

Exploratory 5-Week Oral Toxicokinetic Study in Rats (Study # 01-106-0):

Methods: The toxicokinetic profile of two MK-0869 formulations with different particle size was determined after oral administration to Sprague-Dawley rats for 5 weeks. MK-0869 formulation M had average particle size of _____ and formulation NB had average particle size of _____ nanometers. Formulation M was administered at a dose of 125 mg/kg b.i.d (250 mg/kg/day) and formulation NB at doses of 125, 250, 500 and 750 mg/kg b.i.d. (250, 500, 1000 and 1500 mg/kg/day) by oral gavage. The dosing volume was 5 ml/kg with the exception of the 1500 mg/kg/day group that received 5.3 ml/kg. Each group consisted of 5 male and 5 female animals. Blood samples were collected from 4 rats/sex/group/time point at approximately 2, 6, 10, 24 and 48 hours following the first daily dose on Day 29. On the final day of dosing, 4 animals/sex in the formulation M and formulation NB groups received b.i.d doses of the respective formulations of [¹⁴C]MK-0869. Plasma concentration of drug-related substances was determined by measuring the total radioactivity. The AUC_{0-48h} for drug-related substances was determined using the Trapezoidal rule.

Results: There was no dose-related increase in the plasma exposure levels of MK-0869-related substances in the male and female rats receiving the NB formulation. The plasma exposure levels in females were higher than that of males (about 1.5 times). Animals receiving 125 mg/kg b.i.d. doses of Formulation NB had higher exposure levels than those receiving the same dose of Formulation M (97% and 52% higher in males and females, respectively). There was a saturation of absorption at 125 mg/kg b.i.d dose of the NB formulation. The plasma exposure levels of the male and female animals receiving different doses of MK-0869 are shown in the sponsor's Table below.

Plasma MK-0869 Systemic Exposure (AUC) - Drug Week 5

MK-0869 (Form M)	AUC _{0-48h} (ng·h/mL)	
	Females	Males
125 mg/kg b.i.d.	244	140
MK-0869 (Form NB)		
125 mg/kg b.i.d.	373	276
250 mg/kg b.i.d.	306	293
500 mg/kg b.i.d.	466	315
750 mg/kg b.i.d.	302	287

a [¹⁴C]MK-0869 and its related substances
Form M = Formulation M, average MK-0869 particle size of _____
Form NB = Formulation NB, average MK-0869 particle size of _____

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In Vitro Metabolism of MK-0869 in Microsomes and Primary Cultures of Rat and Human Hepatocytes.

Methods: The metabolism of [morpholine-2-¹⁴C]MK-0869 was studied in hepatic microsomes and primary cultures of rat and human hepatocytes. Two primary metabolites of MK-0869, L-755446 (formed by an initial step of N-dealkylation) and L-809861 (an imine metabolite) were used as alternate substrates. For induction of cytochrome P₄₅₀ enzymes in male rats, dexamethasone (200 mg/kg) was administered by oral gavage for 4 days before preparation of microsomes or isolation of the hepatocytes. For studying the oxidative metabolism in liver microsomes, microsomal proteins were incubated with [¹⁴C]MK-0869, [¹⁴C]L-755446 and [¹⁴C]L-809861 for 37°C for 5 to 45 minutes. Primary cultures of rat or human hepatocytes were incubated with these substrates for 4, 6, 24 or 48 hrs (for rat hepatocytes) and 48 hrs (for human hepatocytes). For induction of metabolic enzymes, rat hepatocytes were treated with 10 µM dexamethasone and human hepatocytes were treated with 50 µM rifampicin for 48 hrs prior to the addition of the test compounds. The metabolite profiles of the incubates were determined by  and nuclear magnetic resonance (NMR) spectroscopy.

Results:

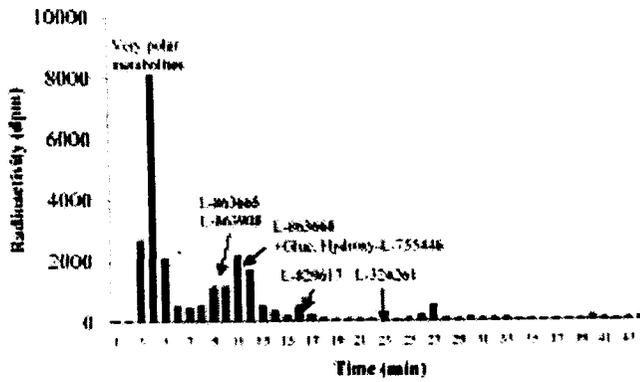
Metabolism in rat liver microsomes: In liver microsomes prepared from untreated rats, the metabolism of MK-0869 was slow. Two nonpolar metabolites, identified as L-755446 and L-809861 were detected. In addition, three polar (L-829615, L-829617 and L-324261) and four very polar (L-294569, L-596064, L-872939 and L-872712) metabolites were also detected. MK-0869 underwent more extensive metabolism when incubated with liver microsomes prepared from dexamethasone-treated rats.

When L-755446 (the primary metabolite of MK-0869) was used as a substrate, the major metabolite was L-809861. Three very polar metabolites, identified as L-294569, L-872939 and L-872712, were also detected by  [¹⁴C]L-809861 underwent slow metabolism in the presence of rat liver microsomes (<5% conversion in 10 min). Most of the metabolites identified were very polar metabolites.

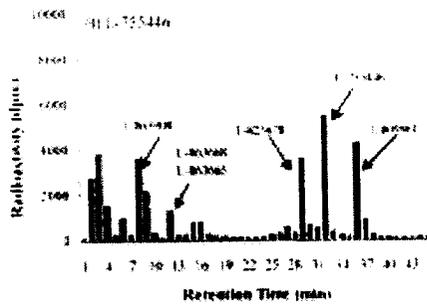
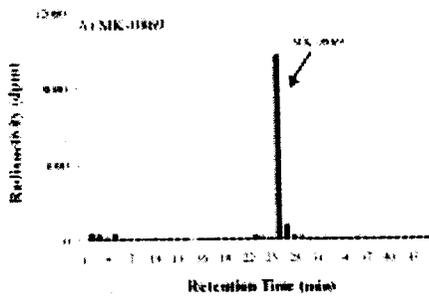
The structures of the phase I and phase II metabolites formed after incubation of [¹⁴C]MK-0869 with rat and human liver microsomes are shown in the sponsor's Figure below.

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Representative Radioactivity Profiles of [¹⁴C]MK-0893 Metabolites After 72 hr Incubation With Dexamethasone-Treated Rat Hepatocytes



Representative Radioactivity Profiles of [¹⁴C]MK-0893 and [¹⁴C]L-755446 Metabolites After 48 hr Incubation With Bilirubin-Induced Human Hepatocytes



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Mass Balance and Metabolism Studies of [¹⁴C]L-758298 and [¹⁴C]MK-0869 in Humans.

