mg. b(4)

Methods:

Dosing:

Species/strain: Sprague Dawley rat ——— BR) b(4)

#/sex/group or time point (main study): 15/sex/group

Satellite groups used for toxicokinetics or recovery: None

Age: 39 days

Weight: Males: 138 to 189 grams; Females: 114 to 150 grams

Doses in administered units: 2, 5, and 10 mg/kg/day. The basis of dose selection was not mentioned.

Route, form, volume, and infusion rate: Intravenous, solution, 5 ml/kg, and 2 ml/min

The following table presents the study design:

Group No.	Treatment	Dose (mg/kg/day)	Concentration (mg/ml)	Dose Volume (ml/kg)	No. of Males	No. of Females
Control 1	Saline	0	0	5	15	15
Control 2	TSCD	0	0	5	15	15
3	L-758, 298	2	0.4	5	15	15
4	L-758, 298	5	1.0	5	15	15
5	L-758, 298	10	2	5	15	15

Observations and times:

Clinical signs: Daily

Body weights: Weekly

Food consumption: Once or twice weekly by visual inspection

Ophthalmoscopy: Week 4

Hematology: Week 2 and 4

Clinical chemistry: Week 2 and 4

Urinalysis: Week 4

Gross pathology: At necropsy

Organs weighed: At necropsy. The following organs were weighed: heart, spleen, brain, pituitary, kidneys, testes, prostate, thyroid, liver, adrenal, and ovaries.

Histopathology: At necropsy from control-2 and 10 mg/kg/day groups. The following organs or tissues were examined: lung, heart, liver, kidneys, urinary bladder, spleen, thymus, lymph nodes, adrenals, thyroid with parathyroid, pituitary, salivary gland, stomach, small intestine, injection site (tail), pancreas, spinal cord, peripheral nerves, eye with optic nerve and Harder's gland, skin (with mammary gland), bone (including joint), bone marrow, testes and epididymides, prostate, ovaries, uterus, brain, skeletal muscle, large intestine and esophagus.

Toxicokinetics: Plasma samples were taken from 3 rats/sex/timepoint at Week 4.

Results:

1. **Clinical Signs:** Treatment-induced salivation was observed in all groups during week 4.

2. **Mortality:** None.

3. **Body Weight:** The mean initial and final body weights of the Control-2 (vehicle-treated) males were 170 g and 266 g, respectively. The mean initial and final body weights of the Control-2 (vehicle-treated) females were 129 g and 176 g, respectively. There were no treatment-related changes in the body weight in either sex.

4. **Food Consumption:** Food consumption was observed visually and there were no apparent treatment-related changes in food consumption.

5. **Hematology:** No treatment-related changes were observed.

6. **Blood Chemistry:** No treatment-related changes were observed.

7. **Urinalysis:** No treatment-related changes were observed.

8. **Ophthalmology:** No treatment-related changes were observed.

9. **Organ Weights:** Liver weights were increased in a dose-related manner in the females (absolute: 104, 117, and 122% of control at 2, 5, and 10 mg/kg/day, respectively, control = 5.78 g; relative (% body weight): 106, 116, and 122% of control at 2, 5, and 10 mg/kg/day, respectively, control = 3.43%; relative (% brain weight): 103, 116, and 120% of control at 2, 5, and 10 mg/kg/day, respectively, control = 320%) as well as in the males (absolute: 104, 110, and 115% of control at 2, 5, and 10 mg/kg/day, respectively, control = 8.13 g; relative (% body weight): 104, 107, and 113% of control at 2, 5, and 10 mg/kg/day, respectively, control = 3.26%; relative (% brain weight): 103, 108, and 114% of control at 2, 5, and 10 mg/kg/day, respectively, control = 418%). These changes in the liver weight were considered to be due to hepatocellular hypertrophy seen in the females in these groups. However, no such histopathological changes in the liver were observed in the males. The apparent cause of increased liver weight in males was not clear. However, it is to be mentioned here that in a previous i.v. 5-week toxicity study in rats at 0.25, 1, and 4 mg/kg (TT#95-607-0), the females showed 2 to 4-fold higher exposure to active drugs than males. It is possible

that in the present study, the sex-specific histopathological changes in the liver (hepatocellular hypertrophy in females) could be partially attributed to the sex-related difference in exposure to active drug.

10. **Gross Pathology:** No treatment-related changes were observed.

11. **Histopathology:** Hepatocellular hypertrophy was seen in the livers of 5 of 15 females and 6 of 15 females at 5 and 10 mg/kg/day, respectively. No treatment-related changes in the liver were seen in the males. There were no other significant treatment-related changes.

12. **Toxicokinetics:** Blood samples were collected from 3 rats/sex/timepoint at approximately 4 min, 8 min, 1, 2, 5, 7, 10 and 24 hours postdose. The sponsor stated that the results of toxicokinetic analysis would be submitted later.

Five-Week Oral Range-Finding and Toxicokinetic Study in Rats (Report Date/Number #97-060-0).

Testing Laboratory: Merck Institute for Therapeutic Research
Merck Research Laboratories
Merck & Co., Inc.
West Point, PA 19486

Date Started: July 10, 1997

Date Completed: March 4, 1998

GLP Compliance: Statements of compliance with GLP Regulations and the Quality Assurance Unit were included.

Animals: Sprague Dawley [redacted] BR] rats were used in these studies. At the start of treatment, animals were 46 days of old and body weight ranges were 132-213 g for male rats and 111-147 g for female rats. b(4)

Drug Batch: L-754,030-004H (Lot #21).

Methods: Rats received L-754,030 by oral gavage at doses of 0 mg/kg/day, 0 mg/kg/day-B.I.D., 250 mg/kg/day, 250 mg/kg/day-B.I.D., 500 mg/kg/day-B.I.D., and 1000 mg/kg/day-B.I.D. for 28-29 days. There were 15 rats/sex/group. Control rats received the vehicle, 0.5% aqueous methylcellulose containing 0.02% sodium lauryl sulfate. The dose volume was 5 mL/kg, once or twice per day. For B.I.D. groups, the vehicle or L-754,030 was administered a minimum of 5-6 hr apart. Rats were examined daily for mortality and clinical signs of toxicity. Body weight was measured prior to the

start of treatment, once/week in weeks 1 and 4, and twice/week in weeks 2 and 3. Food consumption was measured twice per week. Ophthalmic examinations were performed during week 3 on control-B.I.D. and 1000 mg/kg/day B.I.D. groups. Blood for determination of hematological and clinical chemistry parameters was collected in week 4. Urine for analysis was collected from 10 rats/sex/group during week 4. Blood for determination of plasma drug levels was collected in week 4 at 2, 4, 6, 8, 10, 16, and 24 hr after the first dose. For B.I.D. groups, the second dose was administered immediately after collection of blood at the 6-hr time point. Four rats/sex/group were bled at 2 and 8 hr, 4 and 10 hr, or 6 and 16 hr and 3 rats/sex/group were bled at 24 hr. Blood samples (2 mL) were also collected from control groups to match treatment groups. L-754,030 was isolated by solid phase extraction and quantified by Liquid Chromatography/Mass Spectrometry/Mass Spectrometry. At the termination of treatment, all animals were sacrificed and a complete gross examination was performed on each animal. Organ weights were measured for the heart, spleen, brain, pituitary, kidneys, testes, prostate, thyroid gland, liver, adrenal glands, and ovaries. Organ weights were expressed as absolute, percent of body weight, and percent of brain weight. A complete microscopic examination for organs and tissues from the control-B.I.D. and 1000 mg/kg/day-B.I.D. groups was performed as follows: lung, heart, liver, kidneys, urinary bladder, spleen, thymus, lymph nodes, adrenal glands, thyroid gland (with parathyroid), pituitary gland, salivary gland, stomach, small intestine, pancreas, spinal cord, peripheral nerve, eye (with optic nerve and Harder's gland), skin (with mammary gland), bone (including joint), bone marrow, testes and epididymides, prostate, ovaries, esophagus, uterus, brain, skeletal muscle, and large intestine. In addition, all grossly observed changes in the liver and thyroid gland from all animals and all grossly changes in the pituitary gland from all male rats were subjected to microscopic examination.

Results:

1. **Observed Effects:** There were no treatment-related observed effects.

2. **Mortality:** There were no treatment-related deaths. One male rat in the 1000 mg/kg/ day-B.I.D. died during the blood collection procedure during week 4 at the 16 hr time point.

3. **Body Weight and Food Consumption:** There were no treatment-related changes of body weight gain or food consumption. Body weights for the male control group during weeks –1 and 4 were 176 and 185 g, respectively. Body weights for the male control-B.I.D. group during weeks –1 and 4 were 177 and 281 g, respectively. Body weight gains for the male 250 mg/kg/day, 250 mg/kg/day-B.I.D., 500 mg/kg/day-B.I.D., and 1000 mg/kg/day-B.I.D. groups were 110.9, 111.9, 109.9, and 104.2% of controls, respectively. Body weights for the female control group during weeks –1 and 4 were 134 and 185 g, respectively. Body weights for the female control-B.I.D. group during weeks –1 and 4 were 130 and 184 g, respectively. Body weight gains for the female 250 mg/kg/day, 250 mg/kg/ day-B.I.D., 500 mg/kg/day-B.I.D., and 1000 mg/kg/day-B.I.D. groups were 99.8, 100.6, 103.2, and 96.3% of controls, respectively.

4. **Hematology:** There were no treatment-related changes of hematological parameters.

5. **Blood Biochemistry and Urinalysis:** Serum protein levels for the male 250 mg/kg/day, 250 mg/kg/day-B.I.D., 500 mg/kg/day-B.I.D., and 1000 mg/kg/day-B.I.D. groups were increased to 103.4, 103.4, 105.2, and 106.9% of controls (Control and Control-B.I.D. values were both 5.8 g/dL), respectively. Serum protein levels for the female 250 mg/kg/ day, 250 mg/kg/day-B.I.D., 500

mg/kg/day-B.I.D., and 1000 mg/kg/day-B.I.D. groups were increased to 106.8, 111.7, 108.3, and 108.3% of controls (Control and Control-B.I.D. values were 5.9 and 6.0 g/dL, respectively), respectively. The albumin to globulin ratio for the male 250 mg/kg/day, 250 mg/kg/day-B.I.D., 500 mg/kg/day-B.I.D., and 1000 mg/kg/ day-B.I.D. groups were decreased to 92.3, 92.3, 84.6, and 84.6% of controls (Control and Control-B.I.D. values were both 1.3), respectively. The albumin to globulin ratio for the female 250 mg/kg/day, 250 mg/kg/day-B.I.D., 500 mg/kg/day-B.I.D., and 1000 mg/kg/day-B.I.D. groups were decreased to 86.7, 80, 80, and 80% of controls (Control and Control-B.I.D. values were both 1.5), respectively. Serum calcium levels for the female 250 mg/ kg/day, 250 mg/kg/day-B.I.D., 500 mg/kg/day-B.I.D., and 1000 mg/kg/day-B.I.D. groups were increased to 103, 105, 102, and 104% of controls (Control and Control-B.I.D. values were both 10 mg/mL), respectively. Cholesterol levels for the male 1000 mg/kg/day-B.I.D. group were increased to 108.6% of the control (58 mg/dL). Cholesterol levels for the female 250 mg/kg/day, 250 mg/kg/day-B.I.D., 500 mg/kg/day-B.I.D., and 1000 mg/kg/day-B.I.D. groups were increased to 112.5, 178.9, 162, and 171.8% of controls (Control and Control-B.I.D. values were 80 and 71 mg/dL, respectively), respectively. Triglyceride levels for the male 250 mg/kg/day, 250 mg/kg/day-B.I.D., 500 mg/kg/day-B.I.D., and 1000 mg/kg/ day-B.I.D. groups were decreased to 68.75, 57.7, 57.7, and 52.1% of controls (Control and Control-B.I.D. values were 64 and 71 mg/dL, respectively), respectively. There were no treatment-related changes of urinalysis parameters.

6. Ophthalmic Examination: There were no treatment-related ophthalmic effects.

7. Organ Weights: Treatment-related increases in absolute and relative liver and thyroid gland weights were observed for all male and female treatment groups. Increased liver weight was correlated with hepatocellular hypertrophy. Increased thyroid gland weight was correlated with thyroid follicular cell hyperplasia.

Liver: Absolute liver weights for the male 250 mg/kg/day, 250 mg/kg/day-B.I.D., 500 mg/kg/day-B.I.D., and 1000 mg/kg/day-B.I.D. groups were increased to 125.2, 141.1, 148, and 148.8% of controls (Control and Control-B.I.D. values were 9.37 and 9.17 g, respectively), respectively. Relative liver weights for the male 250 mg/kg/day, 250 mg/kg/ day-B.I.D., 500 mg/kg/day-B.I.D., and 1000 mg/kg/day-B.I.D. groups were increased to 125.2, 136.2, 142.4, and 149.6% of control (Control and Control-B.I.D. values were 3.41 and 3.37%, respectively), respectively. Absolute liver weights for the female 250 mg/kg/ day, 250 mg/kg/day-B.I.D., 500 mg/kg/day-B.I.D., and 1000 mg/kg/day-B.I.D. groups were increased to 146.2, 194.4, 170.6, and 187.3% of controls (Control and Control-B.I.D. values were 6.51 and 6.78 g, respectively), respectively. Relative liver weights for the female 250 mg/kg/day, 250 mg/kg/day-B.I.D., 500 mg/kg/day-B.I.D., and 1000 mg/kg/day-B.I.D. groups were increased to 143.3, 192.7, 173.3, and 181.7% of control (Control and Control-B.I.D. values were 3.55 and 3.71%, respectively), respectively.

Thyroid Gland: Absolute thyroid gland weights for the male 250 mg/kg/day, 250 mg/kg/day-B.I.D., 500 mg/kg/day-B.I.D., and 1000 mg/kg/day-B.I.D. groups were increased to 112.7, 135.2, 139.3 and 133.1% of controls (Control and Control-B.I.D. values were 0.0158 and 0.0145 g, respectively), respectively. Relative thyroid gland weights for the male 250 mg/kg/day, 250 mg/kg/day-B.I.D., 500 mg/kg/day-B.I.D., and 1000 mg/kg/ day-B.I.D. groups were increased to 114, 129.6, 131.5, and 133.3% of control (Control and Control-B.I.D. values were 0.0057 and 0.0054%, respectively), respectively. Absolute thyroid gland weights for the female 250 mg/kg/day, 250

mg/kg/day-B.I.D., 500 mg/kg/day-B.I.D., and 1000 mg/kg/day-B.I.D. groups were increased to 122.8, 125.8, 119.6, and 135.8% of controls (Control and Control-B.I.D. values were 0.0123 and 0.0120 g, respectively), respectively. Relative liver weights for the female 250 mg/kg/day, 250 mg/kg/ day-B.I.D., 500 mg/kg/day-B.I.D., and 1000 mg/kg/day-B.I.D. groups were increased to 119, 124.2, 119.7, and 131.8% of control (Control and Control-B.I.D. values were 0.0068 and 0.0066%, respectively), respectively.

8. Gross Pathology: Necropsy examination found that the livers of several female rats in the 1000 mg/kg/day B.I.D. group were enlarged and/or red/yellow in color. The sponsor did not provide a summary table or individual line listings for gross pathological changes.

9. Histopathology: Target organs of toxicity were the liver, thyroid gland, and pituitary gland. For the liver, very slight to slight hepatocellular hypertrophy was observed in all male and female treatment groups. Hepatocyte hypertrophy most likely represents an induction of cytochrome P-450 enzymes and has no toxicological significance. Very slight to slight diffuse vacuolation was observed with an increased incidence for male and female rats that received doses of 250, 500, and 1000 mg/kg/day-B.I.D. For the thyroid gland, very slight to slight thyroid follicular cell hyperplasia was observed was observed in all male and female treatment groups. Follicular cell hyperplasia of the thyroid gland is most likely due to increased catabolism of thyroxine (T_4) and triiodothyronine (T_3) by the liver. For the pituitary gland, a very slight to slight dose-dependent vacuolation of individual cells in the pars distalis was observed for all male treatment groups. This change was characterized by enlargement of individual pituitary cells due to formation of large cytoplasmic vacuoles and occasional protein droplets. This change may represent a degeneration or exhaustion of Thyroid stimulating hormone-producing pituitary cells secondary to hepatic enzyme induction and increased catabolism of T_3 and T_4.

Histopathological changes for rats that received L-754,030 by oral gavage at doses of 250 mg/kg/day, 250 mg/kg/day-B.I.D., 500 mg/kg/day-B.I.D., and 1000 mg/kg/day-B.I.D. for 28 or 29 days (n = 15/group).

Organ/Tissue	0		0-B.I.D.		250		250-B.I.D.		500-B.I.D.		1000-B.I.D.	
	F	M	F	M	F	M	F	M	F	M	F	M
Liver -hepatocyte hypertrophy -diffuse vacuolation	 0 1	 0 0	 0 3	 0 0	 14 2	 8 1	 15 5	 15 3	 15 8	 15 4	 15 8	 14 3
Thyroid gland -follicular cell, diffuse hyperplasia	0	0	0	0	8	4	15	9	14	11	10	10
Pituitary gland -vacuolation	-	0	0	0	-	1	-	5	-	8	-	10

10. Plasma Drug Levels: A plateau in plasma C_{max} and $AUC_{0\text{-}24hr}$ values for L-754,030 was evident in male and female treatment groups in week 4 at doses ≥250 mg/kg/day B.I.D. Plasma C_{max} and $AUC_{0\text{-}24hr}$ values for L-754,030 in female rats were significantly higher than in male rats.

Toxicokinetic parameters for plasma L-754,030 levels in weeks for rats that received L-754,030 by oral gavage at doses of 250 mg/kg/day, 250 mg/kg/day-B.I.D., 500 mg/kg/ day-B.I.D., and 1000 mg/kg/day-B.I.D.