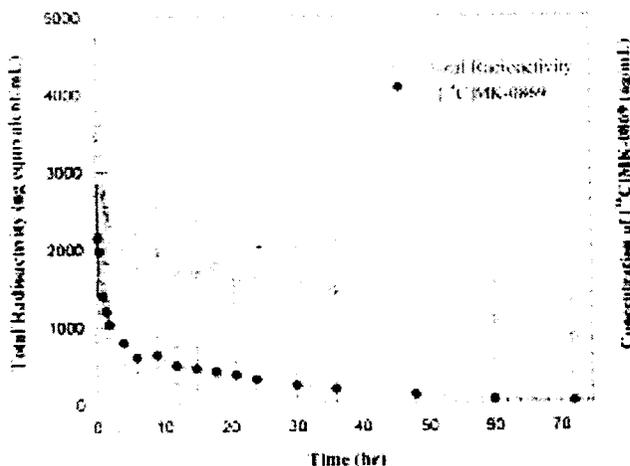
Methods: The pharmacokinetics and metabolism of L-758298 (a water-soluble phosphoramidate prodrug of MK-0869) were studied in healthy male human subjects after i.v. dosing. Each subject received 100 mg (88.8 µCi) of [¹⁴C]L-758298, administered by infusion for 30 minutes via an arm vein. For the mass balance study, blood samples were collected at -1, 0.5, 1, 1.5, 2, 4, 6, 9, 12, 15, 18, 21, 24, 30, 36, 48, 60 and 72 hr post-dose and each morning on days 4 to 21. For the metabolism study, blood samples were collected at 0.25, 0.5, 1, 1.5, 2, 4, 6, 9, 12, 15, 18, 21, 24, 30, 36, 48, 60, 72, 96, 120, 144 and 168 hr post-dose. Excreta were collected daily for 30 days.

[¹⁴C]MK-0869 was administered orally as capsules containing 100 mg (28.72 µCi) of the active drug in each capsule. Each of the 8 subjects received 3 capsules at a dose level of 300 mg/person. Blood samples were collected at 0.5, 1, 1.5, 2, 4, 6, 9, 12, 15, 18, 21, 24, 30, 36, 48, 60, 72, 96, 120, 144 and 168 hr post-dose. Excreta were collected daily for 10 days. By comparison with authentic metabolite standards and ¹⁴C characteristics, the metabolite profiles in the plasma, urine and feces were confirmed by ¹⁴C and/or NMR analyses.

Results:

[¹⁴C]L-758298: Following i.v. administration of [¹⁴C]L-758298 to healthy human males, about 58% of the radioactivity was recovered in the urine and 45% in the feces during the 28-day collection period. Only 7% of the radioactivity were excreted in the urine collected in days 8-28. In the plasma, L-758298 was rapidly converted to MK-0869. Plasma levels of [¹⁴C]MK-0869 were higher at the earliest time point (0.25 hr). At 0.5 hr, [¹⁴C]MK-0869 accounted for for 56% of the plasma radioactivity that declined to 40, 19 and 4% at 4, 24 and 60 hr post-dose, respectively. The mean radioactivity concentrations (total and MK-0869) in the plasma of healthy human subjects after i.v. administration of [¹⁴C]L-758298 during the 72-hr period are shown in the sponsor's Figure below.

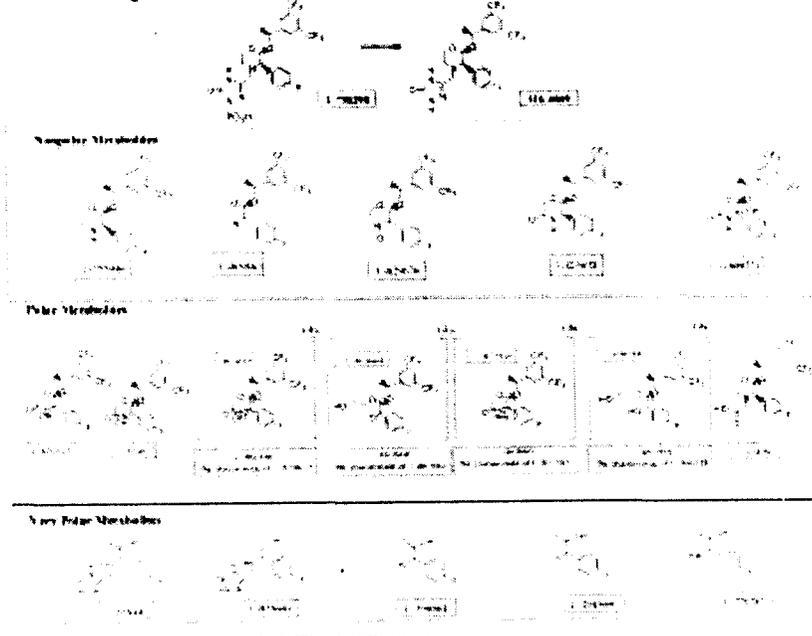
Mean (±SD) Concentrations of Total Radioactivity and [¹⁴C]MK-0869 in Plasma of Eight Healthy Human Subjects following Intravenous Administration of [¹⁴C]L-758298 at 100 mg (Protocol # 013-01)



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Several nonpolar (L-755446, L-809861, L-829674, L-825678 and L-809771), polar (L-829615, L-829617 and L-324261) and very polar (L-294569, L-596064 and L-770787) metabolites were identified in the plasma. The two major metabolites in the human plasma were L-829615 and L-829617. L-829674, L-324261 and L-809771 were present at very low concentrations, and L-863664, L-846188 and L-872945 were not detected in human plasma. In feces, several metabolites were identified and those include: L-829617, L-829771, L-825678, L-755446 and L-809771, L-829774 and L-829615. In the urine samples of humans receiving intravenous [¹⁴C]L-758298, five very polar metabolites (UM-1 or L-858442, UM-2 or L-858443, UM-3 that had 2 components, L-596064 and L-294569 and UM-4 and UM-5 which consisted of the glucuronides L-86366, L-863665 and L-863908) were detected by . — The major metabolites detected in the plasma, urine and feces of humans following i.v. administration of [¹⁴C]L-758298 to healthy human subjects are shown in the sponsor's Figure below.

The Major Metabolites of MK-0869 in Plasma and/or Excreta of Healthy Human Subjects Following Intravenous Administration of 100 mg [¹⁴C]L-758298 (Protocol #013-01)



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[¹⁴C]MK-0869: After oral administration of a 300 mg dose of [¹⁴C]MK-0869 to healthy human subjects, about 92% of the radioactivity was recovered in the 10-day collection period, of which 5% was excreted in the urine and 86% excreted in the feces. Intact [¹⁴C]MK-0869 accounted for 49% to 64% of the plasma radioactivity during 4 hr following the administration and declined to 30% and 10% by 24 and 60 hr, respectively. By 96 to 168 hr, the plasma levels of the parent drug was below the detection limit. Nonpolar metabolites including L-755446, L-809861, L-829674, L-825678 and L-809771, and polar metabolites including L-829615 and L-829617 were shown to be present in the plasma samples of subjects receiving the oral dose of MK-0869. In the fecal extracts, the major component was the parent compound, MK-0869. In addition, trace levels of the nonpolar and polar metabolites, detected in the plasma, were also detected in the fecal extracts. An additional minor metabolite, identified as L-764120 (an N-oxide of MK-0869), was detected in the feces. Five very polar metabolites (L-596064, L-294569, L-770787, L-858442 and L-858443) and phase II conjugates

L-863665 (glucuronide of L-872945), L-863908 (glucuronide of L-864188) and L-863668 (glucuronide of L-863664) were identified in the urine. Glucuronides of L-755446 (primary nonpolar metabolite of MK-0869) and of hydroxylated L-755446 were also detected in the urine. The structures of the nonpolar, polar and very polar metabolites detected in the plasma, urine and feces of healthy human subjects receiving a 300mg oral dose of [¹⁴C]MK-0869 are shown in the sponsor's Figure below.

MK-0869 and its Metabolites in Plasma and/or Excreta of Healthy Human Subjects Following Oral Administration of [¹⁴C] MK-0869 at 300 mg (Protocol 0013-01)

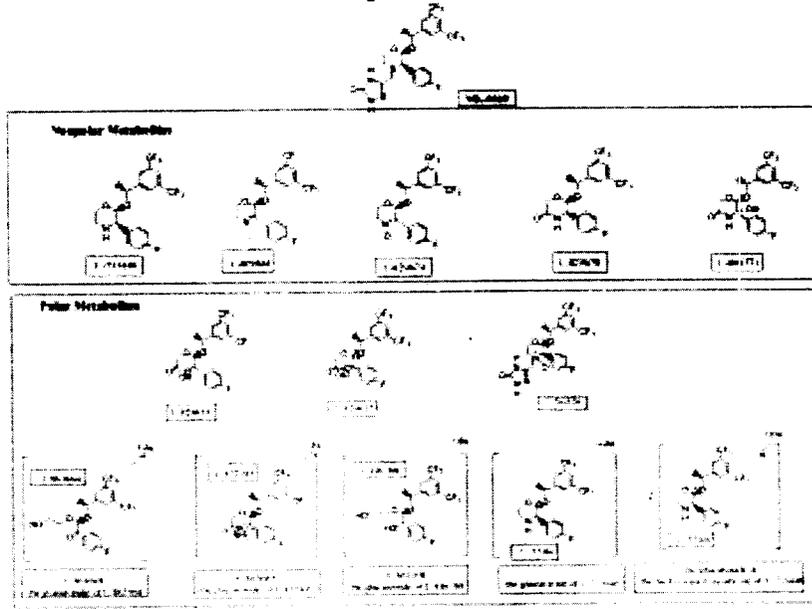
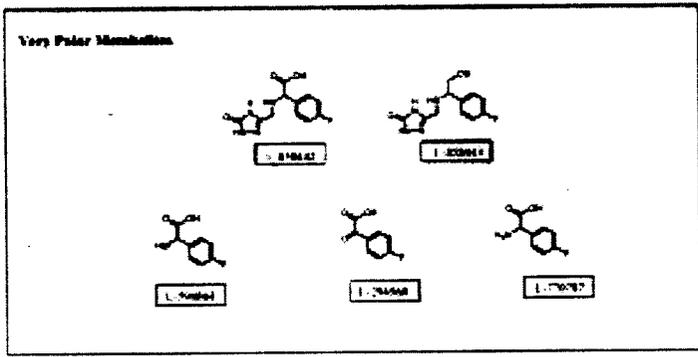


Figure 1 (Cont.)
MK-0869 and its Metabolites in Plasma and/or Excreta of Healthy Human Subjects Following Oral Administration of [¹⁴C] MK-0869 at 300 mg (Protocol 0013-01)



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Thus, the metabolite profiles in the plasma, feces and urine of healthy human subjects were qualitatively similar after i.v administration of the pro-drug, L-758298 and oral administration of MK-0869.

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 P₄₅₀ (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 testosterone 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 _____ methods.

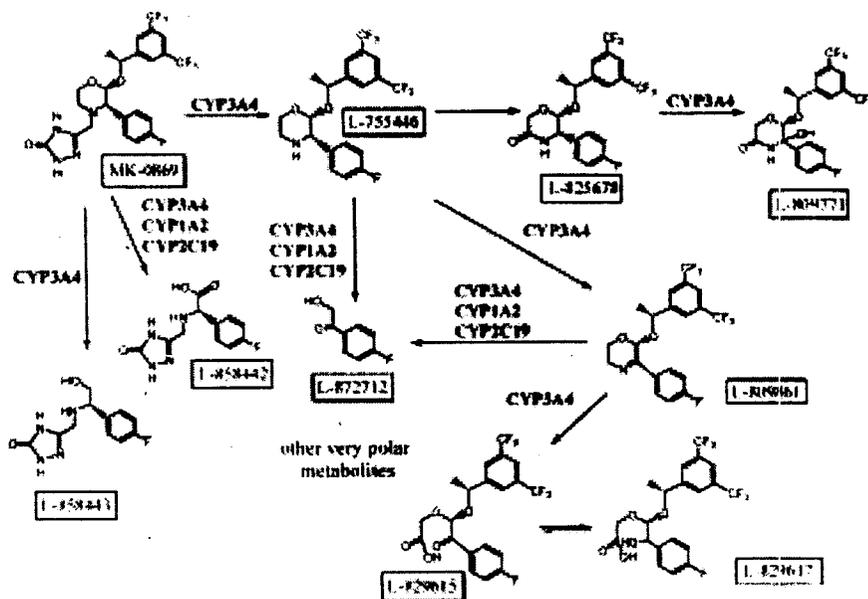
Results: The metabolism of MK-0869 in human liver microsomes was completely inhibited by the CYP3A4-specific inhibitor, ketoconazole (1 µM). No inhibition was observed with CYP2D6, CYP2C9, CYP1A2 or CYP2E1 isozyme-specific inhibitors, quinidine, sulfaphenazole, furaphylline or 4-methylpyrazole, respectively. 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.

[¹⁴C]MK-0869 was also metabolized by Sf21 cells expressing recombinant human CYP1A2, CYP2C19 or CYP3A4 isozymes 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-hydroxylation 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 IC₅₀ 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 μ M), CYP2C9 (IC_{50} = 63 μ M), CYP2D6 (IC_{50} = 75 μ M), CYP2E1 (IC_{50} > 100 μ M) and CYP3A4 (IC_{50} = 30 to 35 μ M). 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 μ M). 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.

Brain Penetration of MK-0869 in Male Sprague-Dawley Rats and Male Ferrets.

Methods: The possibility of [3 H]MK-0869 to penetrate the blood brain barrier was studied in male Sprague-Dawley rats following i.v and oral administration of a 2 mg/kg dose. The metabolism of [3 H]MK-0869 was studied in the plasma and brain collected from the animals. Blood was collected by cardiac puncture at 5, 15 and 30 minutes and 1, 2, 4 and 24 hr after dosing. Brain was collected for determination of brain penetration and isolation of metabolites in brain homogenates. The identity of the MK-0869 metabolites in the plasma and brain were established by comparison with the characteristics of standard authentic metabolites and by 1 H and NMR analyses. In male ferrets, the possibility of blood brain penetration of MK-0869 and its metabolites was studied after oral administration a 3 mg/kg dose of [14 C]MK-0869. Two ferrets were sacrificed at 24 hr after dosing and the remaining 2 ferrets were sacrificed at 48 hr after dosing. Blood and brains were collected at the end of the study to evaluate the brain penetration and isolation of the metabolites.