Dose, mg/kg/day	C_{max}, µg/mL		T_{max}, hr		AUC_{0-24hr}, µg*hr/mL	
	Male	Female	Male	Female	Male	Female
250	0.233	0.819	2	24	3.21	13.9
250-B.I.D.	0.519	2.59	16	24	7.19	33.2
500-B.I.D.	0.444	2.27	16	24	7.22	34.8
1000-B.I.D.	0.722	1.74	16	16	9.37	32.1

Rats received L-754,030 by oral gavage at doses of 0 mg/kg/day, 0 mg/kg/day-B.I.D., 250 mg/kg/day, 250 mg/kg/day-B.I.D., 500 mg/kg/day-B.I.D., and 1000 mg/kg/day-B.I.D. for 28-29 days. A no effect dose was not established. Target organs of toxicity were the liver, thyroid gland, and pituitary gland. For the liver, very slight to slight hepatocellular hypertrophy was in all male and female treatment groups. Hepatocyte hypertrophy most likely represents an induction of cytochrome P-450 enzymes and has no toxicological significance. Very slight to slight diffuse vacuolation was observed with an increased for male and female rats that received doses of 250 mg/kg/day-B.I.D., 500 mg/kg/day-B.I.D., and 1000 mg/kg/day-B.I.D. For the thyroid gland, very slight to slight thyroid follicular cell hyperplasia was observed for all male and female treatment groups. Follicular cell hyperplasia of the thyroid gland is most likely due to increased catabolism of thyroxine (T_4) and triiodothyronine (T_3) by the liver. For the pituitary gland, a very slight to slight dose-dependent vacuolation of individual cells in the pars distalis was observed for all male treatment groups. This change was characterized by enlargement of individual pituitary cells due to formation of large cytoplasmic vacuoles and occasional protein droplets. This change may represent a degeneration or exhaustion of Thyroid stimulating hormone-producing pituitary cells secondary to hepatic enzyme induction and increased catabolism of T_3 and T_4. A plateau in plasma C_{max} and AUC_{0-24hr} values for L-754,030 was evident in male and female treatment groups in week 4 at doses ≥250 mg/kg/day B.I.D. Plasma C_{max} and AUC_{0-24hr} values for L-754,030 in female rats were significantly higher than in male rats.

Study Title: MK-0869: Five-Week Oral Toxicity Study in Rats.

Report No: TT 00-001-0,-1
Conducting laboratory and location: Merck Institute for Therapeutic Research
Merck Research Laboratories
Merck & Co., Inc.
West Point, PA 19486

Date Started: January 6, 2000

Date Completed: August 8, 2000
GLP compliance: A statement of compliance with GLP regulations was included.
QA- Report Yes (X) No ( )
Methods: The toxicology and toxicokinetic profiles of two MK-0869 formulations of different particle size, were assessed in Sprague-Dawley rats during a 5-week treatment period (28 to 29 days). MK-0869 Formulation M (average particle size, —— n) was administered to 16 rats/sex/group at oral doses of 0 and 125 mg/kg B.I.D. (total daily doses of 0 and 250 mg/kg/day, respectively). MK-0869 Formulation NB (average particle size, —— nm) was administered to 16 rats/sex/group at oral doses of 0, 5, 125, 250, 500, and 750 mg/kg B.I.D. (total daily doses of 0, 10, 250, 500, 1000, and 1500 mg/kg, respectively). The first and second daily doses were administered a minimum of 4 hr apart for vehicle-control groups and a minimum of 6 hr apart for all drug-treated groups. A total of 56 to 58 doses were administered. b(4)

Dosing:

- **species/strain:** Sprague-Dawley rats —— BR] were obtained from b(4)
- **#/sex/group or time point:** 16 rats/sex/group
- **age:** Male and female rats were 42 and 38 days old, respectively, at the start of treatment.
- **weight:** Body weight ranges were 131 to 200 g for male rats and 93 to 135 g for female rats at the start of treatment.
- **satellite groups used for toxicokinetics or recovery:** None.
- **dosage groups in administered units:** MK-0869 Formulation M (average particle size, —— m) was administered at doses of 0 and 125 mg/kg B.I.D. (total daily doses of 0 and 250 mg/kg/day, respectively). Formulation NB (average particle size, —— m) was administered at doses of 0, 5, 125, 250, 500, and 750 mg/kg B.I.D. (total daily doses of 0, 10, 250, 500, 1000, and 1500 mg/kg, respectively). The first and second daily doses were administered a minimum of 4 hr apart for vehicle-control groups and a minimum of 6 hr apart for all drug-treated groups. b(4)
- **route, form, volume, and infusion rate:** Vehicle or drug suspension was administered by oral gavage using a dose volume of 5 mL/kg.

Drug, lot#, radiolabel, and % purity: MK-0869 Formulation M, lot number L-754030-004H031, had an average particle size of —— with 99.5% purity. MK-0869, Formulation NB (MK-0869 blended ——), batch #X0869OPP015C001 (also known as L-754030-016S001) with an average particle size of —— m, was obtained by blending 3 batches of MK-0869 —— with purity ranging from 99.6 to 100.0%. b(4)
Formulation/vehicle: The vehicle for MK-0869 Formulation M was 5% methylcellulose and 0.02% sodium lauryl sulfate in deionized water. The vehicle for MK-0869 Formulation NB was 3% hydroxypropyl cellulose, 15% sucrose, and 0.14% sodium lauryl sulfate in deionized water.
Observations and times:

- **Clinical signs:** Animals were monitored daily for clinical signs of toxicity and mortality before and after dosing.
- **Body weights:** Body weights were measured prior to the start of treatment, once per week during weeks 1 and 2, and 2 per week thereafter.

- **Food consumption:** Cages were examined twice per week at 24 hr after dosing to determine if any food was remaining.
- **Ophthalmoscopy:** Ophthalmic examinations were conducted in control and high dose groups during week 4.
- **EKG:** Not performed.
- **Hematology:** Blood for determination of hematology parameters was collected during weeks 2 and 4.
- **Clinical chemistry:** Blood for determination of clinical chemistry parameters was collected during weeks 2 and 4.
- **Urinalysis:** Urine samples for analysis were collected from approximately 10 rats/sex/group during week 4.
- **Gross pathology:** Rats were sacrificed at the termination of the study and submitted to complete necropsy.
- **Organs weighed:** Absolute and relative organ weights were determined for the adrenal glands, brain, heart, ovaries, kidneys, liver, pituitary gland, prostate gland, spleen, testes, and thyroid gland.
- **Histopathology:** Tissue sections from the vehicle-control groups, Formulation M high dose group, Formulation NB high dose, and deaths or moribund sacrifices during the treatment period were prepared, stained with hematoxylin and eosin, and submitted to microscopic examination. Tissues with gross pathological changes from all animals were examined at the discretion of the pathologist. Sections of liver and thyroid gland were examined from all animals. Tissues examined were as follows: salivary gland (submandibular/sublingual), esophagus, stomach (glandular and nonglandular portions), small intestines (duodenum, jejunum, ileum), large intestine (colon), liver, pancreas, adrenal glands, parathyroid (when present in thyroidal sections), pituitary gland, thyroid gland, kidneys, skin (from mammary region), mammary gland (when present in skin section), lung, heart, spleen, lymph nodes (cervical and mesenteric), thymus, bone marrow, bone (femur, including femorotibial joint), skeletal muscle, brain (including cerebral cortex and subcortical white matter, basal ganglia, cerebellum, and pons), urinary bladder, ovaries, uterus, testes and epididymides, prostate, spinal cord (cervical) peripheral nerve (sciatic), eye (with optic nerve), and Harder's gland.
- **Toxicokinetics:** Blood for determination of plasma drug levels was collected during week 4 at 2, 4, 6, 8, 10, 16, and 24 hr after dosing. Four rats/sex/group were used for each time point. Levels of MK-0869 were quantified by liquid chromatography/tandem mass spectrometry. The lower limit of quantitation was 0.0253 µg/mL.
- **Other:** None.

Results:

- **Clinical signs:** There were no treatment-related clinical signs of toxicity.

- **Mortality:** There was no treatment-related mortality. Two animals died during the treatment period due to a dosing accident or from anesthesia associated with blood collection. Animal #00-0165F in the Formulation NB 250 mg/kg B.I.D. group was found dead during week 4. Death was attributed to a dosing accident. Animal #00-0237F in the

Formulation NB 750 mg/kg B.I.D. group was found dead during week 4. Death was attributed to an anesthesia accident associated with blood collection

- **Body weights:** There were no treatment-related effects on body weight gains with MK-0869 Formulation M or NB. Body weights for male controls, which received the vehicle for Formulation M, at weeks -1 and 4 were 167 and 278 g, respectively. Body weights for female controls, which received vehicle for Formulation M, at weeks -1 and 4 were 118 and 171 g, respectively. Body weight gains for male and female rats that received MK-0869 Formulation M at 125 mg/kg B.I.D. were 96 and 115% of the control, respectively. Body weights for male controls, which received vehicle for Formulation NB, at weeks -1 and 4 were 172 and 291 g, respectively. Body weight gains for male rats that received MK-0869 Formulation NB at doses of 5, 125, 250, 500, and 750 mg/kg B.I.D. were 103.2, 100, 105.4, 103.75, and 103.1% of the control, respectively. Body weights for female controls, which received vehicle for Formulation NB, at weeks -1 and 4 were 120 and 179 g, respectively. Body weight gains for female rats that received MK-0869 Formulation NB at doses of 5, 125, 250, 500, and 750 mg/kg B.I.D. were 100.8, 106.9, 110.3, 102.5, and 107.8% of the control, respectively.

- **Food consumption:** There were no treatment-related changes of food consumption.

- **Ophthalmoscopy:** Ophthalmic examinations of vehicle-control groups, the high dose MK-0869 Formulation M group, and the high dose MK-0869 Formulation NB group apparently revealed no treatment-related changes; however, no data was provided for independent analysis.

- **Hematology:** Decreased platelet counts were observed for male treatment groups at week 2 that received MK-0869 Formulation NB and female treatment groups at weeks 2 and 4 that received MK-0869 Formulation NB.

MK-0869 Formulation NB, Week 2: Platelet counts for male rats that received MK-0869 Formulation NB at 125, 250, 500, and 750 mg/kg B.I.D. were increased to 122.2, 121.6, 124.8, and 124.8% of the control (1540 x 10^3/mm^3), respectively. Platelet counts for female rats that received MK-0869 Formulation NB at 5, 125, 250, 500, and 750 mg/kg B.I.D. were increased to 112.4, 135.7, 147.7, 151.3, and 140.1% of the control (1311 x 10^3/mm^3), respectively.

MK-0869 Formulation NB, Week 4: Platelet counts for female rats that received MK-0869 Formulation NB at 5, 125, 250, 500, and 750 mg/kg B.I.D. were increased to 114.2, 120.5, 124, 122.7, and 116.0% of the control (1222 x 10^3/mm^3), respectively.

- **Clinical chemistry:** Clinical chemistry changes observed for rats that received MK-0869 Formulation M or NB included alterations of total protein levels, A/G ratios, cholesterol levels, and triglyceride levels, which may be correlated with histopathological changes observed in the liver.

MK-0869 Formulation M, Week 2: Total protein levels for male rats at 125 mg/kg B.I.D. were increased to 105.7% of the control (5.3 g/dL). A/G ratios for male and female rats that received 125 mg/kg B.I.D. were decreased to 80 and 87.5% of control values (1.6 and 1.5), respectively. Cholesterol levels for female rats at 125 mg/kg B.I.D. were increased to 116.4% of the control (67 mg/dL), respectively. Triglyceride levels for male rats that received 125 mg/kg B.I.D. were decreased to 82.8% of the control (64 mg/dL).

MK-0869 Formulation M, Week 4: Total protein levels for male rats at 125 mg/kg B.I.D. were increased to 105.45% of the control (5.5 g/dL). Total protein levels for female rats that received 125 mg/kg B.I.D. were increased to 110.5% of the control (5.7 g/mL). A/G ratios for male and female rats that received 125 mg/kg B.I.D. were decreased to 85.7 and 75% of control values (1.4 and 1.6), respectively. Cholesterol levels for female rats at 125 mg/kg B.I.D. were increased to 135.3% of the control (68 mg/dL), respectively. Triglyceride levels for male rats that received 125 mg/kg B.I.D. were decreased to 62.2% of the control (90 mg/dL).

MK-869 Formulation NB, Week 2: Glucose levels for female rats that received 250, 500, and 750 mg/kg B.I.D. were decreased to 84.1-90.85% of the control (164 mg/dL). Glucose levels for male rats that received 125, 250, 500, and 750 mg/kg B.I.D. were decreased to 89.1-91.5% of the control (165 mg/dL). Total protein levels for male rats that received 125, 250, 500, and 750 mg/kg B.I.D. were increased to 105.5-109.3% of the control (5.4 g/dL). A/G ratios for female rats that received 125, 250, 500, and 750 mg/kg B.I.D. were decreased to 84.1-90.85% of the control (1.6). A/G ratios for male rats that received 5, 125, 250, 500, and 750 mg/kg B.I.D. were decreased to 80-86.7% of the control (1.5). Cholesterol levels for female rats that received 125, 250, 500, and 750 mg/kg B.I.D. were increased to 111.5-124.4% of the control (78 mg/dL). Cholesterol levels for male rats that received 750 mg/kg B.I.D. were increased to 112.1% of the control (58 mg/dL). Potassium levels for female rats that received 125, 250, 500, and 750 mg/kg B.I.D. were increased to 106.25-110.4% of the control (4.8 mEq/L). Triglyceride levels for male rats that received 5, 125, 250, 500, and 750 mg/kg B.I.D. were decreased to 85.9, 56.25, 73.4, 54.7, and 64.1% of the control (64 mg/dL), respectively.

MK-0869 Formulation NB, Week 4: Glucose levels for male rats that received 125, 250, 500, and 750 mg/kg B.I.D. were decreased to 82-87.4% of the control (183 mg/dL). Total protein levels for female rats that received 125, 250, 500, and 750 mg/kg B.I.D. were increased to 110.3-113.8% of the control (5.8 g/mL). Total protein levels for male rats that received 125, 250, 500, and 750 mg/kg B.I.D. were increased to 105.45-110.9% of the control (5.5 g/dL). A/G ratios for female rats that received 125, 250, 500, and 750 mg/kg B.I.D. were decreased to 75% of the control (1.6). A/G ratios for male rats that received 125, 250, 500, and 750 mg/kg B.I.D. were decreased to 85.7% of the control (1.4). Cholesterol levels for female rats that received 125, 250, 500, and 750 mg/kg B.I.D. were increased to 152-169% of the control (77 mg/dL). Cholesterol levels for male rats that received 750 mg/kg B.I.D. were increased to 117.9% of the control (56 mg/dL). Potassium levels for female rats that received 125, 250, 500, and 750 mg/kg B.I.D. were increased to 104.2-106.25% of the control (4.8 mEq/L). Triglyceride levels for male rats that received 5, 125, 250, 500, and 750 mg/kg B.I.D. were decreased to 82.9, 43.9, 63.4, 52.4, and 59.8% of the control (82 mg/dL), respectively.

- **Urinalysis:** There were no treatment-related changes of urinalysis parameters.

- **Organ Weights:** Treatment-related changes of absolute and relative liver and thyroid gland weights were observed with MK-0869 Formulations M and NB.

MK-0869 Formulation M: Absolute and relative liver weights for male rats that received 125 mg/kg B.I.D. were increased to 144.1 and 138.2% of control values (9.87 g and 3.61% B.W.), respectively. Absolute and relative liver weights for female rats that received 125 mg/kg B.I.D. were increased to 177 and 170.6% of control values (6.35 g and 3.74% B.W.), respectively. Absolute and relative thyroid gland weights for male rats that received 125 mg/kg B.I.D. were increased to 136 and 130% of control values (0.0164 g and 0.060% B.W.), respectively. Absolute and relative thyroid gland weights for female rats that received 125 mg/kg B.I.D. were increased to 128.7 and 123.7% of control values (0.0129 g and 0.0076% B.W.), respectively.

MK-0869 Formulation NB: Absolute liver weights for male rats that received 5, 125, 250, 500, and 750 mg/kg B.I.D. were increased to 109.7, 146.4, 150.7, 150.7, and 156.3% of the control (10.47 g), respectively. Relative liver weights for male rats that received 5, 125, 250, 500, and 750 mg/kg B.I.D. were increased to 111.5, 148.4, 154.4, 158.5, and 156.3% of the control (3.66% B.W.), respectively. Absolute liver weights for female rats that received 5, 125, 250, 500, and 750 mg/kg B.I.D. were increased to 121.1, 186.1, 206.2, 218.9, and 202.3% of the control (6.62 g), respectively. Relative liver weights for female rats that received 5, 125, 250, 500, and 750 mg/kg were increased to 122.25, 188.2, 206.2, 214.5, and 203.2% of the control (3.73% BW), respectively. Absolute thyroid gland weights for male rats that received 5, 125, 250, 500, and 750 mg/kg B.I.D. were increased to 108.5, 129.5, 133, 129.5, and 128.4% of the control (0.0176 g), respectively. Relative thyroid gland weights for male rats that received 5, 125, 250, 500, and 750 mg/kg B.I.D. were increased to 111.5, 132.8, 137.7, 137.7, and 129.5% of the control (0.0061% B.W.), respectively. Absolute thyroid gland weights for female rats that received 5, 125, 250, 500, and 750 mg/kg B.I.D. were increased to 108.9, 126.7, 127.4, 136.3, and 141.5% of the control (0.0135 g), respectively. Relative thyroid gland weights for female rats that received 5, 125, 250, 500, and 750 mg/kg B.I.D. were increased to 110.5, 128.9, 128.9, 134.2, and 142.1% of the control (0.0076% B.W.), respectively.

- **Gross Pathology:** Treatment-related liver enlargement, often with prominent lobular architecture, was observed for all MK-0869 Formulation M or NB groups. Treatment-related thyroid gland enlargement was observed for all MK-0869 Formulation M or NB groups.