Results: In the plasma of rats receiving the i.v. dose, 88% of the radioactivity corresponded to the unchanged drug at 5 min post-dose and the plasma concentration of unchanged drug decreased to 1.2% at 24 hr post-dose. In the plasma of rats receiving the oral dose, the unchanged drug represented approximately 74% of the radioactivity at 15 min post-dose, and similar to animals receiving the i.v. dose, the plasma concentration of MK-0869 decreased to 2.5% at 24 hr post-dose. Two metabolites, L-829617 and L-829615, were identified in the plasma of rats receiving the i.v. dose.

Following i.v. dosing, MK-0869 was detected in the brain of rats. The concentration of the parent compound in the brain showed a steady decrease from 116.4 ng/g at 5 min to 46.7 ng/g at 4 hr post-dose (60% decrease). However, the decrease in the concentration of the parent compound was associated with an increase in total radioactivity in the brain (from 185 ng equiv/g at 5 min to 529.7 ng equiv/g at 4 hr post-dose). Rats, receiving an oral dose of 3 mg/kg, had a steadily increase in the brain MK-0869 concentrations from 1.6 ng equiv/g at 15 min post-dose to 21.5 ng/g at 4 hr post-dose. Total MK-0869-related radioactivity increased approximately 46-fold from 15 min (5.7 ng equiv/g) to 4 hr (265.5 ng equiv/g). Three major metabolites were identified in the brain extracts of rats receiving the i.v. or oral doses of MK-0869, and the metabolites were identified as L-825678, L-755446 and L-809861. The plasma and brain total radioactivity and MK-0869 concentrations of rats receiving oral and i.v doses are shown in the sponsor's Table below.

Mean Total Radioactivity (n=3) and Concentrations of [³H]MK-0869 in Plasma and Brain of Sprague-Dawley Rats Dosed Orally or Intravenously With 2 mg/kg [³H]MK-0869

Dosing Route	Time (hr)	Plasma		Brain	
		Total Radioactivity (ng equiv/mL)	MK-0869 (ng/mL)	Total Radioactivity (ng equiv/g tissue)	MK-0869 (ng/g tissue)
P.O	0.083	2.3±1	BLQ ^a	3.6±0.6	01.0 ^b
	0.25	11±8	8.2	5.7±2.5	1.6
	0.5	54±15	44.6	17.7±0.2	2.4
	1	118±44	77.2	63.6±1.7	6.6
	2	149±27	98.1	103.9±3.5	10.2
	4	281±47	146.1	265.5±1.8	21.5
	24	132±42	3.3	196.2±33.2	BLQ
IV	0.083	785±87	687.7	185.0±2.3	116.4
	0.25	602±33	459.1	219.5±2.2	101.6
	0.5	670±33	457.6	329.4±29.6	89.3
	1	404±241	262.5	275.0±4.6	45.4
	2	567±45	308.3	499.8±20.5	77.0
	4	482±39	181.6	529.7±35.6	46.7
	24	133±9	1.9	151.9±3.5	BLQ

Values represent mean ± SD

^a The limit of quantification was ng/mL.

^b The limit of quantification was ng/g tissue.

In the brain extracts of ferrets receiving a 3 mg/kg oral dose of [¹⁴C]MK-0869, MK-0869 was the major component at both 24 hr and 48 hr post-dose, and represented >60% of the radioactivity. Two minor metabolites, identified as L-825678 and L-755446 were detected in the brain at 48 hr post-dose. In the plasma, [¹⁴C]MK-0869 was the major component at both the time points and accounted for 90 to 96% of the plasma radioactivity. In addition three metabolites (L-755446 and L-825678 at 24 hr and L-829617 at 48 hr post-dose) were detected in the plasma of ferrets receiving the oral dose. The total radioactivity and MK-0869 concentrations in the plasma and brain of ferrets receiving an oral dose of [¹⁴C]MK-0869 are shown in the sponsor's Table below.

Distribution of [¹⁴C]MK-0869 and its Metabolites in Plasma and Brain of Ferrets Dosed Orally With 3 mg/kg [¹⁴C]MK-0869

Ferret #	Time, hr	¹⁴ C Radioactivity (ng-eq/g tissue)		% ¹⁴ C Radioactivity as MK-0869 ^b	
		Plasma ^a	Brain	Plasma	Brain
1	24	610	469	96.1	80
2	24	570	460	96.8	70.5
3	48	393	370	85.4	61.9
4	48	493	332	95.2	68.5

^a ng-eq/mL plasma.

^b Estimated from chromatographic profile using

NB/page: 15184/115

Study period: Jul-Oct-1998

Thus, in contrast to rats, MK-0869 was the major component in both the plasma and the brain of ferrets at 24 hr post-dose.

L-754,030: Single-Dose Oral Toxicokinetic Study in Rats (Report Date/Number TT #97-036-0).

Methods: Toxicokinetic parameters of plasma L-754,030 levels were determined in Sprague Dawley rats following single dose oral administration of two drug formulations of different particle sizes. For Formulation M, the mean L-754,030 particle size was _____ and drug was suspended in 0.5% aqueous methylcellulose/0.02% sodium lauryl sulfate. L-754,030 in Formulation M was administered at a dose of 25 and 125 mg/kg using a dose volume of 5 mL/kg. For Formulation N, the mean L-754,030 particle size was _____ and drug was suspended in a 5 to 1 ratio of L-754,030 to hydroxypropylcellulose. L-754,030 in Formulation N was administered at doses of 25 and 125 mg/kg using a dose volume of 5 mL/kg. There were 10 rats/sex/dosing group. Blood for determination of plasma L-754,030 levels was collected at 2, 4, 6, 8, 10, 16, and 24 hr after dosing. Blood was collected from 3-4 rats/sex/group for each time point. L-754,030 was isolated by _____

Results: Exposure (i.e., AUC) to L-754,030 with Formulation N was almost twice that observed with Formulation M at both dose levels. Increased exposure resulted from smaller particle size that may have allowed increased surface area for absorption. Plasma C_{max} values for L-754,030 with Formulation N were almost twice that observed with Formulation M. Plasma C_{max} and AUC values for L-754,030 increased with ascending dose for both Formulations N and M; although, values were less than proportional to dose. For both formulations, plasma C_{max} and AUC values for L-754,030 were higher in female rats than male rats. T_{max} values for both formulations indicate that drug absorption was slower at higher doses.

Plasma toxicokinetic parameters of L-754,030 in male and female rats following single oral dosing with L-754,030 in Formulations N and M.

Dose, mg/kg	Formulation N						Formulation M					
	C_{max} , $\mu\text{g/mL}$		T_{max} , hr		AUC_{0-24hr} , $\mu\text{g}\cdot\text{hr/mL}$		C_{max} , $\mu\text{g/mL}$		T_{max} , hr		AUC_{0-24hr} , $\mu\text{g}\cdot\text{hr/mL}$	
	M	F	M	F	M	F	M	F	M	F	M	F
25	1.62	1.77	2.00	2.00	11.4	21.4	0.769	1.39	2.00	4.00	6.50	15.3
125	1.98	3.57	6.00	4.00	19.7	47.8	1.22	2.33	6.00	6.00	11.5	26.2

MK-0869: Exploratory 16-Day Oral Toxicokinetic Study in Rats (TT #99-054-0).

Methods: Plasma toxicokinetic parameters for 5 very polar metabolites of MK-0869 (i.e., L-294,569, L-596,064, L-770,787, L-858,422, and L-858,443) were assessed in Sprague-Dawley rats that received MK-0869 at oral doses of 125, 250, 500, and 750 mg/kg B.I.D. (total daily doses of 250, 500, 1000, and 1500 mg/kg, respectively) for 15 days. The second daily dose was administered approximately 6 hr after the first dose. The MK-0869 drug particle size used in these studies was μm (i.e., Formulation M). There were 25 male and 15 female rats per treatment group. The vehicle was 0.5% methylcellulose with 0.02% sodium lauryl sulfate in deionized water. Drug solutions were administered by oral gavage using a dose volume of 5 mL/kg. On the last day of drug treatment (day 15), 10 male rats/group received a single oral dose of [morpholine-2- ^{14}C] MK-0869. These animals did not receive a second dose on day 15. Blood samples for measurement of plasma levels of very polar metabolites were collected from 8 male rats/group at 2, 6, 10, or 24 hr after dosing. Only four male rats/group were bled at each time point. Plasma levels of polar metabolites were quantified by HPLC . Urine samples were collected over a 24 hr period after dosing for 2 male rats/group that received [^{14}C]MK-0869. No blood samples were collected from these animals. Blood samples were collected from the remaining 15 rats/sex/group at 2, 4, 6, 8, 10, 16, or 24 hr after the first dose of unlabeled MK-0869 on day 15. The second daily dose was administered after the 6-hr blood sample collection. Blood was collected from 3 or 4 rats/sex/group at each time point.

Results: Plasma AUC levels for three very polar metabolites, L-294,569, L-596,064, and L-770,787, increased <2-fold as the dose of MK-0869 was increased 6-fold from 125 to 750 mg/kg B.I.D. Results appear to be consistent with a saturation absorption for MK-0869 as plasma levels of the parent drug and its metabolites plateaued at doses ≥ 125 mg/kg B.I.D.

Plasma levels of two other very polar metabolites, L-858,442 and L-858,443, were below the limit of detection. The major urinary metabolites at all doses were L-596,064 and L-294,569.

Plasma AUC_{0-24hr} levels ($\mu\text{M}\cdot\text{hr}$) of 5 very polar metabolites in male rats that received MK-0869 at oral doses of 125, 250, 500, and 750 mg/kg B.I.D. for 15 days. On the last day of drug treatment (day 15), rats received an oral dose of [morpholine-2-¹⁴C] MK-0869.

Metabolite	MK-0869 (mg/kg B.I.D.)			
	125	250	500	750
L-294,569	6.92	6.80	8.74	10.99
L-596,064	3.88	3.74	4.81	6.11
L-770,787	3.20	2.83	4.03	5.87
L-858,442 ^a	-	-	-	-
L-858,443 ^a	-	-	-	-

a. No AUC values were calculated for L-858,442 or L-858,443, as plasma levels at all time points

were below the limit of quantitation.

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

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} , $\mu\text{g}/\text{mL}$	T _{max} , hr	AUC _{0-24hr} , $\mu\text{g}\cdot\text{hr}/\text{mL}$
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.

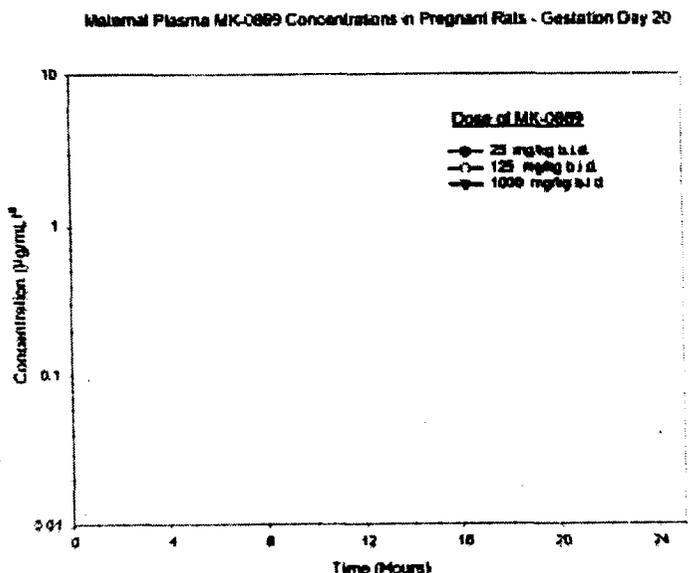
Dose, mg/kg/day	125	250	500	1000
Fetal plasma, µg/mL	0.166	0.142	0.131	0.219
Maternal plasma, µg/mL	1.41	1.37	1.25	1.58
Fetal/Maternal Ratio	0.118	0.104	0.105	0.139

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 —, 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 —

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_{0-24hr} 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.

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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)**

Maternal Toxicokinetic Parameters	MK-0869 (mg/kg b.i.d.)		
	25	125	1000
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

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

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 \pm SEM)

	MK-0869 (mg/kg b.i.d.) 1000
Gestation Day 20	
Maternal Plasma Conc. ($\mu\text{g/mL}$) ^a	
8 hours	2.59 \pm 0.428
24 hours	0.104 \pm 0.0348
Fetal Plasma Conc. ($\mu\text{g/mL}$) ^a	
8 hours	0.228 \pm 0.0348
24 hours	0.0223 \pm 0.00409
Fetal Maternal Plasma Ratio ^b	
8 hours	0.0910 \pm 0.0124
24 hours	0.265 \pm 0.0727
Lactation Day 14	
Maternal Plasma Conc. ($\mu\text{g/mL}$) ^a	
8 hours	5.42 \pm 0.702
Milk Conc. ($\mu\text{g/mL}$) ^a	
8 hours	4.84 \pm 0.582
Milk Maternal Plasma Ratio ^c	0.898 \pm 0.0295
^a Values are the mean \pm SEM.	
^b Values are the mean \pm SEM of the individual fetal plasma concentration divided by the corresponding maternal plasma concentration.	
^c Values are the mean \pm 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

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 1/10th 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
 The AUC_{0-24h}, 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 — 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_{0-24hr}) 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_{0-24hr} (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.

Exploratory 5-Week Oral Toxicokinetic Study in Mice (Study #01-096-0)

Methods: The toxicokinetic profiles of two MK-0869 formulations were examined after oral administration to CD-1 mice for 5 weeks. Formulation M (Lot # L-754030-004H031), with a particle size of approximately — and formulation NB, with a particle size of approximately — were used in the study. Formulation M was dispersed in deionized water containing 0.5% methylcellulose and 0.02% sodium lauryl sulfate (SLS) and administered at a dose of 500 mg/kg s.i.d. Formulation NB was dispersed in deionized water containing 3% hydroxypropylcellulose, 15% sucrose and 0.14% SLS and administered at 500, 1000 and 1500 mg/kg s.i.d. and 250, 500 and 750 mg/kg b.i.d. doses (at least 6 hours apart). On the day of blood collection, 24 animals/sex/group in each group received [^{14}C]MK-0869 Formulation M or Formulation NB (0.87 to 1.0 µCi/mg). Blood samples were collected from 6 mice/sex/group/time point at approximately 2, 6, 10 and 24 hours following the first daily dose on Day 29. Plasma concentrations of [^{14}C]MK-0869 -related substances were determined by measuring the plasma radioactivity.