- **Histopathology:** Histopathological changes were observed in the liver and thyroid gland. Treatment-related hypertrophy of hepatocytes and diffuse vacuolation of hepatocytes were observed in the liver for all MK-0869 Formulation M or NB groups. Treatment-related diffuse follicular cell hyperplasia was observed in the thyroid gland for all MK-0869 Formulation M or NB groups. Benign parafollicular cell adenomas were observed for two animals, one that received MK-0869 Formulation M at 125 mg/kg B.I.D. and one that received MK-0869 Formulation NB at 250 mg/kg B.I.D.

Histopathological changes for rats that received MK-0869 Formulation M at oral doses of 0 or 125 mg/kg B.I.D. for 28 or 29 days.

Tissue	0 mg/kg B.I.D.		125 mg/kg B.I.D.	
	F	M	F	M
Liver				
-hepatocyte hypertrophy	0	0	16	14
-hepatocyte, diffuse vacuolation	0	0	8	2
Thyroid gland				
-parafollicular cell, benign adenoma	0	0	0	1
-follicular cell, diffuse hyperplasia	0	2	11	12

Histopathological changes for rats that received MK-0869 Formulation NB at oral doses of 0, 5, 125, 250, 500, or 750 mg/kg B.I.D. for 28 or 29 days.

Tissue	0		5		125		250		500		750	
	F	M	F	M	F	M	F	M	F	M	F	M
Liver												
-hepatocyte hypertrophy	0	0	9	4	16	16	15	16	16	16	16	16
-hepatocyte,diffuse vacuolation	0	0	0	0	9	4	6	2	3	5	4	3
Thyroid gland												
-parafollicular cell, benign adenoma	0	0	0	0	0	0	1	0	0	0	0	0
-follicular cell,diffuse hyperplasia	0	0	2	6	14	14	12	14	15	16	12	16

- **Toxicokinetics:** Toxicokinetic analysis was restricted to the parent compound, MK-0869. Plasma AUC values for female treatment groups that received MK-0869 Formulation M or NB were significantly higher than AUC values for corresponding male treatment groups. Plasma AUC values for male and female rats that received MK-0869 Formulation M at 125 mg/kg B.I.D. were similar to AUC values for male and female rats that received MK-0869 Formulation NB at 125 mg/kg B.I.D. For male and female rats that received MK-0869 Formulation N at doses of 5 to 750 mg/kg B.I.D., C_{max} and AUC increased in a manner that was significantly less than proportional to dose. For male rats that received MK-0869 Formulation NB at doses from 125 to 750 mg/kg B.I.D., AUC values increased by only 1.6-fold over this 6-fold increase in dose. For female rats that received MK-0869 Formulation NB at doses from 125 to 750 mg/kg B.I.D., AUC values increased by only 1.4-fold over this 6-fold increase in dose. A plateau in AUC values for male rats that received MK-0869 Formulation NB was evident at approximately 500 mg/kg B.I.D. A plateau in AUC values for female rats that received MK-0869 Formulation NB was evident at approximately 250 mg/kg B.I.D.

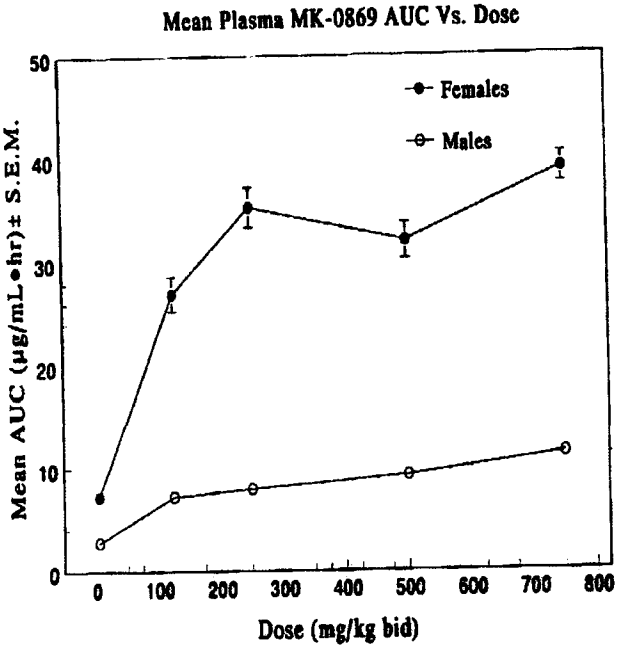
Plasma toxicokinetic parameters of MK-0869 in male and female rats during week 4 following B.I.D. dosing with MK-0869 in Formulation NB

Dose, mg/kg B.I.D.	$AUC_{0\text{-}24hr}$, µg·hr/mL		C_{max}, µg/mL		T_{max}, hr	
	Male	Female	Male	Female	Male	Female
5	2.82	7.20	0.340	0.724	2.0	2.0
125	7.19	26.9	0.485	1.66	8.0	8.0
250	7.91	35.3	0.487	1.91	8.0	24
500	9.21	32.0	0.612	1.63	8.0	8.0
750	11.3	38.9	0.660	2.32	10	10

Plasma toxicokinetic parameters of MK-0869 in male and female rats during week 4 following B.I.D. dosing with MK-0869 in Formulation M.

Dose, mg/kg B.I.D.	$AUC_{0\text{-}24hr}$, µg·hr/mL		C_{max}, µg/mL		T_{max}, hr	
	Male	Female	Male	Female	Male	Female
125	5.28	19.0	0.345	1.32	24	2.0

Dose and Sex Comparison of Drug Week 4 Mean Plasma MK-0869 AUC Values in Rats Treated With B.I.D. Doses of MK-0869 NB Formulation



Key Study Findings: The toxicology and toxicokinetic profiles of two MK-0869 formulations of different particle size, were assessed in Sprague-Dawley rats during a 5-week treatment period (28 to 29 days). MK-0869 Formulation M (average particle size. —— m) was administered to 16 rats/sex/group at oral doses of 0 and 125 mg/kg B.I.D. (total daily doses of 0 and 250 mg/kg/day, respectively). MK-0869 Formulation NB (average particle size, —— nm) was administered to 16 rats/sex/group at oral doses of 0, 5, 125, 250, 500, and 750 mg/kg B.I.D. (total daily doses of 0, 10, 250, 500, 1000, and 1500 mg/kg/day, respectively). b(4)

The first and second daily doses were administered a minimum of 4 hr apart for vehicle-control groups and a minimum of 6 hr apart for all drug-treated groups. A total of 56 to 58 doses were administered. A no effect dose was not observed with Formulation M or NB. Histopathological changes were observed in the liver and thyroid gland. Treatment-related hypertrophy of hepatocytes and diffuse vacuolation of hepatocytes were observed in the liver for all MK-0869 Formulation M or NB groups. Treatment-related diffuse follicular cell hyperplasia was observed in the thyroid gland for all MK-0869 Formulation M or NB groups. Benign parafollicular cell adenomas were observed for two animals, one that received MK-0869 Formulation M at 125 mg/kg B.I.D. and one that received MK-0869 Formulation NB at 250 mg/kg B.I.D. Toxicokinetic analysis was restricted to the parent compound, MK-0869. Plasma AUC values for female treatment groups that received MK-0869 Formulation M or NB were significantly higher than AUC values for corresponding male treatment groups. Plasma AUC values for male and female rats that received MK-0869 Formulation M at 125 mg/kg B.I.D. were similar to AUC values for male and female rats that received MK-0869 Formulation NB at 125 mg/kg B.I.D. For male and female rats that received MK-0869 Formulation N at doses of 5 to 750 mg/kg B.I.D., C_{max} and AUC increased in a significantly less than proportional manner to dose. For male rats that received MK-0869 Formulation NB at doses from 125 to 750 mg/kg B.I.D., AUC values increased by only 1.6-fold over this 6-fold increase in dose. For female rats that received MK-0869 Formulation NB at doses from 125 to 750 mg/kg B.I.D., AUC values increased by only 1.4-fold over this 6-fold increase in dose. A plateau in AUC values for male rats that received MK-0869 Formulation NB was evident at approximately 500 mg/kg B.I.D. A plateau in AUC values for female rats that received MK-0869 Formulation NB was evident at approximately 250 mg/kg B.I.D.

Study Title: **L-754030: Fourteen (14)-Week Oral Range-Finding Study in Rats**

Study no. TT#96-051-0

Conducting laboratory (and location if not Sponsor): Merck Research Laboratories, West Point, PA. .

Dates of study initiation & completion: July 15, 1996 and March 05, 1997.

GLP compliance: Yes

QA Report Yes (X) **No** ( )

Drug, Lot #, radiolabel (if applicable), and % purity: L-754030; Lot # L-754030-004H002 and L-754030-004H004; purity, 99.9%.

Formulation/vehicle: L-75030 was suspended in 0.5% methylcellulose and 0.03% sodium lauryl sulfate.

Methods:

Dosing:

Species/strain: ————) BR rats b(4)

#/sex/group or time point (main study): 10 animals/sex/group were used in the study.

Satellite groups used for toxicokinetics or recovery: N/A

Weight: males – 104 to 160 g; females – 92 to 124 g.

Age: Approximately 6 weeks

Doses in administered units: L-754030 was administered at oral doses of 125, 250, 500 and 1000 mg/kg/day.

Route, form, volume and infusion rate: The doses were administered once a day by oral gavage at a dosing volume of 10.0 ml/kg. Control animals received the same volume of the vehicle (0.5% methylcellulose and 0.03% sodium lauryl sulfate).

Times at which Observations were made:

Clinical signs- The animals were observed daily for clinical signs and mortality.

Body weights- Body weights were measured prior to initiation of dosing, once in Week 1 and twice a Week thereafter.

Food consumption- Food consumption was recorded twice a week during the dosing period.

Ophthalmoscopy - Ophthalmoscopic examinations were conducted on all rats in Week 5 and Week 12.

Hematology- Blood samples for hematology examinations were collected in Weeks 6 and 10. Hematologic examination of one animal (#96-4485F) from the 1000 mg/kg/day group was not conducted in Week 10 because the blood sample clotted.

Clinical chemistry- Blood samples for clinical chemistry examinations were collected in Week 6 and Week 10.

Urinalysis- Urine samples were collected from all animals in Week 6 and Week 10 of the dosing period.

Gross pathology- At the end of the dosing period, all animals were sacrificed and complete necropsies performed.

Organs weighed- The weights of the following organs were recorded: heart, kidneys, liver, spleen, testes, adrenal brain, prostate, ovaries, pituitary and thyroid.

Histopathology- Histopathological examinations of the following organs from the control and the high dose (1000 mg/kg/day) group were conducted.

Lung, heart, liver, kidneys, urinary bladder, spleen, thymus, lymph nodes, adrenals, thyroid, pituitary, salivary gland, stomach, small intestine, esophagus, pancreas, spinal cord, peripheral nerve, eye, skin, bone, bone marrow, testes and epididymides, prostate, ovaries, uterus, brain, skeletal muscle and large intestine.

In addition, all gross and visible changes and the liver and thyroids from all animals were examined microscopically.

Toxicokinetics- Blood samples were collected from 5 animals/sex/time point in Week 13 at approximately 0.5, 2, 4, 8, 12 and 24 hours after dosing. Plasma concentrations of L-754030 were determined by an LC/MS/MS method.

Results:

Mortality: There were no mortalities in any group.

Clinical signs: No treatment related clinical signs were observed in any group.

Body weights: The mean body weights of the control male and female animals before initiation of dosing were 158 and 119 g and in Week 14 were 440 and 400 g, respectively. No treatment related changes in the body weight were observed in any group.

Food consumption: No treatment-related changes in the food consumption were observed in any group.

Ophthalmoscopy: No treatment-related ophthalmologic changes were observed in any group.

Hematology: Males (35% at both doses) and females (30% and 85% at 500 and 1000 mg/kg/day doses, respectively) receiving 500 and 1000 mg/kg/day doses had increased neutrophil counts when compared with the controls.

Clinical chemistry: Decreases in triglyceride levels (20-60%) were observed in males at 125 mg/kg/day and higher doses. Females receiving 250 mg/kg/day and higher doses had slightly increased serum calcium levels (4-6%), and males (5-8%) and females (8-13%) receiving 125 mg/kg/day and higher doses had slightly higher protein levels.

Gross Pathology: Slight prominent reticulation of the liver parenchyma was occasionally observed in treatment group animals with no dose-relationship.

Organ weight: Treatment group male and female animals had increased liver and thyroid weights when compared with the controls (not dose-dependent). The mean relative (to body weight) liver weights at 125, 250, 500 and 1000 mg/kg/day doses were 19.3%, 19.6%, 26.1% and 26.5% higher for males, and 39.4%, 52.0%, 49.7% and 56.3% higher for females, respectively. The mean relative (to body weight) thyroid weights at 125, 250, 500 and 1000 mg/kg/day doses were 23.8%, 31.0%, 21.4% and 35.7% higher for males, and 15.2%, 25.8%, 15.2% and 22.7% higher for females, respectively.

Histopathology: Very slight to slight centrilobular hypertrophy of the hepatocytes was observed in all groups of male and female rats. This finding corresponds to the increase in liver weights in these groups, and is likely to be related to the induction of CYP P-450 enzymes. In the thyroid gland, follicular cell hyperplasia was observed in males and females at all doses. Five (of 10) males from the high dose group had acinar atrophy of the pancreas. High dose males (5 of 10) had chronic focal inflammation of the prostate and high dose females (3 of 10) had distention of the uterus. The histopathological findings in male and female rats are shown in the Table below.

Organ/Lesion	MALES					FEMALES				
	Control	125 mg/kg	250 mg/kg	500 mg/kg	1000 mg/kg	Control	125 mg/kg	250 mg/kg	500 mg/kg	1000 mg/kg
Liver										
-Centrilobular hypertrophy	0/10	6/10	6/10	9/10	7/10	0/10	5/10	8/10	8/10	9/10
Thyroid										
-Follicular cell hyperplasia	0/10	5/10	3/10	5/10	5/10	0/10	2/10	7/10	10/10	8/10
Pancreas										
-Acinar atrophy	1/10	0/10	0/10	0/10	5/10	0/10	0/10	0/10	0/10	0/10
Uterus										
-Distention	--	--	--	--		1/10	0/10	1/10	1/10	3/10
Prostate										
-Chronic focal inflammation	1/10	0/10	0/10	0/10	4/10	--	--	--	--	--

Toxicokinetics: Following oral administration of L-754030 at doses of 125, 250, 500 and 1000 mg/kg/day to male and female rats for 13 weeks, the maximum plasma concentrations were reached in 2.0 to 4.0 hours following administration. The C_{max} and AUC values for L-754030 did not increase with increasing doses, suggesting a saturation of absorption at 125 mg/kg/day. The mean C_{max} values in male rats at 125, 250, 500 and 1000 mg/kg/day doses were 0.554, 0.524, 0.373 and 0.431 µg/ml, respectively. The AUC values at these doses were 3.79, 6.24, 3.69 and 4.15 µg.hr/ml, respectively. In females, the C_{max} values were 0.916, 1.36, 1.12 and 1.01 µg/ml, and the AUC values were 8.67, 15.1, 8.56 and 8.69 µg.hr/ml at 125, 250, 500 and 1000 mg/kg/day doses, respectively. Thus, in female rats, both C_{max} and AUC values plateaued at the 250 mg/kg/day dose. The toxicokinetic parameters for L-754030 in male and female rats are shown in the Table below.

MEAN PLASMA CONCENTRATIONS AND TOXICOKINETIC PARAMETERS OF L-754,030 IN MALE AND FEMALE RATS IN DRUG WEEK 13 FOLLOWING REPEATED ORAL DOSING

	125 mg/kg/day		250 mg/kg/day		500 mg/kg/day		1000 mg/kg/day	
	Males	Females	Males	Females	Males	Females	Males	Females
C_{max} (µg/ml)	0.554	0.916	0.524	1.36	0.373	1.12	0.431	1.01
T_{max} (hr)	2	2	2	2	2	4	2	4
AUC [µg•hr/ml (0-24 hr)]	3.79	8.67	6.24	15.1	3.69	8.56	4.15	8.69

Summary: In the 14–week oral dose-ranging study with L-754,030 in rats, the drug was administered at doses of 0, 125, 250, 500 and 1000 mg/kg/day. Males and females receiving 500 and 1000 mg/kg/day doses had increased neutrophil counts. Increased liver and thyroid weights were observed in male and female animals receiving the drug. Centrilobular hypertrophy of the liver and thyroid follicular cell

hyperplasia were observed in male and female rats at all doses. High dose males had an increased incidence of pancreatic acinar cell atrophy. The target organs of toxicity were the liver, thyroid gland and pancreas, and the NOAEL was not established.

Fourteen-Week Oral Range-Finding Study in Rats (Report Date/Number TT #97-117-0).

Testing Laboratory: Merck Institute for Therapeutic Research
Merck Research Laboratories
Merck & Co., Inc.
West Point, PA 19486

Date Started: November 3, 1997

Date Completed: July 8, 1998

GLP Compliance: Statements of compliance with GLP Regulations and the Quality Assurance Unit were included.