Results: Female mice had higher plasma radioactivity concentrations (<1.5-fold) as compared with that of male mice. Following oral dosing of M or NB formulation of MK-0869 to mice, drug-related substances appeared in the plasma rapidly and their concentrations were sustained for up to 10 hours. After 24 hours of dosing, the plasma concentrations were approximately 11 to 33% of the respective maximal concentrations. The exposure levels after s.i.d. or b.i.d. dosing of the same doses were similar. The exposure levels following a 750 mg/kg b.i.d. dose of the NB formulation was about 3.5-fold higher than that achieved after a 500 mg/kg s.i.d. dose of Formulation M. In males, s.i.d. dosing of formulation M had slightly lower exposure levels as compared to that achieved following b.i.d. dosing. However, in females, no such differences were observed. The plasma MK-0869 exposure levels in male and female mice following administration of Formulation M or NB are summarized in the sponsor's Table below.

Plasma MK-0869^a Systematic Exposure (AUC) – Drug Week 5

MK-0869 (Form M) 500 mg/kg s.i.d.	AUC _(0-24 hr) (µM·hr)	
	Females	Males
MK-0869 (Form NB) 500 mg/kg s.i.d.	152	123
1000 mg/kg s.i.d.	437	265
1500 mg/kg s.i.d.	399	332
250 mg/kg b.i.d.	425	389
500 mg/kg b.i.d.	375	320
750 mg/kg b.i.d.	402	384
750 mg/kg b.i.d.	435	429

^a [¹⁴C]-MK-0869 and its drug-related substances.
Form M – Formulation M, average MK-0869 particle size of 1
Form NB – Formulation NB, average MK-0869 particle size of 1

Pharmacokinetics and Brain/Plasma Distribution of MK-0869 in CD-1 Mice.

Methods: The pharmacokinetics and brain/plasma distribution of MK-0869 were investigated in the mdr 1a (+/+) CF-1 mice, capable of P-glycoprotein (Pgp) expression and those that are deficient in mdr 1a (-/-), to assess the role of Pgp in the disposition of MK-0869 after i.v. and oral administration. For i.v. administration, the drug was dissolved in control mouse plasma and injected into the tail vein at a dose of 2.35 mg/kg (0.15 ml/mouse). For oral administration, the drug was dissolved in 20% hydroxypropyl β-cyclodextrin and administered by oral gavage at a dose of 5 mg/kg (2.5 ml/kg). Ondansetron and dexamethasone, two antiemetic agents, and known Pgp substrates were administered to determine the potential Pgp-mediated drug-drug interactions. Dexamethasone was given at an i.v. dose of 1 mg/kg and ondansetron was given at an oral dose of 0.5 mg/kg. The concentrations of MK-0869 and its metabolite, L-755446 in the plasma and brain extracts were determined by

Results: Following i.v. or oral administration of MK-0869 to mdr 1a (+/+) and mdr 1a (-/-) mice, the bioavailability of the drug was similar in both groups. However, the peak plasma concentration was slightly higher and the time-to-peak concentration was slightly delayed in the mdr 1a (+/+) mice. There was rapid distribution of MK-0869 into the brain of both groups of mice. The peak plasma concentrations were achieved at 0.5 and 2 hours in mdr 1a (+/+) and mdr 1a (-/-) mice, respectively. The concentrations of MK-0869 in the brain of mdr 1a (-/-) mice were higher than that of mdr 1a (+/+) mice after either i.v. or oral dosing. The ratio of brain to plasma AUC values (AUC_{brain}/AUC_p) in the mdr 1a (-/-) mice was about 13-fold higher after i.v. dosing and about 19-fold higher after oral dosing, as compared with that of mdr 1a (+/+) mice, suggesting that MK-0869 is a substrate for mdr 1a P-glycoprotein. The concentrations of the metabolite, L-755446 in the plasma of both groups of mice were below the limits of detection, and the brain concentrations were similar in both groups (5-20 ng/g of tissue), suggesting that L-755446 may not be a substrate for mdr 1a P-glycoprotein. The plasma and brain concentrations and the pharmacokinetic parameters of MK-0869 in two groups of CF-1 mice are shown in the sponsor's Tables below.

Table 1. Plasma Concentrations (ng/mL) and Pharmacokinetic Parameters of MK-0869 in Pgp (+/-) or (-/-) CF-1 Mice Following a 2.35 mg/kg IV Dose of MK-0869 (Mean ± SD, n=3 per time point) (PDM 1444)

Time (hr)	Pgp (+/+)	Pgp (-/-)
0.083	1173 ± 159.2	1528 ± 94.1
0.5	755.4 ± 42.1	624.1 ^b
2	429.7 ± 37.1	267.0 ^b
6	152.0 ± 19.7	244.6 ± 97.9
8	77.2 ± 14.3	26.3 ^b
AUC _{0-8hr}} (ng·hr/mL) ^a	2433	2310
CL _R (mL/min/kg) ^a	14.4	16.5
Vd _d (L/kg) ^a	2.6	2.2
t _{1/2} (hr) ^a	2.5	1.7

^aParameters were calculated based on mean plasma concentration data.

^bFor this time point n=2.

Notebook page: 15183/121 (Feb-Jun-1998)

Table 2. Brain Concentrations of MK-0869 and L-755446 in Pgp (+/+ or (-/-) CF-1 Mice Following a 2.35 mg/kg IV Dose of MK-0869 (Mean ± SD, n=3 per time point) (PDM1444)

Time (hr)	Brain (ng/g Tissue)			
	MK-0869		L-755446	
	Pgp (+/+)	Pgp (-/-)	Pgp (+/+)	Pgp (-/-)
0.083	56.1 ± 5.52	349.5 ± 8.96	6.49 ± 1.12	6.61 ± 0.69
0.5	92.5 ± 24.7	678.8 ± 25.0	18.8 ± 2.93	18.0 ± 0.41
2	63.0 ± 7.52	798.5 ± 137.5	19.1 ± 0.58	13.9 ± 3.03
6	37.8 ± 3.32	618.1 ± 284.1	12.2 ± 0.84	8.67 ± 2.81
8	19.3 ± 1.84	205.2 ± 76.5	8.43 ± 1.73	5.03 ± 1.17
AUC _{0-8hr}} (ng·hr/g) ^a	488.6	8054	117.2	88.2

When MK-0869 was co-administered orally with ondansetron, the brain MK-0869 concentrations or the brain to plasma concentration ratios were not affected by ondansetron in mdr 1a (+/+) mice. Similarly, when MK-0869 was co-administered with intravenous dexamethasone, the brain concentrations or the brain to plasma concentration ratios for MK-0869 were unchanged in these mice. The plasma and brain concentrations and AUC values for MK-0869 following co-administration with ondansetron or dexamethasone are shown in the sponsor's Table below.