Animals: Sprague Dawley [———— BR] rats were used in these studies. At the start of treatment, animals were 35 days of old and body weight ranges were 98-150 g for male rats and 101-124 g for female rats. b(4)

Drug Batch: L-754,030-004H026

APPEARS THIS WAY
ON ORIGINAL

Methods: Rats received L-754,030 by oral gavage at doses of 0, 5, 25, 125, and 250 mg/ kg/day-B.I.D. for 14 weeks. Male rats received a total of 182 doses and female rats received a total of 184 doses. There were 15 rats/sex/group. Control rats received the vehicle, 0.5% aqueous methylcellulose containing 0.02% sodium lauryl sulfate. The dose volume was 5 mL/kg, twice per day. For B.I.D. groups, the vehicle or L-754,030 was administered a minimum of 5-6 hr apart. Animals were fed between the first and second doses. The initiation of feeding was 2-3 hr after the last drug treatment group received its first daily dose and 3-4 hr before administration of the second daily dose. From week 7 to study termination, the initiation of feeding was 2-2.5 hr after the last drug treatment group received their first daily dose and 3-4.5 hr before administration of the second daily dose. Rats were examined daily for mortality and clinical signs of toxicity. Body weight was measured prior to the start of treatment, and once or twice/week, thereafter. Food consumption was estimated twice per week by visual inspection. Ophthalmic examinations were performed during weeks 6 and 12 on control-B.I.D. and 250 mg/kg/day B.I.D. groups. Blood for determination of hematological and clinical chemistry parameters was collected at weeks 5 and 11. Urine for analysis was collected from 10 rats/sex/group during week 11. Blood for determination of plasma drug levels was collected in week 13 at 2, 4, 6, 8, 10, 16, and 24 hr after the first dose. Three to four rats/sex/group were bled at each time point. Blood samples (3 mL) were also collected from control groups to match treatment groups. L-754,030 was isolated by solid phase extraction and quantified by Liquid Chromatography/Mass Spectrometry/Mass Spectrometry. At the termination of treatment, all animals were sacrificed and a complete gross examination was performed on each animal. Organ weights were measured for the heart, spleen, brain, pituitary, kidneys, testes, prostate, thyroid gland, liver, adrenal glands, and ovaries. Organ weights were expressed as absolute, percent of body weight, and percent of brain weight. A complete microscopic examination for organs and tissues from the control-B.I.D. and 250 mg/kg/day-B.I.D. groups was performed as follows: lung, heart, liver, kidneys, urinary bladder, spleen, thymus, lymph nodes, adrenal glands, thyroid gland (with parathyroid), pituitary gland, salivary gland, stomach, small intestine, pancreas, spinal cord, peripheral nerve, eye (with optic nerve and Harder's gland), skin (with mammary gland), bone (including joint), bone marrow, testes and epididymides, prostate, ovaries, esophagus, uterus, brain, skeletal muscle, and large intestine. In addition, all grossly observed changes in the liver, thyroid gland, and pituitary gland from all animals were subjected to microscopic examination.

Results:

1. **Observed Effects:** There were no treatment-related observed effects.

2. **Mortality:** There were no treatment-related deaths.

3. **Body Weight and Food Consumption:** There were no treatment-related changes of body weight gain or food consumption. Body weights for the male control-B.I.D. group at weeks -1 and 13 were 128 and 388 g, respectively. Body weight gains for the male 5, 25, 125, and 250 mg/kg/day-B.I.D. groups were 100.4, 100.8, 98.14, and 101.2% of the control, respectively. Body weights for the female control-B.I.D. group at weeks -1 and 13 were 111 and 232 g, respectively. Body weight gains for the male 5, 25, 125, and 250 mg/ kg/day-B.I.D. groups were 103.5, 102.5, 90.2, and 100.9% of the control, respectively.

4. **Hematology:** A number of small, statistically significant changes were observed in hematological parameters (i.e., hemoglobin levels, hematocrit, mean corpuscular volume) for male and female

treatment groups; however, changes were generally <5-10% and appeared to have little or no biological significance.

5. Blood Biochemistry and Urinalysis:

Week 5: Protein levels for the female 125 and 250 mg/kg/day-B.I.D. groups were both increased to 108.3% of the control (6.0 g/dL). Protein levels for the male 125 and 250 mg/kg/day-B.I.D. groups were increased to 106.8 and 105.1% of the control (5.9 g/dL), respectively. The albumin to globulin ratio for the female 5, 25, 125, and 250 mg/kg/day-B.I.D. groups were decreased to 92.9, 85.7, 78.6, and 85.7% of the control (1.4), respectively. The albumin to globulin ratio for the male 250 mg/kg/day-B.I.D. group was increased to 92.3% of the control (1.3). Alkaline phosphatase activity for the female 125 and 250 mg/kg/day-B.I.D. groups were decreased to 77.7 and 77.0% of the control (148 U/L), respectively. Cholesterol levels for the female 125 and 250 mg/kg/day-B.I.D. groups were increased to 123.1 and 128.6% of the control (91 mg/dL), respectively. Triglyceride levels for the female 125 and 250 mg/kg/day-B.I.D. groups were decreased to 84.4 and 75% of the control (64 mg/dL), respectively. Triglyceride levels for the male 5, 25, 125, and 250 mg/kg/day-B.I.D. groups were decreased to 85.5, 82.3, 51.6, and 45.2% of the control (62 mg/dL), respectively.

Week 11: Protein levels for the female 5, 25, 125, and 250 mg/kg/day-B.I.D. groups were increased to 103.2, 106.3, 117.5, and 114.3% of the control (6.3 g/dL), respectively. Protein levels for the male 25, 125, and 250 mg/kg/day-B.I.D. groups were increased to 103.3, 106.6, and 108.2% of the control (6.1 g/dL), respectively. Albumin levels for the female 125 and 250 mg/kg/day-B.I.D. groups were increased to 108.3 and 105.6 of the control (3.6 g/dL), respectively. The albumin to globulin ratio for the female 5, 25, 125, and 250 mg/kg/day-B.I.D. groups were decreased to 92.3, 92.3, 84.6, and 84.6% of the control (1.3), respectively. The albumin to globulin ratio for the male 5, 25, 125, and 250 mg/kg/ day-B.I.D. groups were decreased to 91.7, 91.7, 83.3, and 83.3% of the control (1.2), respectively. Alkaline phosphatase activity for the female 125 and 250 mg/kg/day-B.I.D. groups were decreased to 67.5 and 71.1% of the control (83 U/L), respectively. Calcium levels for the female 250 mg/kg/day-B.I.D. group were increased to 105.2% of the control (9.7 mg/dL). Cholesterol levels for the female 25, 125, and 250 mg/kg/day-B.I.D. groups were increased to 121.95, 145.1, and 158.5% of the control (82 mg/dL), respectively. Triglyceride levels for the female 125 and 250 mg/kg/day-B.I.D. groups were decreased to 88.9 and 84.1% of the control (63 mg/dL), respectively. Triglyceride levels for the male 5, 25, 125, and 250 mg/kg/day groups were decreased to 84.8, 63.3, 44.3, and 43% of the control (79 mg/dL), respectively. There were no treatment-related changes of urinalysis parameters.

6. Ophthalmic Examination: There were no treatment-related ophthalmic effects.

7. Organ Weights: Treatment-related increases in absolute and relative liver and thyroid gland weights were observed for all male and female treatment groups. Increased liver weight was correlated with hepatocellular hypertrophy. Increased thyroid gland weight was correlated with thyroid follicular cell hyperplasia.

Liver: Absolute liver weight for the male 5, 25, 125, and 250 mg/kg/day-B.I.D. groups were increased to 111.3, 126.8, 135.4, and 149.9% of the control (10.68 g), respectively. Relative liver weight for the male 5, 25, 125, and 250 mg/kg/day-B.I.D. groups were increased to 109.7, 124.9, 132.1, and 145% of the control (2.89%), respectively. Absolute liver weight for the female 5, 25, 125,

and 250 mg/kg/day-B.I.D. groups were increased to 122.8, 148, 179.6, and 180.4% of the control (6.79 g), respectively. Relative liver weight for the female 5, 25, 125, and 250 mg/kg/day-B.I.D. groups were increased to 121.9, 145.1, 179.1, and 180.7% of the control (3.06%), respectively.

Thyroid Gland: Absolute thyroid gland weight for the male 5, 25, 125, and 250 mg/ kg/day-B.I.D. groups were increased to 127.7, 129.7, 129, and 150% of the control (0.0148 g), respectively. Relative thyroid gland weight for the male 5, 25, 125, and 250 mg/kg/day-B.I.D. groups were increased to 125, 127.5, 127.5, and 145% of the control (0.004%), respectively. Absolute thyroid gland weight for the female 5, 25, 125, and 250 mg/kg/day-B.I.D. groups were increased to 113.7, 119.8, 118.3, and 138.2% of the control (0.0131 g), respectively. Relative thyroid gland weight for the female 5, 25, 125, and 250 mg/kg/day-B.I.D. groups were increased to 113.6, 118.6, 118.6, and 139% of the control (0.0059%), respectively.

8. Gross Pathology: The sponsor did not provide a summary table or individual line listings for gross pathological changes.

9. Histopathology: Target organs of toxicity were the liver, thyroid gland, and pituitary gland. For the liver, very slight to slight hepatocellular hypertrophy was observed in all male and female treatment groups. Hepatocyte hypertrophy most likely represents an induction of cytochrome P-450 enzymes and has no toxicological significance. Very slight to slight diffuse hepatocellular vacuolation was observed with an increased incidence for male rats that received a dose of 250 mg/kg/day-B.I.D. For the thyroid gland, very slight to slight diffuse thyroid follicular cell hyperplasia was observed for all male and female treatment groups. Follicular cell hyperplasia of the thyroid gland is most likely due to increased catabolism of thyroxine (T_4) and triiodothyronine (T_3) by the liver. For the pituitary gland, a very slight to slight vacuolation of individual cells in the pars distalis was observed for male rats that received doses of 125 and 250 mg/kg/day-B.I.D. This change was characterized by enlargement of individual pituitary cells due to formation of large cytoplasmic vacuoles and occasional protein droplets. This change may represent a degeneration or exhaustion of Thyroid stimulating hormone-producing pituitary cells secondary to hepatic enzyme induction and increased catabolism of T_3 and T_4. For the adrenal gland, diffuse vacuolation was observed in the cortex.

Histopathological changes for rats that received L-754,030 by oral gavage at doses of 0, 5, 25, 125, and 250 mg/kg/day-B.I.D. for 14 weeks (n = 15/group except where noted).

Organ/Tissue	0		5		25		125		250	
	F	M	F	M	F	M	F	M	F	M
Liver										
-hepatocyte hypertrophy	0	0	12	5	15	12	15	15	15	15
-diffuse vacuolation	5	4	6	3	7	4	10	6	6	11
Thyroid gland										
-follicular cell, diffuse hyperplasia	0	0	1	4	4	7	7	9	12	12
Pituitary gland										
-vacuolation	0	0	0	0	0	0	0	2	0	2
-cyst	0	0	0	0	0	0	0	0	4	0
Adrenal gland	n=15	n=15	n=1	n=2	n=1	n=0	n=0	n=1	n=15	n=15
-cortex,diffuse vacuolation	0	0							0	2

10. Plasma Drug Levels: Plasma C_{max} and AUC values for L-754,030 in male and female rats plateaued at doses ≥125 mg/kg/day. Plasma C_{max} and AUC values for L-754,030 in female rats were 3 to 4 times greater than corresponding values in male rats.
Toxicokinetic parameters for plasma levels of L-754,030 at week 13 in rats that received L-754,030 by oral gavage at dose of 5, 25, 125, and 250 mg/kg/day-B.I.D.

Dose, mg/kg/day-B.I.D.	C_{max}, µg/mL		T_{max}, hr		$AUC_{0\text{-}24hr}$, µg*hr/mL	
	Male	Female	Male	Female	Male	Female
5	0.334	0.560	8.00	8.00	2.31	6.81
25	0.325	0.905	8.00	8.00	5.04	13.7
125	0.385	1.62	8.00	16.00	6.04	27.3
250	0.333	1.64	24.00	8.00	6.37	25.7

Rats received L-754,030 by oral gavage at doses of 0, 5, 25, 125, and 250 mg/kg/ day-B.I.D. for 14 weeks. A no effect dose was not established. Target organs of toxicity were the liver, thyroid gland, and pituitary gland. For the liver, very slight to slight hepatocellular hypertrophy was observed in all male and female treatment groups. Hepatocyte hypertrophy most likely represents an induction of cytochrome P-450 enzymes and has no toxicological significance. Very slight to slight diffuse hepatocellular vacuolation was observed with an increased incidence for male rats that received a dose of 250 mg/kg/day-B.I.D. For the thyroid gland, very slight to slight diffuse thyroid follicular cell hyperplasia was observed for all male and female treatment groups. Follicular cell hyperplasia of the thyroid gland is most likely due to increased catabolism of thyroxine (T_4) and triiodothyronine (T_3) by the liver. For the pituitary gland, a very slight to slight vacuolation of individual cells in the pars distalis was observed for male rats that received doses of 125 and 250 mg/kg/day-B.I.D. This change was characterized by enlargement of individual pituitary cells due to formation of large cytoplasmic vacuoles and occasional protein droplets. This change may represent a degeneration or exhaustion of Thyroid stimulating hormone-producing pituitary cells secondary to hepatic enzyme induction and increased catabolism of T_3 and T_4. For the adrenal gland, diffuse vacuolation was observed in the cortex for male rats that received 250 mg/kg/day-B.I.D. Plasma C_{max} and AUC values for L-754,030 during week 13 in male and female rats plateaued at doses ≥125 mg/kg/day. Plasma C_{max} and AUC values for L-754,030 in female rats were 3 to 4 times greater than corresponding values in male rats.

Study Title: L-754030: Fourteen (14)-Week Oral Toxicity Study in Rats

Study no. TT#95-627-0

Conducting laboratory (and location if not Sponsor): Merck Research Laboratories, Route de Marsat, Riom, 63963 Clermont-Ferrand Cedex 9, France.

Dates of study initiation & completion: November 16, 1995 and June 10, 1997.

GLP compliance: Yes

QA Report Yes (X) **No** ( )

Drug, Lot #, radiolabel (if applicable), and % purity: L-754030; Lot # L-754030-000Z; purity >99%.

Formulation/vehicle: L-75030 was suspended in 0.5% methylcellulose and 0.03% sodium lauryl sulfate.

Methods:

Dosing: All male and female animals received a total of 94 doses.

Species/strain: Sprague Dawley rats

#/sex/group or time point (main study): 15 animals/sex/group were used in the study.

Satellite groups used for toxicokinetics or recovery: N/A

Weight: males – 157 to 180 g; females – 121 to 145 g.

Age: Approximately 6 weeks

Doses in administered units: L-754030 was administered at oral doses of 0.2, 1.0 and 5.0 mg/kg/day.

Route, form, volume and infusion rate: The doses were administered by oral gavage at a dosing volume of 5.0 ml/kg. Control animals received the same volume of the vehicle (0.5% methylcellulose and 0.03% sodium lauryl sulfate).

Times at which Observations were made:

Clinical signs- The animals were observed daily for clinical signs and mortality.

Body weights- Body weights were measured prior to initiation of dosing, once in Week 1 and twice a Week thereafter.

Food consumption- Food consumption was recorded twice a week during the dosing period.

Gross pathology- At the end of the dosing period, all animals were sacrificed and a partial necropsy, limited to the liver, was performed.

Organs weighed- The weights of only the liver were recorded.

Histopathology- Only the liver from the control and high dose males and females, and the mid dose females were examined microscopically following staining with hematoxylin and eosin.

Toxicokinetics- Blood samples were collected from all drug-treated animals (3-4 animals/time point) on Week 13 at 30 minutes, and 2, 4, 6, 8, 10 and 24 hours after dosing. Plasma concentrations of L754030 were determined by an LC/MS/MS method.

Results:

Mortality: There were no mortalities in any group.

Clinical signs: No treatment related clinical signs were observed in any group.

Body weights: The mean body weights of the control male and female animals before initiation of dosing were 167 and 134 g and in Week 14 were 440 and 246 g, respectively. No treatment related changes in the body weight were observed in any group.

Food consumption: No treatment-related changes in the food consumption were observed in any group.

Gross Pathology: No treatment related gross pathological changes were observed in the liver of any treatment group animals.

Organ weight: The absolute and relative weights of the liver of females from all treatment groups were slightly higher than that of controls. The percent increases in the liver weight in female rats are shown in the Table below.

Percent increases in liver weights above control values

L-754,030	0.2 mg/kg/day	1.0 mg/kg/day	5.0 mg/kg/day
Females			
Liver Weight (gm)	+4.7 NS	+6.0 S	+11.4 S
Liver Weight (% B.W.)	+3.7 NS	+6.7 S	+10.7 S

(gm) = absolute organ weights
(% B.W.) = weights expressed as a percent of body weight
NS = trend not statistically significant through the indicated dose ($P > 0.05$)
S = trend statistically significant through the indicated dose ($P \leq 0.05$)

Histopathology: Slight centrilobular hypertrophy of the hepatocytes was observed in two (of 15) high dose (5 mg/kg/day) male rats.

Toxicokinetics: Following oral administration of L-754030 to male and female rats, the C_{max} and AUC values increased with increasing doses. The maximum plasma concentrations were reached in 4.0 hours in both males and females. The C_{max} and AUC values for L-750030 in females were higher than that of males at all doses. The toxicokinetic parameters for L-754030 in male and female rats are shown in the Table below.

Summary of Mean Plasma L-754,030 Toxicokinetic Parameters in Male and Female Rats in Drug Week 13 After Repeated Oral Dosing of L-754,030 (0.2, 1, and 5 mg/kg/day)

	0.2 mg/kg/day		1 mg/kg/day		5 mg/kg/day	
	Males	Females	Males	Females	Males	Females
C_{max}(µg/ml)	0.0275	0.0598	0.122	0.207	0.332	0.633
T_{max} (hr)	4	4	4	4	4	4
AUC 0-24 hr (µg•hr/ml)	0.275	0.964	1.05	2.97	3.20	6.96

Summary: In the 14–week oral toxicity study with L-754030 in rats, the drug was administered at doses of 0, 0.2, 1.0 and 5.0 mg/kg/day. This was not a complete toxicology study, because hematology, clinical chemistry and urinalysis were not performed and gross pathology, organ weight and histopathology examinations were limited to only the liver. Slight increases in liver weights were observed in female animals receiving the drug, and a slight centrilobular hypertrophy of the hepatocytes was observed in two (of 15) high dose (5 mg/kg/day) male rats.

Study title: **Twenty Seven (27)-Week Oral Toxicity Study in Rats.**

b(4)

Key study findings: In the 27-week oral toxicity study with MK-0869 —— n particle size) in Sprague-Dawley rats, treatment-related alterations of the hematology and clinical chemistry parameters were observed at all doses in both males and females. Hepatocellular hypertrophy and thyroid follicular cell hyperplasia were observed in both sexes in all treatment groups. The no effect dose was not identified, and the target organs of toxicity were the liver and the thyroid gland.

Study no: 01-092-0
Volume #, and page #: Vol #15, page # A4938

Conducting laboratory and location: Merck Research Laboratories, Merck & Co., Inc., West Point, PA.
Date of study initiation: July 11, 2001
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Four batches of MK-0869 blended ——— were used in the study. Batches #X0869OPP015C001 (also known as L-754030-016S001) and #F0869OPP018C004 (also known as L-754030-016S002) were obtained by blending 3 and 2 batches, respectively, of MK-0869 ——— Batches #X0869OPP023C001 (also known as L-754030-018W005) and #X0869OPP024C001 (also known as L-754030-018W006) were used without blending multiple batches of ——— The purity of the batches ranged from 99.6% to 100%. b(4)

Formulation/vehicle: Dosing formulations were prepared by dispersing the ——— in 4% hydroxypropylcellulose, 20% sucrose and 0.19% sodium lauryl sulfate (SLS) in deionized water. The average particle size of MK-0869 in the dispersion was ——— n. Control 1 animals received 0.5% methylcellulose in deionized water and control 2 animals received 4% hudroxypropylcellulose, 20% sucrose and 0.19% SLS in deionized water. b(4)

Methods: There were two control groups in the study, and the treatment groups received 125, 500 and 1000 mg/kg b.i.d. doses of the drug. The second dose was administered approximately 6 hours after the first dose.

Dosing:

Species/strain: ——— BR Sprague-Dawley rats. b(4)

#/sex/group or time point (main study): 20 animals/sex/group were used in the study, except in the 500 mg/kg group which had 21 males and 19 females due to a gender identification error.

Satellite groups used for toxicokinetics or recovery: None

Age: 37 days old at study initiation.

Weight: Males: 122 to 178 g; Females: 87 to 147 g.

Doses in administered units: The treatment groups received the drug at 125, 500 and 1000 mg/kg b.i.d. (250, 1000 and 2000 mg/kg/day) doses.

Route, form, volume, and infusion rate: Dosing formulations were prepared by dispersing the ——— in 4% hydroxypropylcellulose, 20% sucrose and 0.19% sodium lauryl sulfate, and the doses were administered by oral gavage (5 ml/kg b.i.d). b(4)

Observations and times:

Clinical signs: The animals were observed daily for clinical signs and mortality.

Body weights: Body weights were recorded once prior to initiation of dosing and once or twice a week during the dosing period.

Food consumption: Food consumption was measured twice per week during weeks 1 to 13 and twice every 4th week thereafter.

Ophthalmoscopy: Ophthalmoscopic examinations were performed of all control 1, control 2 and high dose animals in weeks 12 and 26.

Hematology: Blood samples for hematological examinations were collected in dosing weeks 4, 12 and 25. The following hematological parameters were determined: erythrocytes, reticulocytes,

hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelets, leukocytes, leukocytes differential count and cell morphology.

Clinical chemistry: Blood samples for clinical chemistry analyses were collected in Drug Weeks 4, 12 and 25.

Urinalysis: Urinalysis was performed on urine samples collected from 10 rats/sex/group in Dosing Weeks 12 and 25.

Gross pathology: Complete necropsies of all animals were conducted at termination. Animals that died or were sacrificed moribund were also examined macroscopically.

Organs weighed: The weights of the following organs from all animals were recorded: adrenals, brain, heart, ovaries, kidneys, liver, pituitary, prostate, spleen, testes, thyroids.

Histopathology: The following tissues from the vehicle control, high dose and animals found dead/sacrificed early were examined histologically.
Salivary gland, esophagus, stomach, small intestine (duodenum, jejunum, ileum), large intestine, liver, pancreas, adrenals, parathyroid, pituitary, thyroid, kidneys, urinary bladder, ovaries, uterus, skin, mammary gland, lung, heart, spleen, lymph nodes (cervical and mesenteric), thymus, bone marrow, bone, skeletal muscle, brain, spinal cord, peripheral nerve, eye, testes and epididymides, prostate, harderian gland.

Toxicokinetics: Blood samples for toxicokinetic analysis were collected from 4 animals/sex/treatment group/sampling time at 2, 4, 6, 8, 10, 16 and 24 hours post-dose in Week 13. Plasma concentrations of MK-0869 were determined by Liquid chromatography – tandem mass spectrometry.

Results:

Mortality: One high dose female was found dead in Week 4. The cause of the death was not known, however, it could be related to treatment with the drug. Another high dose female died from possible anesthesia accident in Week 25. One high dose male died in Week 12 and the cause of the death was stated to be an intubation accident. One mid-dose male was found dead following anesthesia in Week 4 and one low dose male was sacrificed because of trauma.

Clinical signs: No treatment-related clinical signs were observed in any group.

Body weights: The mean body weights of the control 1 and control 2 males before initiation dosing were 155±11 and 157±9 g and at the end of the dosing period were 485±20 and 522±26 g, respectively. The mean body weights of the control 1 and control 2 females before initiation of dosing were 124±8 and 117±10 g and at the end of dosing were 269±13 and 283±20 g, respectively. Treatment with MK-0869 was not associated with any changes in the body weights in any group.

Food consumption: The sponsor stated that there were no treatment-related changes in the food consumption any group (no data provided).

Ophthalmoscopy: No treatment-related abnormal ophthalmic changes were observed in any group.

Hematology: Increases in the platelet levels were observed in treatment group males at Weeks 4, 12 and 25 and in females at Week 4. In treatment group females, slight decreases in hemoglobin, hematocrit, MCV, MCH and MCHC were observed in Weeks 12 and 25. Hematological changes observed in the male and female animals at different times of the treatment period are shown in the Table below.

Parameter	Treatment Week	Control values	Percent changes from control		
			250 mg/kg/day	1000 mg/kg/day	2000 mg/kg/day
Males:					
Platelets (1,000/MM^3)	Week-4	1278 ± 161	+20	+15	+14
	Week-12	1247 ± 220	+18	+12	+14
	Week-25	1240 ± 163	+19	+17	+18
Females:					
Platelets (1,000/MM^3)	Week-4	1383 ± 167	+27	+23	+30
Hemoglobin (g/100 ml)	Week-12	14.4 ± 0.5	-8	-8	-8
	Week-25	14.0 ± 0.6	-11	-12	-11
Hematocrit (%)	Week-12	40.7 ± 1.3	-6	-6	-7
	Week-25	40.2 ± 1.7	-10	-10	-9
MCV (Cubic micron)	Week-12	53.0 ± 1.1	-4	-5	-5
	Week-25	53.8 ± 1.2	-6	-6	-6
MCH (micro gram)	Week-12	18.7 ± 0.4	-5	-7	-6
	Week-25	18.8 ± 0.5	-8	-8	-8
MCHC (g/dl)	Week-25	18.8± 0.5	-2	-3	-2

Clinical chemistry: There were increases in the serum protein and albumin levels, and decreases in albumin/globulin ratio in both males and females receiving MK-0869. Treatment group females had slight decreases in the alkaline phosphatase levels. Both males and females had slightly higher serum potassium and calcium levels. Treatment group females had higher cholesterol levels and both males and females had lower triglyceride levels. Males also had slightly higher cholesterol levels during Week 12. However, none of the effects were dose-dependent, except the cholesterol levels in the males. The changes in the clinical chemistry parameters in the male and female animals are summarized in the Table below.

Parameter	Treatment Week	Control values	Percent changes from control		
			250 mg/kg/day	1000 mg/kg/day	2000 mg/kg/day
Males:					
Protein (g/dL)	Week-4	5.6 ± 0.2	+4	+7	+5
	Week-12	6.2 ± 0.2	+6	+10	+6
	Week-25	6.5 ± 0.2	+8	+9	+8
Albumin (g/dL)	Week-12	3.5 ± 0.1	+3	+6	+3
	Week-25	3.5 ± 0.2	+6	+6	+6
A/G Ratio	Week-4	1.4 ± 0.1	-14	-14	-14
Potassium (meq/L)	Week-4	5.1 ± 0.3	+6	+8	+8
	Week-12	4.9 ± 0.2	+8	+6	+6
	Week-25	5.0 ± 0.2	+10	+12	+10
Cholesterol (mg/dL)	Week-12	64 ± 9	+20	+28	+29
Triglycerides (mg/dL)	Week-4	65 ± 36	-48	-48	-55
	Week-12	96 ± 43	-56	-61	-63
	Week-25	88 ± 41	-65	-69	-75
Females:					
Protein (g/dL)	Week-4	6.0 ± 0.3	+12	+16	+12
	Week-12	6.6 ± 0.3	+19	+23	+19
	Week-25	7.1 ± 0.6	+19	+20	+17
Albumin (g/dL)	Week-12	3.8 ± 0.2	+11	+16	+11
	Week-25	4.2 ± 0.4	+9	+12	+7
A/G Ratio	Week -4	1.6 ± 0.1	-19	-19	-25
	Week-12	1.4 ± 0.1	-14	-7	-14

	Week-25	1.5 ± 0.2	-19	-19	-19
Alkaline Phosphatase (U/L)	Week-4	146 ± 30	-20	-20	-20
	Week-12	67 ± 14	-21	-23	-15
	Week-25	45 ± 18	-23	-17	-9
Potassium (meq/L)	Week-4	4.6 ± 0.2	+9	+4	+7
	Week-12	4.5 ± 0.2	+9	+9	+9
	Week-25	4.4 ± 0.3	+7	+12	+12
Cholesterol (mg/dL)	Week-4	83 ± 10	+50	+68	+59
	Week12	79 ± 11	+85	+105	+92
	Week25	97 ± 20	+88	+107	+88
Triglycerides (mg/dL)	Week-4	66 ± 24	-13	-26	-43
	Week-12	56 ± 19	-17	-17	-37
	Week-25	80 ± 37	-30	-16	-37

Urinalysis: No significant treatment-related changes were observed in any group.

Organ weights: Treatment-related increases in the liver and thyroid weights (both absolute and relative) were observed in both males and females receiving MK-0869. The increases in the liver and thyroid weights were not dose-related and appeared to plateau between 250 and 1000 mg/kg/day doses. The liver and thyroid weights (in grams) and the changes in absolute and relative weights in different groups of animals are summarized in the Table below.

Organs	Control Weights		Percent Changes From Control					
			250 mg/kg/day		1000 mg/kg/day		2000 mg/kg/day	
	Males	Females	Males	Females	Males	Females	Males	Females
Liver (absolute)	12.73±0.97 g	8.04±0.74 g	57%	97%	70%	116%	65%	107%
Liver (relative)	2.70±0.18	3.09±0.27	58%	98%	72%	120%	67%	112%
Thyroid (absolute)	0.024±0.0033 g	0.014±0.0016 g	60%	56%	61%	74%	44%	74%
Thyroid (relative)	0.0051±0.0007	0.0056±0.0006	61%	56%	63%	77%	46%	77%

Gross pathology: Males and females of all treatment groups had enlarged liver and thyroid glands. The enlargement of the liver was associated with hepatocellular hypertrophy, characterized by enlarged eosinophilic hepatocytes surrounding the central vein.

Histopathology: Hepatocellular hypertrophy, characterized by enlarged eosinophilic hepatocytes was observed in all treatment group animals. In the mid- and the high-dose groups, enlarged hepatocytes extended into the midzonal region. The severity of the hepatocellular hypertrophy appeared to plateau at 500 mg/kg b.i.d. in females and 125 mg/kg b.i.d. in males. Individual hepatocytes, in all dose groups, underwent single cell necrosis and had increased mitotic activity. Diffuse thyroid follicular cell hypertrophy, characterized by tall columnar follicular cells with an expanded cytoplasm was observed in both males and females of all treatment groups. The incidences of histopathological changes observed in the liver and thyroid glands of males and females of different groups are shown in the sponsor's Table below.

Histomorphologic Changes
(Incidence, n=20)

	MK-0869 (mg/kg b.i.d.)									
	Females					Males				
	Control 1	Control 2	125	500	1000	Control 1	Control 2	125	500	1000
Liver										
Eosinophilic cellular alteration	1	-	1[a]	1[a]	-	-	-	2[a]	-	1[a]
Centrilobular hypertrophy	-	-	20[a]	20[a]	20[a]	-	-	20[a]	20[a]	20[a]
Midzonal vacuolation	-	-	8[a]	12[a]	9[a]	-	-	20[a]	19[a]	20[a]
Single-cell necrosis	3	-	12[a]	16[a]	17[a]	3	3	19[a]	19[a]	18[a]
Mitotic activity	3	-	10[a]	13[a]	8[a]	-	1	5[a]	9[a]	5[a]
Multinucleated hepatocytes	-	-	17[a]	16[a]	17[a]	-	-	2[a]	4[a]	2[a]
Thyroid										
Hypertrophy, diffuse, follicular cell	-	-	18[a]	19[a]	19[a]	-	-	20[a]	19[a]	20[a]
Hyperplasia, Focal, cystic, follicular cell	-	-	-	-	-	-	-	2[a]	-	-

[a] Treatment-related changes based on incidence and/or severity.
- = Not observed.

Toxicokinetics: Following multiple dosing to male and female rats, the maximum plasma concentration (C_{max}) and the plasma exposure (AUC) values were up to 4-fold higher in females as compared to that of males. The C_{max} was achieved in 8 to 12 hours after dosing in both sexes. The exposure levels appeared to plateau at the 250 mg/kg bi.d. dose in both males and females. Plasma toxicokinetic parameters in male and female animals receiving MK-0869 are shown in the sponsor's Table below.

Mean Plasma MK-0869 Toxicokinetic Parameters - Drug Week 13

	MK-0869 (mg/kg b.i.d.)		
	Females		
	125	500	1000
$AUC_{0\text{-}24\ hr}$ (µg•hr/mL)[a]	27.9 ± 3.12	26.0 ± 2.74	31.3 ± 2.22
C_{max} (µg/mL)[b]	1.84	1.57	1.97
T_{max} (hr)[c]	10	8.0	10
	Males		
	125	500	1000
$AUC_{0\text{-}24\ hr}$ (µg•hr/mL)[a]	6.51 ± 0.386	7.38 ± 0.546	8.02 ± 0.588
C_{max} (µg/mL)[b]	0.456	0.410	0.511
T_{max} (hr)[c]	10	10	10

[a] Mean ± SEM calculated using all individual plasma concentrations.
[b] Maximum mean plasma concentrations.
[c] Time at which C_{max} occurred.

Summary: In the 27-week oral toxicity study MK-0869 in Sprague-Dawley rats, groups of animals received 125, 500 and 1000 mg/kg b.i.d. (250, 1000 and 2000 mg/kg/day) doses of the drug.

Treatment-related changes in the hematology (increased platelet levels in males and increased platelets, decreased hemoglobin and hematocrit values in females) and clinical chemistry (increased protein and cholesterol and decreased triglycerides in both sexes) parameters were observed in all treatment group animals. Hepatocellular hypertrophy and thyroid follicular cell hyperplasia were observed in both males and females of all treatment groups. Thus, the target organs of toxicity were the liver and the thyroid glands, and the no effect dose was not established.

53-Week Oral Toxicity Study in Rats with a 27-Week Interim Necropsy (TT #97-071-0).

Testing Laboratory: Merck Institute for Therapeutic Research
Merck Research Laboratories
Merck & Co., Inc.
West Point, PA 19486

Date Started: July 7, 1997

Date Completed: November 18, 1998

GLP Compliance: Statements of compliance with GLP regulations and the Quality Assurance Unit were included.

Animals: Sprague-Dawley rats [BR] were used in this study. At the start of treatment, animals were 35 days of age and had body weight ranges of 100 to 174 g for male rats and 93 to 130 g for female rats. b(4)

Drug Batch: L-754,030-004H021 and L-754,030-004H026 (drug particle size)

Methods: In a 52-week oral toxicity study, rats received L-754,030 (drug particle size) at doses of 0, 0.25, 25, and 250 mg/kg/day. There were 30 rats/sex/group. An interim evaluation was conducted with 10 rats/sex/group, which were sacrificed after treatment for 26 weeks. For animals sacrificed at 27 weeks for interim evaluation, male and female rats received 182 and 183 doses, respectively. For animals sacrificed at the termination of the study at 53 weeks, male and female rats received 364-365 and 365-366 doses, respectively. Control animals received the vehicle, 0.5% aqueous methylcellulose containing 0.02% sodium lauryl sulfate. Vehicle or drug suspension was administered by oral gavage using a dose volume of 5 mL/kg. Animals were observed daily for clinical signs of toxicity and moribundity/mortality. Body weight was measured once in week 1, twice per week through week 13, and once per week thereafter. Food consumption was evaluated twice per week from weeks 1 through 13 and twice per week every 4 weeks thereafter. Ophthalmic examinations were conducted in all surviving animals from the control and 250 mg/kg/day groups during weeks 12, 24, 39, and 52. Blood for determination of hematology and serum biochemical parameters was collected during weeks 4, 12, and 25 from 9 to 10 rats/sex/group (rats designated for the interim necropsy) and during weeks 39 and 51 from all surviving animals (rats designated for the final necropsy). Urinalysis was conducted during weeks 12 and 25 with 7 to 10 rats/sex/group (rats designated for interim sacrifice) and during weeks 39 and 51 with surviving animals (rats designated for the final necropsy). At the scheduled interim sacrifice (i.e., week 27), 9 or 10 rats/sex/group were sacrificed, and at the scheduled terminal sacrifice b(4)

(i.e., week 53), 19 or 20 rats/sex/group were sacrificed. Complete gross examinations were conducted on all animals, including those that died or were sacrificed in a moribund condition during the treatment period prior to scheduled necropsy. Absolute and relative organ weights were determined for the adrenal gland, brain, heart, ovaries, kidneys, liver, pituitary gland, prostate, spleen, testes, and thyroid gland. Organs and tissues were collected and preserved from all animals as follows: lung, heart, liver, kidneys, urinary bladder, spleen, thymus, lymph nodes, adrenal glands, thyroid gland, parathyroid gland, pituitary gland, salivary gland, stomach, small intestine, esophagus, pancreas, spinal cord, peripheral nerve, eye (with optic nerve and Harder's gland), skin (from mammary region), bone, bone marrow, testes and epididymides, prostate, ovaries, uterus, brain, skeletal muscle, and large intestine. Complete histopathological examinations were conducted on all animals in the control and 250 mg/kg/day groups sacrificed at interim and final necropsies and from all animals which died or were sacrificed in a moribund condition prior to scheduled termination. Further all gross changes and sections of liver and thyroid gland were submitted to histopathological examination from all animals.