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Table 3. Plasma Concentrations of MK-0869 in Pgp (+/+) and (-/-) CF-1 Mice Following a 5 mg/kg Oral Dose of MK-0869, in the Presence or Absence of 0.5 mg/kg Ondansetron (OND) (Mean ± SD, n=3 per time point) (PDM 1682)

Time (hr)	Plasma (ng/mL)			
	Pgp (+/+)	Pgp (+/+) with OND	Pgp (-/-)	Pgp (-/-) with OND
0.5	122.7 ± 46.9	165.8 ± 45.0	208.0 ± 48.6	255.0 ± 16.3
2	442.4 ± 74.8	612.0 ± 146.2	566.2 ± 82.6	793.4 ± 137.8
4	932.7 ± 61.5	573.8 ± 24.3	432.5 ± 209.0	366.8 ± 69.1
6	309.5 ± 37.8	414.5 ± 179.2	351.2 ± 63.1	284.4 ± 61.0
10	157.5 ± 18.8	158.3 ± 17.8	121.2 ± 27.3	168.3 ± 43.7
AUC _{0-10hr}} (ng·hr/mL) ^a	4005.8	3944.5	3360.5	3566.9
F _{abs} ^b	77%	76%	68%	73%

^aBased on mean plasma concentration data.

^bBioavailability (F_{abs}) was calculated based on the mean AUC value (dose normalized) following the IV dose (Table 1).

Notebook page: 15183/239-240 (Sep-Oct-1998).

Table 4. Brain Concentrations of MK-0869 in Pgp (+/+) and (-/-) CF-1 Mice Following a 5 mg/kg Oral Dose of MK-0869, in the Presence or Absence of 0.5 mg/kg Ondansetron (OND) (Mean ± SD, n=3 per time point) (PDM 1682)

Time (hr)	Brain (ng/g tissue)			
	Pgp (+/+)	Pgp (+/+) with OND	Pgp (-/-)	Pgp (-/-) with OND
0.5	4.8 ¹	3.7 ± 1.1	26.6 ± 8.2	33.7 ± 5.0
2	13.7 ± 3.8	20.4 ± 4.0	192.0 ± 45.4	309.1 ± 82.4
4	36.6 ± 1.2	28.8 ± 0.7	346.5 ± 79.6	353.9 ± 20.3
6	28.7 ± 1.8	29.1 ± 9.3	398.6 ± 184.0	475.4 ± 64.5
10	11.8 ± 2.8	13.5 ± 2.4	283.8 ± 35.0	334.1 ± 73.1
AUC _{0-10hr}} (ng·hr/g) ^a	211.7	211.3	3419	3377

^aBased on mean brain concentration data.

¹For this time point n=2.

Table 5. Plasma and Brain Concentrations of MK-0869 in Pgp (+/-) CF-1 Mice Following a 0.5 mg/kg IV Dose of MK-0869, in the Presence or Absence of 1 mg/kg IV Dexamethasone (DEX) (Mean ± SD, n=3 per time point) (PDM 1773)

Time (hr)	Plasma (ng/mL)		Brain (ng/g tissue) ^a	
	Pgp (+/-)	Pgp (+/-) with DEX	Pgp (+/-)	Pgp (+/-) with DEX
0.5	205.5 ± 113.9	322.4 ± 99.3	38.6	19.5
4	80.9 ± 6.0	82.6 ± 11.3	34.3	47.6
10	10.7 ± 0.4	18.6 ± 6.8	44.0	33.1

^aValues obtained from pooled sample.

Thus, ondansetron or dexamethasone had no significant inhibitory effect on P-glycoprotein-mediated transport of MK-0869 in CF-1 mice.

Thus, the data suggest that the brain distribution of MK-0869, but not its metabolite L-755444, was dependent on the P-glycoprotein-dependent transport mechanism. The two commonly used antiemetic agents, dexamethasone and ondansetron does not appear to affect the P-glycoprotein-mediated transport of MK-0869 in CF-1 mice.

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L-754,030: Five-Week Oral Toxicokinetic Study In Mice (Report Date/Number TT #96-068-0).

Methods: Toxicokinetic parameters of plasma L-754,030 levels in male and female CD-1 mice were determined following treatment with L-754,030 for 29-30 days at doses of 25, 50, 125, 500, and 1000 mg/kg/day administered by oral gavage. The vehicle was 0.5% methylcellulose/0.02% sodium lauryl sulfate. The dose volume was 10 mL/kg. There were 30 mice/sex/group. Blood for determination of plasma L-754,030 levels was collected at 0.5, 2, 4, 6, 8, 10, and 24 hr after dosing in week 5. There were 4 mice/sex/group for each time point. Livers were collected from all animals, weighted, and frozen at -20°C for possible determination of tissue drug levels. L-754,030 was isolated by

Results: Plasma C_{\max} and $\text{AUC}_{0-24\text{hr}}$ values for L-754,030 in female mice increased with ascending doses; however, values were less than proportional to dose. Plasma $\text{AUC}_{0-24\text{hr}}$ values for L-754,030 in male mice increased with ascending doses between 25 and 500 mg/kg/day; however, values plateaued at doses ≥ 500 mg/kg/day and increases between doses of 25 and 500 mg/kg/day were less than proportional to dose. Plasma C_{\max} values for L-754,030 in male mice did not increase with ascending doses. Plasma C_{\max} and $\text{AUC}_{0-24\text{hr}}$ values for female mice observed at doses of 500 and 1000 mg/kg/day were higher than corresponding values observed for male mice. A plateau of plasma $\text{AUC}_{0-24\text{hr}}$ values for L-754,030 in female mice was not evident. T_{\max} values indicated a slow absorption of drug, particularly at higher doses of 500 and 1000 mg/kg/day in both male and female mice. At doses of 500 and 1000 mg/kg/day in male and female mice, substantial plasma drug levels were evident at 2 hr after dosing; however, peak plasma levels did not occur until 8 to 10 hr after dosing.

Plasma C_{\max} , T_{\max} , and $\text{AUC}_{0-24\text{hr}}$ values for L-754,030 in male and female CD-1 mice following treatment with L-754,030 for 29-30 days at doses of 25, 50, 125, 500, and 1000 mg/kg/day administered by oral gavage.

Dose, mg/kg/day	C_{\max} , $\mu\text{g/mL}$		T_{\max} , hr		$\text{AUC}_{0-24\text{hr}}$, $\mu\text{g}\cdot\text{hr/mL}$	
	Male	Female	Male	Female	Male	Female
25	1.38	1.41	4	2	18.0	21.4
50	1.83	1.76	2	2	19.8	26.6
125	1.74	2.65	4	8	26.3	24.9
500	2.97	4.07	10	10	42.9	58.0
1000	3.91	6.01	8	8	42.1	74.6

L-754,030: Five-Week Oral Toxicokinetic Study in Female Mice (Report Date/Number TT #97-018-0).