Results:

1. **Observed Effects:** No treatment-related observed effects.

2. **Mortality:** Death or moribund sacrifice during the treatment period occurred for 1 female rat at 0.25 mg/kg/day, 1 female rat at 25 mg/kg/day, and 1 male rat and 3 female rats at 250 mg/kg/day. None of the deaths or moribund sacrifices were considered to be treatment-related.

Death and moribund sacrifice during the treatment period.

Animal #	Dose, mg/kg/day	Number of doses	Death/ Moribund Sacrifice	Cause of Death/ Moribund Sacrifice
97-4201F	25	29	Moribund Sacrifice	Urinary obstruction attributed to pyelonephritis
97-4294M	250	67	Moribund Sacrifice	Bladder filled with blood and markedly enlarged kidney (i.e., pyelonephritis)
97-4275F	250	97	Moribund Sacrifice	Intubation accident
97-4247F	250	309	Moribund Sacrifice	Gastrointestinal distention
97-4123F	0.25	320	Death	Esophageal impaction with food
97-4265F	250	351	Moribund Sacrifice	Pituitary adenoma

3. **Body Weight and Food Consumption:** There were no treatment-related effects on body weight gain, final body weight, or food consumption. Mean body weight of male controls prior to the start of treatment and at weeks 26 and 52 were 137, 471, and 531 g, respectively. Mean body weights of female controls prior to the start of treatment and at weeks 26 and 52 were 111, 257, and 282 g, respectively.

Body weight gain and final body weight, expressed as a percentage of the control, for male and female rats that received L-754,030 at oral doses of 0, 0.25, 25, and 250 mg/kg/day for 26 or 52 weeks.

Week	0.25 mg/kg/day		25 mg/kg/day		250 mg/kg/day	
	Male	Female	Male	Female	Male	Female
Week 26						
-body weight gain (wk 0-26), % of control	98.4	96.4	98.6	103.7	100.3	98.9
-body weight, % of control	99.6	98.8	100.4	98.8	100.2	101.2
Week 52						
-body weight gain (wk 0-52), % of control	98.8	95.6	98.8	97.9	100.25	95.3
-body weight, % of control	100.4	97.9	100.6	98.2	100	98.9

4. Hematology: For female rats at 250 mg/kg/day, there were slight decreases of hemoglobin levels (93-97.2% of the control, 13.4-14.3 g/100 mL) and hematocrit (92.8-96.8% of the control, 38.4-40.6%) at weeks 4, 12, 25, 39, and 51; however, the biological significance of these changes is questionable.

5. Blood Biochemistry and Urinalysis: Total cholesterol levels were increased for female rats at 25 and 250 mg/kg/day during weeks 4, 12, 25, 39, and 51. Triglyceride levels were decreased for male rats at 250 mg/kg/day during weeks 4, 12, 25, 39, and 51, male rats at 25 mg/kg/day during weeks 12, 25, 39, and 51, and male rats at 0.25 mg/kg/day during weeks 39 and 51. There were no treatment-related changes of urinalysis parameters.

Male rats: Triglyceride levels for male rats at 250 mg/kg/day during weeks 4, 12, 25, 39, and 51 were decreased to 72.7, 79.1, 80.3, 55.9, and 50.4% of the control (44-131 mg/dL), respectively. Triglyceride levels for male rats at 25 mg/kg/day during weeks 12, 25, 39, and 51 were decreased to 91.0, 90.9, 61.3, and 55% of the control, respectively. Triglyceride levels for male rats at 0.25 mg/kg/day during weeks 39 and 51 were decreased to 82 and 76.3% of the control, respectively.

Female rats: Total cholesterol levels for female rats at 25 mg/kg/day during weeks 4, 12, 25, 39, and 51 were increased to 111.7, 117.65, 124.4, 130.3, and 126% of the control (68-96 mg/dL), respectively. Total cholesterol levels for female rats at 250 mg/kg/day during weeks 4, 12, 25, 39, and 51 were increased to 116.9, 127.9, 126.9, 136, and 127% of the control, respectively. Triglyceride levels for female rats at 25 and 250 mg/kg/day during week 4 were decreased to 81.5 and 74.1% of the control (54 mg/dL), respectively; however, no changes were observed at later time points in the study. Alanine aminotransferase activities for female rats at 25 and 250 mg/kg/day during week 51 were increased to 129.3 and 148.3% of the control (58 U/L), respectively. Aspartate aminotransferase activity for female rats at 250 mg/kg/day was increased to 116.7% of the control (108 U/L). Albumin/globulin ratios for female rats at 250 mg/kg/day during weeks 4, 12, 25, 39, and 51 were decreased to 86.7, 92.3, 84.6, 91.7, and 92.3% of the control (1.2-1.5), respectively.

6. Ophthalmic Examinations: Ophthalmic examinations conducted in all surviving animals from the control and 250 mg/kg/day groups during weeks 12, 24, 39, and 52 revealed no apparent treatment-related findings. The sponsor provided no data for independent analysis.

7. Organ Weights: Changes in liver and thyroid gland weights were observed that appeared to correlate with histopathological findings.

Liver: At interim necropsy (week 27), absolute liver weights for male rats at 25 and 250 mg/kg/day were increased to 118.5 and 123.5% of the control (11.79 g), respectively. Relative liver weight for male rats at 25 and 250 mg/kg/day were increased to 120 and 126.2% of the control (2.60% B.W.), respectively. Absolute liver weights for female rats at 25 and 250 mg/kg/day were increased to 131.35 and 145.25% of the control (7.05 g), respectively. Relative liver weights for female rats at 25 and 250 mg/kg/day were increased to 126.7 and 143.4% of the control (2.88%), respectively. At terminal necropsy (week 53), absolute liver weights for male rats at 25 and 250 mg/kg/day were increased to 116.9 and 128% of the control (12.95 g), respectively. Relative liver weights for male rats at 25 and 250 mg/kg/day were increased to 117.2 and 129% of the control (2.62% B.W.), respectively. Absolute liver weights for female rats at 25 and 250 mg/kg/day were increased to 126.9 and 142.9% of the control (7.46 g), respectively. Relative liver weights for female rats at 25 and 250 mg/kg/day were increased to 127.3 and 144.2% of the control (2.85% B.W.), respectively.

Thyroid gland: At interim necropsy (week 27), absolute and relative thyroid gland weights for male rats at 250 mg/kg/day were increased to 114.6 and 117% of control values (0.0275 g and 0.0062% B.W.), respectively. Absolute and relative thyroid gland weights for female rats at 250 mg/kg/day were increased to 120 and 118% of control values (0.0210 g and 0.0085% B.W.), respectively. At terminal necropsy (week 53), absolute and relative thyroid glands for male rats at 250 mg/kg/day were increased to 109.6 and 111.3% of control values (0.0286 g and 0.0059% B.W.), respectively. Absolute thyroid gland weights for female rats at 25 and 250 mg/kg/day were increased to 110.7 and 117.7% of the control (0.0187 g), respectively. Relative thyroid gland weights for female rats at 25 and 250 mg/kg/day were increased to 111.1 and 118.1% of the control (0.0072% B.W.), respectively.

8. Gross Pathology: Not reported.

9. Histopathology: Target organs of toxicity were the liver and thyroid gland following treatment for either 26 or 52 weeks. For the liver, slight centrilobular hypertrophy was observed for both male and female rats at 25 and 250 mg/kg/day following treatment for 26 or 52 weeks. For the thyroid gland, slight diffuse follicular cell hyperplasia was observed for both male and female rats at 25 and 250 mg/kg/day following treatment for 26 or 52 weeks. For the liver, organ weight and histological changes appear to be consistent with an induction of cytochrome P-450.

Histopathological findings for rats that received L-754,030 by the oral route at doses of 0, 0.25, 25, and 250 mg/kg/day for 26 weeks (Interim Sacrifice).

Organ/Tissue	0 mg/kg/day		0.25 mg/kg/day		25 mg/kg/day		250 mg/kg/day	
	Female	Male	Female	Male	Female	Male	Female	Male
Liver, n =	10	10	10	10	11	10	11	10
-hemorrhage	0	0	0	0	0	0	0	1
-centrilobular hypertrophy	0	0	0	0	6	6	10	10
-focal necrosis	0	0	0	0	0	0	1	2
Thyroid gland, n =	10	10	10	10	11	10	11	10
-follicular cell diffuse hyperplasia	0	0	0	0	5	5	10	8
Pituitary gland, n =	10	10	1	-	1	-	11	10
-hyperplasia	1	-	-	-	-	-	2	1
Kidney, n =	10	10	1	-	1	1	11	10
-transitional epithelium, hyperplasia	-	-	1	-	-	-	-	2
-mineralization	1	-	1	-	-	-	3	3
-pyelonephritis	-	-	-	-	1	-	-	1

Histopathological findings for rats that received L-754,030 by the oral route at doses of 0, 0.25, 25, and 250 mg/kg/day for 53 weeks (Terminal Sacrifice).

Organ/Tissue	0 mg/kg/day		0.25 mg/kg/day		25 mg/kg/day		250 mg/kg/day	
	Female	Male	Female	Male	Female	Male	Female	Male
Liver, n =	20	20	20	20	19	20	19	20
-centrilobular hypertrophy	0	0	0	0	16	13	18	19
-focal necrosis	-	-	-	-	-	-	1	2
Thyroid gland, n =	20	20	18	20	19	20	19	20
-follicular cell diffuse hyperplasia	-	-	-	-	2	2	10	12
-follicular cell, focal cystic hyperplasia	-	-	-	-	1	-	-	-
Adrenal gland, n=	20	20	4	1	4	1	19	20
-benign pheochromocytoma	-	-	-	-	-	-	-	1
Parathyroid gland, n=	20	20	1	-	-	-	19	20
-benign adenoma	-	-	-	-	-	-	0	1
Pituitary gland, n =	20	20	6	1	3	2	19	20
-benign adenoma	1	1	-	-	1	2	1	1
Large Intestine, n =	20	20	1	1	-	-	19	20
-rectum, malignant leiomyosarcoma	-	-	-	1	-	-	-	-
Skin, n =	20	20	1	-	-	-	19	20
-benign papilloma	-	-	-	-	-	-	-	1

In a 52-week oral toxicity study, rats received L-754,030 ([redacted] drug particle size) at doses of 0, 0.25, 25, and 250 mg/kg/day. An interim evaluation was conducted after treatment for 26 weeks. The no effect dose was 0.25 mg/kg/day. There was no treatment-related mortality. Target organs of toxicity were the liver and thyroid gland following treatment for either 26 or 52 weeks. For the liver, slight centrilobular hypertrophy was observed for both male and female rats at 25 and 250 mg/kg/day following treatment for 26 or 52 weeks. For the thyroid gland, slight diffuse follicular cell hyperplasia was observed for both male and female rats at 25 and 250 mg/kg/day following treatment for 26 or 52 weeks. For the liver, increased absolute and relative organ weights as well as histological changes appeared to be consistent with an induction of cytochrome P-450. This study used the [redacted] drug particle size and selection of the high dose was based upon a saturation of absorption for the parent compound. Subsequent toxicokinetic studies suggested that observed saturation of absorption was actually a saturation of dissolution related to the drug particle size as exposure (i.e., AUC) was found to increase with a [redacted] rug particle size formulation. The sponsor is conducting a 5 week oral toxicity/toxicokinetic study in rats designed as a bridging study to link the [redacted] drug particle size formulations as communicated in Amendment #115 submitted on January 7, 2000. If this bridging study fails to demonstrate saturation for the [redacted] particle size formulation, dose selection for this 52-week oral toxicity study may be deemed to be inadequate and the study might have to be repeated. b(4)

Study Title: L-000758298: Seventeen (17)-Day Intravenous Toxicity Study in dogs

Study no. TT#04-6004

Conducting laboratory (and location if not Sponsor): Merck Research Laboratories, Route de Marsat, Riom, 63963 Clermont-Ferrand Cedex 9, France.

Dates of study initiation & completion: February 23, 2004 and July 27, 2004.

GLP compliance: Yes

QA Report Yes (X) **No** ( )

Drug, Lot #, radiolabel (if applicable), and % purity: L-000758298; Lot # L-000758298-010A001 (also known as #0517HLS007F001); purity 99.6%.

Formulation/vehicle: L-000758298 lyophilized formulation was reconstituted in sterile 0.9% NaCl solution for injection.

Methods: L-000758298 was administered intravenously to beagle dogs at dose levels of 2, 4 or 6 mg/kg/day for about 2 weeks. The males received 14 or 15 doses, and the females received 15 or 16 doses depending on the schedule of sacrifice.

Dosing:

Species/strain: Beagle dogs

#/sex/group or time point (main study): 4 animals/sex/group were used in the study.

Satellite groups used for toxicokinetics or recovery: N/A

Weight: males – 7.3 to 9.9 kg; females – 5.4 to 8.6 kg.

Age: 29 to 32 weeks

Doses in administered units: L-000758298 was administered at i.v. doses of 2, 4 and 6 mg/kg/day.

Route, form, volume and infusion rate: The doses were administered intravenously into a cephalic and/or saphenous vein at dosing volumes of 0.08, 0.16 and 0.32 ml/kg for low, mid and high doses, respectively. The injections were made at a rate of 40 ml/min.

Times at which Observations were made:

Clinical signs- The animals were observed daily for clinical signs and mortality. In addition, each animal was given a detailed physical examination once a week.

Body weights- Body weights were measured pre-test once a week thereafter.

Food and water consumption- Daily food consumption was measured 4 times per week.

Ophthalmoscopy: Ophthalmologic examinations were performed on all animals before initiation of dosing and in week 2 of the dosing period.

Electrocardiography: ECG recordings were performed on all animals before initiation of dosing and in week 2.

Hematology: Blood samples for hematology were collected pre-test and in week 2 of the dosing period.

Clinical chemistry- Blood samples for clinical chemistry analyses were collected pre-test and in week 2 of the dosing period.

Urinalysis- Urine samples were collected pre-dose and in week 2 of the dosing period.

Gross pathology- At the end of the dosing period, the animals were sacrificed and a complete macroscopic examination was performed on all animals.

Organs weighed- The weights of the following organs were recorded.

Adrenals, brain, heart, kidneys, liver, ovary, pituitary, prostate, spleen, testes and thyroid.

Histopathology- Following organs from the control and high dose animals were fixed and examined microscopically.

Salivary gland, esophagus, stomach, small intestine (duodenum, jejunum, ileum), large intestine (colon), liver, gallbladder, mammary gland, lung, heart, spleen, lymph nodes, thymus, pancreas, adrenals, pituitary, thyroid, parathyroid, kidneys, urinary bladder, ovaries, uterus, testes and epididymides, prostate, skin, bone marrow, bone, skeletal muscle, brain, spinal cord, peripheral nerve, optic nerve, injection sites (cephalic and cephaneous vein).

Toxicokinetics- Blood samples were collected from all animals at approximately 2, 5, 15 and 30 minutes and 1, 4, 8 and 24 hours post-dose after the first dose and in week 2 of the dosing period.

Results:

Mortality: One high dose female (#04-0005) was sacrificed on dosing day 13 because dosing of the animal was not feasible because of changes at the injection sites.

Clinical signs: Beginning dosing day 4, there was a treatment-related increased incidence of swelling and/or cutaneous discoloration at mid and high doses (38-88% and 38-100% of the animals were affected, respectively/day). No treatment-related clinical signs were observed in the low dose group.

Body weights: The mean body weights of the control animals before initiation of dosing and in week 2 were 7.8±1.3 and 7.8±1.4 kg, respectively. Animals receiving the mid and high dose had a loss in body weight during treatment week 2 when compared with the pre-treatment (week -1) body weights (-0.2±0.1 and -0.1±0.2 kg, respectively).

Food consumption: The mean food intake of the control animals in weeks 1 and 2 were 298 and 286 g/day, respectively. Compared to control, the mean food intake of the high dose females was lower in week 2 (10%). High dose males had lower food intake in weeks 1 and 2 (11% and 14%, respectively), and the high dose males had lower food intake in week 2 (12%).

Ophthalmoscopy: No ophthalmologic abnormalities were observed in any of the animals.

Electrocardiography: No treatment related changes in the ECG parameters were observed in any group.

Hematology: No treatment related changes in the hematology parameters were observed in any group.

Clinical chemistry: No treatment related changes in clinical chemistry were observed in any group.

Urinalysis: No treatment-related changes were observed in any group.

Gross Pathology: Treatment related gross pathological changes were observed at the injection sites of mid and high dose males and females. The changes were characterized by the presence of obstructive thrombosis and an increased incidence and/or severity of fibroplasia.

Organ weight: No treatment related changes in any organ weight were observed.

Histopathology: Histopathological examinations of all organs were conducted only of the control and the high dose animals, except injection sites. Treatment related histopathological changes were limited to the injection site of mid and high dose animals, and consisted of venous thrombosis, fibroplasia and necrosis, as well as subcutaneous exudation. Histopathological changes observed at the injection sites of male and female dogs are shown in the Table below.

Dose Group	**Males**				**Females**			
	Control	2 mg/kg	4 mg/kg	6 mg/kg	Control	2 mg/kg	4 mg/kg	6 mg/kg
Vein								
-cellular infiltration	0/4	1/4	1/4	0/4	0/4	1/4	0/4	3/4
-fibroplasia	1/4	3/4	3/4	4/4	2/4	1/4	4/4	3/4
-necrosis	1/4	¼	2/4	2/4	1/4	1/4	2/4	4/4
-obstructive thrombosis	0/4	0/4	3/4	3/4	0/4	0/4	3/4	4/4
Dermis								
-cellular infiltration	0/4	1/4	1/4	2/4	0/4	0/4	1/4	1/4
Subcutis								
-cellular infiltration	2/4	4/4	2/4	3/4	2/4	3/4	3/4	3/4
-exudation	1/4	3/4	3/4	3/4	0/4	2/4	3/4	3/4
-fibroplasia	0/4	4/4	3/4	4/4	3/4	2/4	4/4	4/4
-hemorrhage	1/4	3/4	4/4	4/4	3/4	3/4	4/4	4/4
Nerve								
-degeneration	0/4	0/4	1/4	1/4	0/4	1/4	1/4	2/4
Venous intima								
-cellular proliferaation	1/4	2/4	3/4	4/4	0/4	3/4	3/4	1/4

Toxicokinetics: On Drug Day 1, the C_{max} and AUC values of L-000758298 were consistently slightly higher in males than in females. No sex differences in the C_{max} and AUC values were observed in week 2. following i.v. administration of L-000758298 to dogs, the C_{max} was observed in 2 minutes, and the plasma concentrations declined rapidly on day 1 and week 2 of dosing. No measurable plasma L-000758298 concentrations were observed following the 5 min time point on day 1. L-000754030 appeared in the plasma at the first sampling time with a T_{max} of 0.0333 for all dose groups, suggesting a very rapid conversion of L-000758298 to L-000754030. Systemic exposures of L-000758298 and L-000754030

were approximately dose-proportional at all doses on day 1 and week 2. The AUC values for L-000754030 were approximately 2-fold higher in week 2 compared with day 1 of dosing. This could be due to accumulation of L-000754030 due to its moderate elimination. The toxicokinetic parameters for L-000758298 (pro-drug) and L-000754030 (active compound) are shown in the Tables below.

Mean Plasma L-000758298 Toxicokinetic Parameters – Drug Day 1

	L-000758298 (mg/kg/day)		
	Females		
	2	4	6
$AUC_{0\text{-}24\ hr}$ (µg•hr/mL)	0.0898 ± 0.0208	0.198 ± 0.0484	0.578 ± 0.227
C_{max} (µg/mL)	2.09 ± 0.466	4.61 ± 1.10	13.2 ± 5.12
T_{max} (hr)	0.0333 ± 0	0.0333 ± 0	0.0333 ± 0
	Males		
	2	4	6
$AUC_{0\text{-}24\ hr}$ (µg•hr/mL)	0.199 ± 0.0574	0.331 ± 0.0569	0.635 ± 0.110
C_{max} (µg/mL)	4.52 ± 1.37	7.31 ± 1.32	14.5 ± 2.47
T_{max} (hr)	0.0333 ± 0	0.0333 ± 0	0.0333 ± 0
	Sexes Combined		
	2	4	6
$AUC_{0\text{-}24\ hr}$ (µg•hr/mL)	0.145 ± 0.0350	0.265 ± 0.0426	0.607 ± 0.117
C_{max} (µg/mL)	3.30 ± 0.813	5.96 ± 0.945	13.8 ± 2.64
T_{max} (hr)	0.0333 ± 0	0.0333 ± 0	0.0333 ± 0
Values are the mean ± SEM.			

Mean Plasma L-000754030 Toxicokinetic Parameters – Drug Day 1

	L-000758298 (mg/kg/day)		
	Females		
	2	4	6
$AUC_{0\text{-}24\ hr}$ (µg•hr/mL)	17.2 ± 3.35	32.0 ± 3.47	55.0 ± 3.44
C_{max} (µg/mL)	3.60 ± 0.145	5.81 ± 0.463	8.44 ± 0.707
T_{max} (hr)	0.0333 ± 0	0.0333 ± 0	0.0333 ± 0
	Males		
	2	4	6
$AUC_{0\text{-}24\ hr}$ (µg•hr/mL)	15.7 ± 2.64	39.3 ± 3.73	53.4 ± 7.18
C_{max} (µg/mL)	3.95 ± 0.478	6.66 ± 0.352	9.02 ± 0.739
T_{max} (hr)	0.0333 ± 0	0.0333 ± 0	0.0333 ± 0
	Sexes Combined		
	2	4	6
$AUC_{0\text{-}24\ hr}$ (µg•hr/mL)	16.4 ± 1.99	35.6 ± 2.74	54.2 ± 3.70
C_{max} (µg/mL)	3.78 ± 0.241	6.24 ± 0.314	8.73 ± 0.486
T_{max} (hr)	0.0333 ± 0	0.0333 ± 0	0.0333 ± 0
Values are the mean ± SEM.			

Mean Plasma L-000758298 Toxicokinetic Parameters – Drug Week 2

	L-000758298 (mg/kg/day)		
	Females		
	2	4	6
$AUC_{0\text{-}24\ hr}$ (µg•hr/mL)	0.169 ± 0.0434	0.447 ± 0.171	0.784 ± 0.133
C_{max} (µg/mL)	3.92 ± 1.02	9.31 ± 4.18	17.7 ± 2.94
T_{max} (hr)	0.0333 ± 0	0.0333 ± 0	0.0333 ± 0
	Males		
	2	4	6
$AUC_{0\text{-}24\ hr}$ (µg•hr/mL)	0.140 ± 0.0249	0.438 ± 0.114	0.725 ± 0.309
C_{max} (µg/mL)	3.11 ± 0.522	8.41 ± 1.85	16.1 ± 6.88
T_{max} (hr)	0.0333 ± 0	0.0333 ± 0	0.0333 ± 0
	Sexes Combined		
	2	4	6
$AUC_{0\text{-}24\ hr}$ (µg•hr/mL)	0.155 ± 0.0238	0.442 ± 0.0950	0.755 ± 0.156
C_{max} (µg/mL)	3.51 ± 0.551	8.86 ± 2.12	16.9 ± 3.48
T_{max} (hr)	0.0333 ± 0	0.0333 ± 0	0.0333 ± 0
Values are the mean ± SEM.			

Mean Plasma L-000754030 Toxicokinetic Parameters – Drug Week 2

	L-000758298 (mg/kg/day)		
	Females		
	2	4	6
$AUC_{0\text{-}24\ hr}$ (µg•hr/mL)	32.7 ± 6.79	64.8 ± 7.52	125 ± 11.1
C_{max} (µg/mL)	4.72 ± 0.154	8.21 ± 0.810	12.7 ± 0.657
T_{max} (hr)	0.0333 ± 0	0.0333 ± 0	0.0333 ± 0
	Males		
	2	4	6
$AUC_{0\text{-}24\ hr}$ (µg•hr/mL)	29.5 ± 6.52	60.4 ± 12.4	98.4 ± 15.7
C_{max} (µg/mL)	4.23 ± 0.395	7.91 ± 0.655	10.9 ± 1.29
T_{max} (hr)	0.0333 ± 0	0.0333 ± 0	0.0333 ± 0
	Sexes Combined		
	2	4	6
$AUC_{0\text{-}24\ hr}$ (µg•hr/mL)	31.1 ± 4.40	62.6 ± 6.78	112 ± 10.2
C_{max} (µg/mL)	4.47 ± 0.217	8.06 ± 0.486	11.8 ± 0.748
T_{max} (hr)	0.0333 ± 0	0.0333 ± 0	0.0333 ± 0
Values are the mean ± SEM.			

Summary: In the 17–day intravenous toxicity study with L-000758298 in dogs, the drug was administered at doses of 0, 2, 4 and 6 mg/kg/day. Treatment-related changes at the injection sites, consisting of swelling and/or cutaneous discoloration and the presence of hardening above and/or at the site of injection were observed at the mid and high doses. Venous thrombosis, fibroplasia and necrosis, as well as subcutaneous exudation and fibroplasia were observed in male and female animals receiving the mid and high doses. Thus, the injection site was the target organ of toxicity, and the 2 mg/kg/day dose was the no effect dose.

1. 4-Week Intravenous Toxicity Study of L-758298 (Study TT #95-009-0)

Testing Laboratory: Merck Research Laboratories
Merck & Co., Inc.
West Point, PA

Compliance with Good Laboratory Practice and Quality Assurance Requirements: Sponsor provided statements of compliance.

Date Study Started: February 21, 1995

Date Study Completed: July 18, 1995

Animals: Male (body weight range of 7.5 to 14.2 kg; 43 to 56 weeks of age) and female (body weight range of 5.9 to 11.3 kg; 43 to 56 weeks of age) Beagle dogs.

Methods: Five groups of 8 dogs each (4 males and 4 females) were intravenously administered 0, 0.5, 2, 8 and 32 mg/kg/day of L-758,298, respectively, for 4 weeks via the cephalic vein. The basis for dose selection was not provided by the sponsor. Vehicle was 0.9% Sodium Chloride Injection, USP. Dosing volume was 3.2 ml/kg, not exceeding an injection rate of 40 ml/min.

Mortality and clinical signs of toxicity were observed daily. Body weight was measured once during pretest and once weekly during treatment. Food consumption was measured 3-4 days during each week of treatment.

Blood samples for hematology and blood chemistry examination were withdrawn via the jugular sinus from all dogs prior to study initiation and during Weeks 2 and 4 from all remaining dogs. Urine samples for urinalysis were collected from all dogs prior to study initiation and during Week 4 from all remaining dogs.

Ophthalmic examinations were performed on all dogs prior to study initiation and during Week 4 in all remaining dogs. Electrocardiograms were recorded from all dogs prior to study initiation and from all remaining dogs 3-6 hrs post-dose on one occasion during Week 3.

Blood samples for future determination of L-758,298 plasma levels were collected via the jugular vein on Day 1 at 0.033, 0.117, 1, 2, 5, 7, 10 and 24 hrs after dosing in all remaining animals, during Week 4 at 0.033, 0.117, 1, 2, 5, 7 and 10 hrs after dosing in all animals.

All dogs underwent complete necropsies at scheduled termination. (Details were not provided by the sponsor.) Organ weights of brain, pituitary, spleen, heart, kidney, liver, adrenal, thyroid, testis/ovary and prostates were determined for all animals.

Sections of the following tissues from all control and high-dose dogs were prepared by routine methods, stained with hematoxylin and eosin, and examined microscopically: injection site, salivary gland, stomach, small intestine, large intestine, liver, pancreas, adrenal, thyroid, parathyroid, pituitary, kidney, urinary bladder, ovary/testis, uterus/prostate, skin, mammary gland, lung, heart, spleen, lymph node, thymus, skeletal muscle, bone, bone marrow, brain, cervical spinal cord, sciatic nerve, eye and Harder's gland. Tissues with gross pathology were microscopically examined for all animals.

Results:

1. Observed Effects: The 8 and 32 mg/kg i.v. injections produced slight to marked swelling, firmness of the forelimb and reddish discoloration of the skin. Animals displayed decreased weight bearing on the affected limbs, painful limbs and non-palpable veins. In some cases, when i.v. injections could not be administered by the cephalic veins, i.v. injections were delivered by the saphenous veins.

Males and females in the 32 mg/kg group were removed from the study after a total of 3-4 and 1-2 doses, respectively.

2. Mortality: Males and females in the 8 mg/kg group were removed from the study after a total of 4-8 and 3-8 doses, respectively, and were sacrificed in moribund condition.

3. Body Weight: Mean body weights of control males were 11.7 and 11.8 kg during Weeks 1 and 4, respectively. Mean body weights of control females were 9.0 and 9.2 kg during Weeks 1 and 4, respectively. There were no treatment-related effects on mean body weights.

4. Food Consumption: Mean food consumption of control males was 335 and 342 g/day during Weeks 1 and 4, respectively. Mean food consumption of control females was 241 and 282 g/day during Weeks 1 and 4, respectively. There were no treatment-related effects on mean food consumption.

5. Hematology: When one compares the erythrocyte count (millon/mm^3), hemoglobin concentration (g/ml) and hematocrit (%) at pretest and prior to early sacrifice in dogs of the 8 mg/kg group, values were decreased by -9.25%, -9.5% and -11.0%, respectively. These changes may reflect intravascular hemolysis. There were no treatment-related hematological changes in the 0.5 and 2 mg/kg groups.

6. Blood Chemistry: There were no treatment-related effects on blood chemistry parameters.

7. Urinalysis: There were no treatment-related effects on urinalysis parameters.

8. Ophthalmic Examination/Electrocardiograms: There were no treatment-related ophthalmic effects. There were no treatment-related effects on ECGs.

9. Organ Weights: There were no treatment-related effects on organ weights.

10. Gross Pathology: The 8 and 32 mg/kg i.v. injections produced slight to marked swelling, firmness of the forelimb and reddish discoloration of the skin. These effects were not seen in either the 2 mg/kg/day group or the control group.

11. Histopathology: In the 32 mg/kg/day group, 4/4 males and 4/4 females had venous thrombosis, and 2/4 males and 2/4 females had venous vascularization; these effects were not seen in any other group.

12. Plasma Levels of Drug: Sponsor stated that data for plasma levels of L-758,298 will be provided at a future date.

In summary, the no effect i.v. dose of L-758,298 was 2 mg/kg/day in the dog. The 8 and 32 mg/kg/day doses produced slight to marked swelling, firmness of the forelimb and reddish discoloration of the skin. The 32 mg/kg/day i.v. dose produced venous thrombosis and vascularization. No target organs of toxicity were identified.

5-Week intravenous toxicity study in dogs (TT#95-009-0)

This study has previously been reviewed under IND 48, 924 original submission dated September 28, 1995 (pharmacologist's review of IND 48, 924 dated April 15, 1996). In this amendment, the sponsor submitted the toxicokinetic data for this study, which is reviewed below.

Toxicokinetics: In this study, animals were treated with L-758, 298 at 0.50, 2.0, 8.0, and 32.0 mg/kg i.v. doses. The plasma concentrations were studied at 0.5 and 2.0 mg/kg. On Day 1, animals were bled at 2 min, 7 min, 1, 2, 5, 7, 10, and 24 hours post-dose. At week 4, samples were collected at 0, 2, and 7 min, and at 1, 2, 5, 7 and 10 hours post-dose. Plasma concentrations of pro- and active drug were determined by LC/MS/MS method. Maximum mean plasma concentrations of the active drug, L-754, 030, were attained very rapidly (within 2 minutes post-dose). The systemic exposure or AUC_{0-24h} increased in a slightly greater than dose-proportional manner. There were no gender-related differences in toxicokinetics at 0.5 or 2.0 mg/kg doses. The following table (from vol. 2, pg. 256 and 257 of sponsor's submission) summarizes the mean toxicokinetic parameters of L-754, 030 after i.v. administration of L-758, 298 at 0.5 and 2.0 mg/kg, i.v. in dogs.

SUMMARY OF MEAN PLASMA LEVELS AND TOXICOKINETIC PARAMETERS OF L-754,030 IN MALE AND FEMALE DOGS FOLLOWING INTRAVENOUS DOSING (L-758,298) AT 0.5 MG/KG/DAY - DRUG DAY 1 AND DRUG WEEK 4

	Drug Day 1 - L- 754,030			
Toxicokinetic	Males		Females	
Parameters	Mean	S.E.M.	Mean	S.E.M.
AUC (µg•hr/ml)	2.46	0.42	2.71	0.73
C_{max} (µg/ml)	0.63	0.05	0.72	0.14
T_{max} (hr)	0.03	0.00	0.03	0.00

	Drug Week 4 - L- 754,030			
Toxicokinetic	Males		Females	
Parameters	Mean	S.E.M.	Mean	S.E.M.
AUC (µg•hr/ml)	3.49	0.63	4.50	0.50
C_{max} (µg/ml)	0.74	0.08	0.71	0.06
T_{max} (hr)	0.03	0.00	0.03	0.00

SUMMARY OF MEAN PLASMA LEVELS AND TOXICOKINETIC PARAMETERS OF L-754,030 IN MALE AND FEMALE DOGS FOLLOWING INTRAVENOUS DOSING (L-758,298) AT 2 MG/KG/DAY - DRUG DAY 1 AND DRUG WEEK 4

	Drug Day 1 - L- 754,030			
Toxicokinetic	Males		Females	
Parameters	Mean	S.E.M.	Mean	S.E.M.
AUC (µg•hr/ml)	17.4	3.46	18.36	3.26
C_{max} (µg/ml)	2.54	0.29	2.64	0.34
T_{max} (hr)	0.27	0.24	0.05	0.02

	Drug Week 4 - L- 754,030			
Toxicokinetic	Males		Females	
Parameters	Mean	S.E.M.	Mean	S.E.M.
AUC (µg•hr/ml)	31.81	8.93	24.78	4.44
C_{max} (µg/ml)	3.05	0.28	2.69	0.32
T_{max} (hr)	0.03	0.00	0.05	0.02

Study Title: L-758,298: Five (5)-Week Intravenous Toxicity Study in dogs

Study no. TT#95-025-0

Conducting laboratory (and location if not Sponsor): Merck Research Laboratories, West Point, PA.

Dates of study initiation & completion: February 02, 1995 and November 02, 1995.

GLP compliance: Yes

QA Report Yes (X) **No** ( )

Drug, Lot #, radiolabel (if applicable), and % purity: L-758298; Lot # L-758298-003C009; purity 98.7%.

Formulation/vehicle: L-758298 (bis-N-methyl-D-glucamine salt) was dissolved in sterile 0.9% NaCl solution for injection, and the solutions were filtered through a ——————— filter. b(4)

Methods: L-758298 was administered intravenously to beagle dogs at a dose of 8.0 mg/kg/day (12.8 ml/kg; approximately 30-40 ml/min) for 29 (females) to 30 (males) days. The control animals were administered the vehicle (0.9% sodium chloride).

Dosing:

Species/strain: Beagle dogs

Age: 38 to 41 weeks

Weight: males – 7.9 to 11.3 kg; females – 7.5 to 9.5 kg.

#/sex/group or time point (main study): 4 animals/sex/group were used in the study.

Satellite groups used for toxicokinetics or recovery: N/A

Doses in administered units: L-000758298 was administered at an i.v. dose of 8 mg/kg/day.

Route, form, volume and infusion rate: L-758298 was administered intravenously (cephalic or saphenous vein) at a dosing volume of 12.8 ml/kg, and at a rate of approximately 30-40 ml/min.

Times at which Observations were made:

Clinical signs- The animals were observed daily for clinical signs and mortality.

Body weights- Body weights were measured weekly.

Food consumption- Food consumption was measured 2-4 times per week.

Ophthalmoscopy: Ophthalmologic examinations were performed on all animals before initiation of dosing and in week 4 of the dosing period.

Electrocardiograph: Electrocardiograms were recorded from all animals before initiation of dosing and in Week 4 of the dosing period.

Hematology: Blood samples for hematology were collected pre-dose and in weeks 2 and 4 of the dosing period.

Clinical chemistry- Blood samples for clinical chemistry analyses were collected pre-dose and in weeks 2 and 4 of the dosing period.

Urinalysis- Urine samples were collected from all animals before initiation of dosing and at week 4.

Gross pathology- At the end of the dosing period, all surviving animals were sacrificed and a complete necropsy was performed.

Organs weighed- The weights of the following organs were recorded.

Adrenals, brain, heart, kidneys, liver, ovary, pituitary, prostate, spleen, testes and thyroid.

Histopathology- Following organs from the control and high dose animals were fixed and examined microscopically.

Salivary gland, stomach, small intestine (duodenum, jejunum, ileum), large intestine (colon), liver, gallbladder, lung, heart, spleen, lymph nodes, thymus, pancreas, adrenals, pituitary, thyroid, parathyroid, kidneys, urinary bladder, ovaries, uterus, testes and epididymides, prostate, skin, bone marrow, bone, skeletal muscle, brain, spinal cord, sciatic nerve, eye, injection sites (cephalic and cephaneous vein) and prostate.

Toxicokinetics- Blood samples were collected from all drug-treated animals at approximately 2 minutes after the infusion had started, and approximately 2 and 7 minutes and 1, 2, 5, 7, 10 and 24 hours after the infusion had ended on Day 1 and week 4. Plasma levels of L-759298 (pro-drug) and L-745030 were determined by LC/MS/MS.

Results:

Mortality: There were no mortalities in any group. One male dog receiving L-759298 was sacrificed in Week 4 due to inability to continue dosing as a result of injection site reactions.

Clinical signs: Swelling at the injection site (cephalic vein) was observed in 2 (of 8) control and 7 (of 8) treatment group dogs. Swelling was first observed near the end of Week 1 and continued through Week 5. One male dog receiving L-758298 was sacrificed in Week 4 due to inability to continue dosing as a result of this clinical sign. This dog had a thrombus observed in the right cephalic vein.

Body weights: The mean body weights of the control animals before initiation of dosing (day -1) and in week 4 were 9.2 and 9.4 kg, respectively. Treatment group animals had decreased mean body weight as compared with the controls. Three of the treated dogs lost 0.5 kg, and the dog that was sacrificed in Week 4 lost 0.7 kg. No control dog lost weight during this time. The mean body weights of the control and treatment group animals before dosing and at different times of the dosing period are shown in the Table below.

	Week -1	Week 1	Week 2	Week 3	Week 4
Control	9.2	9.2	9.2	9.4	9.4
8.0 mg/kg/day	8.7	8.6	8.5	8.6	8.4

Food consumption: The mean food intake of the control animals in week 1 and week 4 were 290 and 306 g/day, respectively. Treatment group animals had slightly decreased food consumption (approximately 12% in Week 2 and 11% in Week 4) at different times of the study period.

Ophthalmoscopy: No treat related ophthalmologic changes were observed.

Electrocardiography: No treatment related changes in the ECG parameters were observed.

Hematology: No treatment related changes in hematological parameters were observed in the treated animals.

Clinical chemistry: No treatment related changes in clinical chemistry parameters were observed in any animal.

Urinalysis: No treatment-related changes were observed.

Gross Pathology: Treatment related changes were observed at the injection sites of the treatment group animals. Reddening and thickening were observed at the injection sites were observed in most animals receiving treatment. Injection site changes were characterized by an increased incidence of thrombosis and increased severity of perivascular cellular infiltration, perivascular fibrosis, and perivascular hemorrhage. Thrombosis was observed in 4 (of 8) treatment group animals, as compared to 1 control animal.

Organ weight: No treatment related changes in any organ weight were observed.

Histopathology: Histopathological findings were limited to the injection sites of both the control and the treatment group, with increased incidences and higher severity in the treatment group. The changes included: perivascular cellular infiltration, fobrosis, hemorrhage and thrombosis. Histopathological findings at the injection site of the control and treatment group animals are shown in the Table below.

Incidence	**Control**		**8.0 mg/kg**	
	Females	Males	Females	Males
Perivascular cellular infiltration	3/4	4/4	4/4	4/4
Perivascular fibrosis	2/4	3/4	4/4	4/4
Perivascular hemorrhage	4/4	4/4	4/4	4/4
Thrombosis	0/4	1/4	1/4	3/4

Toxicokinetics: At the 2 minutes time point, the plasma concentrations of L-758298 in male and female dogs were high (25.23 μg/ml in males, 25.23 μg/ml in females). At the end of the infusion, the plasma concentrations of the drug disappeared very rapidly. The mean plasma concentrations at 2 minutes post-dosing were approximately 3% of the concentrations achieved at the first sampling point. The plasma concentrations decreased below the limit of detection (0.005 μg/ml) by 1 hour in males and by 2 hours in females. L-754030 (active drug) was detected at the first sampling point during infusion. Following completion of infusion (2 minutes), the mean plasma concentrations of L-754030 were 5.23 and 5.73 μg/ml for males and females, respectively. The T_{max} value was approximately 18 hours in males and 6 hours in females. The mean plasma L-754030 C_{max} values in males and females were 8.78 and 6.57 μg/ml, respectively. The elimination of L-754030 was slow with plasma clearance values of

approximately 1.3 and 1.9 ml/min/kg in males and females, respectively. Males (103.57 µg.hr/ml) had higher AUC values than females (71.39 µg.hr/ml).

In **Drug Week 4**, the plasma levels of the parent compound (L-758298) at 2 minutes during infusion were slightly higher than those observed on Day 1. Similar to Day 1, by 2 hours post-dose, the plasma concentrations of L-758298 were below the lower limit of quantification. The C_{max} for L-754030 was achieved at approximately 2 min after administration, and were 13.04 µg/ml for males and 10.17 µg/ml for females. Elimination of L-754030 was slow; the mean 24-hour concentrations represented approximately 30% and 53% of the mean C_{max} for males and females, respectively. The AUC value for L-754030 in Week 4 was higher than that on Day 1 (156.24 µg.hr/ml in males and 158.08 µg.hr/ml in females).

The toxicokinetic parameters for L-754030 (active compound) following i.v. administration of an 8.0 mg/kg dose in male and female dogs are shown in the Tables below.

Drug Day 1 - L-754,030

Toxicokinetic	Males		Females	
Parameters	Mean	S.E.M	Mean	S.E.M
AUC (µg•hr/ml)	103.57	23.71	71.39	17.93
C_{max} (µg/ml)	8.78	3.08	6.23	1.11
T_{max} (hr)	18.01	5.99	8.05	7.98

Drug Week 4 - L-754,030

Toxicokinetic	Males		Females	
Parameters	Mean	S.E.M	Mean	S.E.M
AUC (µg•hr/ml)	156.24	22.23	158.08	20.99
C_{max} (µg/ml)	13.04	1.75	10.17	1.16
T_{max} (hr)	0.03	0.00	0.03	0.00

Thus, following i.v. administration of L-758298 to dogs, it is rapidly converted to the active drug (L-754030), and the maximum plasma concentrations of L-754030 were reached at approximately 2.0 minutes after administration.

Summary: In the 5–week intravenous toxicity study with L-758298 in dogs, the drug was administered at dose of 8.0 mg/kg/day. Animals receiving L-758298 had decreased body weight throughout the dosing period. Injection site reactions consisting of perivascular cellular infiltration, fobrosis, hemorrhage and thrombosis, were observed in both control and treatment group animals, with higher incidences and more severity in the treatment group. Thus, the injection site was the target organ of toxicity. L-758298 was rapidly converted to its active metabolite (L-754030) following i.v. administration in dogs, and the maximum plasma concentrations of L-754030 were reached at approximately 2.0 minutes after administration.

Study Title: MK-0869: Five-Week Oral Toxicity Study in Dogs.
Study No: TT #99-082-0, -1

Conducting laboratory and location: Merck Institute for Therapeutic Research
Merck Research Laboratories
Merck & Co., Inc.
West Point, PA 19486

Date Started: October 25, 1999
Date Completed: August 3, 2000
GLP compliance: A statement of compliance with GLP regulations was included.
QA- Report Yes (X) No ( )
Methods: In a 5-week oral dose range finding study, beagle dog received MK-0869 Formulation NB at doses of 5, 25, 125, 250, 500, and 750 mg/kg B.I.D. (total daily doses of 10, 50, 250, 500, 1000, and 1500 mg/kg/day, respectively). The treatment duration was 28 or 29 days. The MK-0869 average particle size in the colloidal dispersion was — n. Two control groups were included in this study. The first control group received deionized water. b(4)

The second control group received the vehicle, 3% hydroxypropyl cellulose, 15% sucrose, and 0.14% sodium lauryl sulfate. In the B.I.D. regimen, the second daily dose was administered approximately 6 hr after the first dose. The total number of doses was 56 or 58.

Dosing:

- **species/strain:** Beagle dogs were obtained from [redacted] b(4)
- **#/sex/group or time point:** 4-beagle dogs/sex/group
- **age:** Dogs were approximately 31 to 35 weeks of age at the start of treatment.
- **weight:** Body weight ranges were 6.1 to 8.6 kg for male dogs and 7.9 to 11.5 kg for female dogs.
- **satellite groups used for toxicokinetics or recovery:** None.
- **dosage groups in administered units:** MK-0869 Formulation NB was administered at doses of 5, 25, 125, 250, 500, and 750 mg/kg B.I.D. (total daily doses of 10, 50, 250, 500, 1000, and 1500 mg/kg/day, respectively). In the B.I.D. regimen, the second daily dose was administered approximately 6 hr after the first dose.
- **route, form, volume, and infusion rate:** Vehicle or drug suspension was administered by oral gavage using a dose volume of 5 mL/kg.

Drug, lot#, radiolabel, and % purity: MK-0869, Formulation NB (MK-0869 blended [redacted]), batch #X0869OPP015C001 (also known as L-754030-016S001) with an average particle size of [redacted], was obtained by blending 3 batches of MK-0869 [redacted] with purity ranging from 99.6 to 100.0%. b(4)

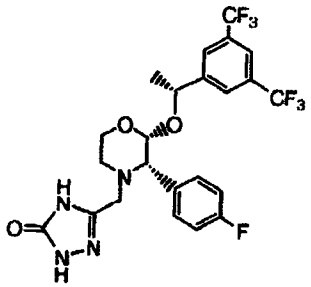
Formulation/vehicle: The vehicle was 3% hydroxypropyl cellulose, 15% sucrose, and 0.14% sodium lauryl sulfate.

Observations and times:

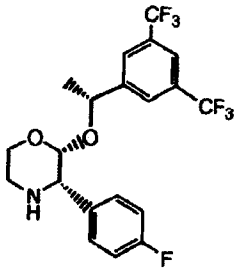
- **Clinical signs:** Dogs were monitored daily for clinical signs of toxicity and mortality before and after dosing.
- **Body weights:** Body weights were measured prior to the start of treatment, once in week 1, and twice per week during weeks 2 through 4.
- **Food consumption:** Daily food consumption was measured 3 to 4 times per week during weeks 2 through 4.
- **Ophthalmoscopy:** Ophthalmic examinations were conducted on all dogs prior to the start of treatment and during week 4.
- **EKG:** Electrocardiograms (ECGs) were recorded from all dogs prior to the start of treatment and during weeks 2 and 4. During weeks 2 and 4, ECGs were recorded approximately 2 to 4 hr after the second daily dose. ECGs were conducted in right lateral recumbency, and recordings were made from leads I, II, III, aVR, aVL, aVF, CV5RL, and V10. The heart rate and PR, QRS, and QT intervals were measured.
- **Hematology:** Blood samples for determination of hematology parameters were collected prior to the start of treatment and during weeks 2 and 4.
- **Clinical chemistry:** Blood samples for determination of serum biochemical parameters were collected prior to the start of treatment and during weeks 2 and 4.
- **Urinalysis:** Urine samples for analysis were collected overnight from all dogs prior to the start of treatment and during week 4 and/or 5.
- **Gross pathology:** Dogs were sacrificed by exsanguinations under barbiturate anesthesia and a complete gross examination was conducted.

- **Organs weighed:** Absolute and relative (% of body weight and % of brain weight) organ weights were determined for the adrenal glands, brain, heart, kidneys, liver, thyroid (with parathyroid), ovaries, pituitary gland, prostate, spleen, and testes.
- **Histopathology:** A complete microscopic examination of paraffin-embedded, hematoxylin- and eosin-stained from all animals in the vehicle-control and 750 mg/kg B.I.D. groups was conducted. The thymus, ovaries (females), prostate and testes (male), and gross and ophthalmic changes from all animals in all groups were also submitted to microscopic examination. Tissues examined were as follows: salivary gland, stomach, small intestine, large intestine, liver, gallbladder, pancreas, adrenal glands, pituitary gland, thyroid gland, parathyroid, kidneys, urinary bladder, ovaries, skin, mammary gland (when present in skin section), lung, heart, spleen, lymph node, thymus, bone, bone marrow, skeletal muscle, brain, spinal cord, nerve - sciatic, eye (with optic nerve), uterus, testes (with epididymides), prostate, and esophagus.
- **Toxicokinetics:** Blood samples for measurement plasma drug and metabolite concentrations were collected in week 4 at 2, 4, 6, 8, 12, 16, and 24 hr after the first daily dose. The second daily dose was administered immediately following the 6-hr time point. Plasma concentrations of MK-0869 were quantified using liquid chromatography with mass spectrometry. Plasma samples from the 250, 500, and 750 mg/kg B.I.D. groups collected at the 4, 8, 16, and 24 hr time points were analyzed for 12 metabolites of MK-0869 by gradient elution reverse-phase HPLC with detection by tandem mass spectrometry. Systemic exposure to each metabolite was expressed as AUC0-24hr of the mass spectrometric response of each metabolite relative to internal standard. Metabolites were categorized into three groups based upon their similarities in molecular structure and polarity. Group1 consisted of 3 very polar metabolites, L-770787, L-858442, and L-858443. Group 2 consisted of 2 polar metabolites, L-294569 and L-596064. Group 3 consisted of 5 nonpolar and 2 polar metabolites, L-809771, L-829674, L-825678, L-755446, L-809861, L-829617, and L-829615.

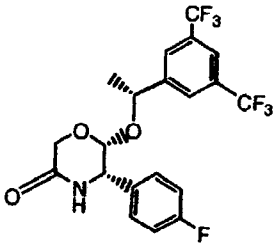
Structures of MK-0869 and 12 Plasma Metabolites Analyzed by LC/MS/MS



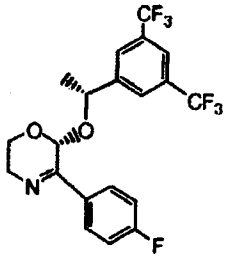
MK-0869



L-755446



L-825678



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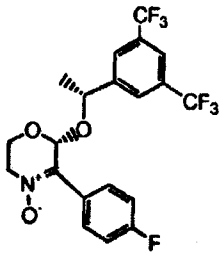

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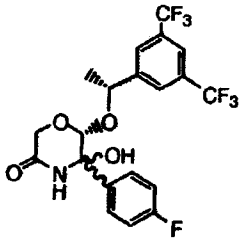
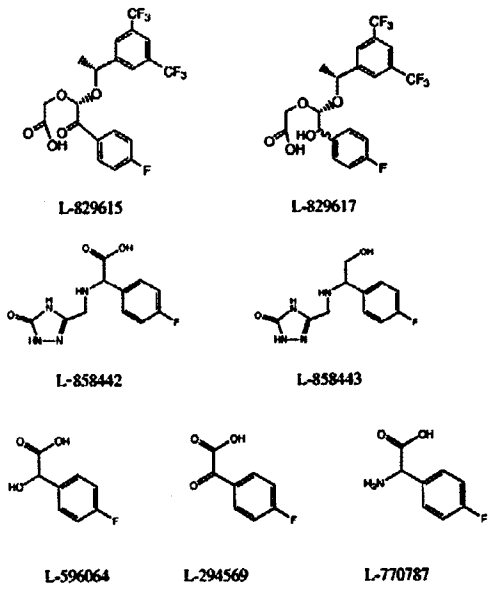


L-809771

Structures of MK-0869 and 12 Plasma Metabolites Analyzed by LC/MS/MS



- **Other:** Body weight, hematology, clinical chemistry, and organ weight data for male and female dogs was combined.

Results:

- **Clinical signs:** Emesis, usually consisting of drug-related material, was observed sporadically during the first 2 weeks of the study in 1 male at 250 mg/kg B.I.D. (#99-0474), 1 male at 500 mg/kg B.I.D. (#99-0466), and 3 females at 750 mg/kg B.I.D. (#99-0431, #99-0457, and #99-0439).

- **Mortality:** None.

- **Body weights:** Suppression of body weight gain was observed for dogs at 25 mg/kg B.I.D. and body weight loss was observed for dogs at doses ≥125 mg/kg B.I.D. Body weights for saline-control dogs at pretest -1 and week 4 were 8.0 and 8.2 kg, respectively, yielding a 2.50% increase of initial body weight. Body weights for vehicle-control dogs at pretest -1 and week 4 were 8.5 and 8.8 kg, respectively, yielding a 3.53% increase of initial body weight. Body weights for dogs at 5 mg/kg B.I.D. were increased by 4.8% over initial body weight. Body weights for dogs at 25 mg/kg B.I.D. were unchanged. Body weights for dogs at 125, 250, 500, and 750 mg/kg B.I.D. were decreased by 2.5, 6.9, 8.4, and 10.7% from initial body weights.