Methods: Toxicokinetic parameters of plasma L-754,030 levels in female CD-1 mice were determined following treatment with L-754,030 for 29 days at doses of 1250, 1500, and 2000 mg/kg/day administered by oral gavage. The vehicle was 0.5% methylcellulose/0.02% sodium lauryl sulfate. The dose volume was 10 mL/kg. There were 30 female mice/group. Blood for determination of plasma L-754,030 levels was collected at 2, 4, 6, 8, 10, 16, and 24 hr after dosing in week 5. There were 4 female mice/group for each time point. L-754,030 was isolated by quantified by

Results: Plasma C_{max} and AUC_{0-24hr} values for L-754,030 in female mice did not increase with ascending doses and had plateaued over the dose range of 1250 to 2000 mg/kg/day. T_{max} values indicated a slow absorption of drug. The sponsor speculated that due to variability in plasma drug levels and poor aqueous solubility of the drug found at higher doses between 500 and 2000 mg/kg/day, exposure (i.e., AUC) had actually plateaued at or near a dose of 500 mg/kg/day in female male.

Plasma C_{max} , T_{max} , and AUC_{0-24hr} values for L-754,030 in female CD-1 mice following treatment with L-754,030 for 29 days at doses of 1250, 1500, and 2000 mg/kg/day administered by oral gavage.

Dose, mg/kg/day	C_{max} , µg/mL	T_{max} , hr	AUC_{0-24hr} , µg*hr/mL
1250	3.50	6	30.6
1500	4.03	8	33.5
2000	2.97	6	25.5

L-754,030: Five-Week Oral Toxicokinetic Study in Mice (Report Date/Number TT #97-070-0).

Methods: Toxicokinetic parameters for plasma L-754,030 levels were determined in CD-1 mice that received drug by the oral route of administration at a dose of 500 mg/kg/day or at doses of 500 and 1000 mg/kg/day B.I.D. (total daily doses of 1000 and 2000 mg/kg, respectively) for 28-29 days. Mice in the B.I.D. 500 and 1000 mg/kg/day groups were dosed twice per day, with second dose administered 6 hr after the initial dose. The vehicle was 0.5% methylcellulose/0.02% sodium lauryl sulfate. There were 30 mice/sex/group. The dosing volume was 10 mL/kg. Blood for determination of plasma L-754,030 levels was collected at 2, 4, 6, 8, 10, 16, and 24 after the first daily dose during week 5. Mice that received drug B.I.D., and had blood drawn at 8, 10, 16, or 24 hr after the first daily dose, received their second dose immediately after the 6 hr blood collection time point. Blood was collected from 4 mice/sex/group for each time point. L-754,030 was isolated by

Results: Plasma C_{max} and AUC values for L-754,030 in male mice did not increase with ascending total daily doses and had plateaued over the dose range of 500 to 2000 mg/kg/day. Plasma C_{max} and AUC values for L-754,030 in female mice increased with ascending total daily doses; however, increases were substantially less than proportional to dose and appeared to also indicate that these parameters had plateaued over the dose range of 500 to 2000 mg/kg/day (total daily dose). B.I.D. treatment at 500 and 1000 mg/kg/day appeared to provide little or no increase in systemic drug exposure (i.e., AUC) as compared to treatment with 500 mg/kg/day. Plasma C_{max} and AUC values for L-754,030 were slightly higher in female mice as compared to male mice. T_{max} values indicated a slow absorption of drug.

Toxicokinetic parameters for plasma L-754,030 levels in CD-1 mice that received drug by the oral route of administration at a dose of 500 mg/kg/day or at doses of 500 and 1000 mg/kg/day B.I.D. (total daily doses of 1000 and 2000 mg/kg, respectively) for 28-29 days.

Dose	C_{max} , $\mu\text{g/mL}$		T_{max} , hr		AUC_{0-24hr} , $\mu\text{g}\cdot\text{hr/mL}$	
	Male	Female	Male	Female	Male	Female
500 mg/kg/day (Total=500 mg/kg/day)	3.19	3.48	8	6	30.7	33.4
500 mg/kg/day B.I.D. (Total=1000 mg/kg/day)	3.06	4.24	8	8	27.7	39.1
1000 mg/kg/day B.I.D. (Total=2000 mg/kg/day)	3.84	4.52	8	8	31.6	44.3

L-754,030: Single-Dose Oral Toxicokinetic Study in Mice (Report Date/Number TT #97-035-0).

Methods: Toxicokinetic parameters of plasma L-754,030 levels were determined in CD-1 mice following single dose oral administration of two drug formulations of different particle sizes. For Formulation M, the mean L-754,030 particle size was — and drug was suspended in 0.5% aqueous methylcellulose/0.02% sodium lauryl sulfate. L-754,030 in Formulation M was administered at a dose of 125 mg/kg using a dose volume of 10 mL/kg. For Formulation N, the mean L-754,030 particle size was — and drug was suspended in a 5 to 1 ratio of L-754,030 to hydroxypropylcellulose. L-754,030 in Formulation N was administered at doses of 125, 500, and 1000 mg/kg using a dose volume of 10 mL/kg. For administration of L-754,030 at a dose of 125 mg/kg in Formulations M and N, there were 30 mice/sex/group. For administration of L-754,030 at a dose of 500 mg/kg in Formulations M and N, there were 30 male mice/group. For administration of L-754,030 at a dose of 1000 mg/kg in Formulations M and N, there were 30 female mice/group. Blood for determination of plasma L-754,030 levels was collected at 2, 4, 6, 8, 10, 16, and 24 hr after dosing. Blood was collected from 4 mice/sex/group for each time point.

L-754,030 was isolated by

Results: Exposure (i.e., AUC) to L-754,030 with Formulation N was almost twice that observed with Formulation M at all dose levels. Increased exposure resulted from smaller particle size that may have allowed increased surface area for absorption. Plasma C_{max} values for L-754,030 with Formulation N were almost twice that observed with Formulation M. Plasma C_{max} and AUC values for L-754,030 increased with ascending dose for both Formulations N and M; although, values were less than proportional to dose. T_{max} values indicated a slow absorption of drug for both formulations.

Plasma toxicokinetic parameters of L-754,030 in male and female mice following single oral dosing with L-754,030 in Formulations N and M.

Dose, mg/kg	Formulation N						Formulation M					
	C _{max} , µg/mL		T _{max} , hr		AUC _{0-24hr} , µg*hr/mL		C _{max} , µg/mL		T _{max} , hr		AUC _{0-24hr} , µg*hr/mL	
	M	F	M	F	M	F	M	F	M	F	M	F
125	7.32	8.20	8	8	78.8	86.6	4.40	5.35	6	6	38.8	46.9
500	13.7	-	8	-	142	-	7.90	-	6	-	69.8	-
1000	-	16.0	-	8	-	201	-	8.23	-	6	-	99.3

L-754,030: Five Week Oral Toxicokinetic Study in Mice: Metabolite Concentrations in Mouse Plasma (Report Date/Number #96-068-0).

Methods: Following administration of ¹⁴C-754,030 to mice, the major forms of radioactivity in the systemic circulation were the parent compound and a series of oxidative metabolites (Data not shown). Oxidative metabolism proceeds by way of dealkylation to L-755,446. This metabolite subsequently undergoes a series of oxidations to form several different metabolites. The structures of parent compound and 7 of the circulating metabolites are shown below. Plasma samples from mice that received L-754,030 by oral gavage at doses of 125, 500, and 1000 mg/kg/day groups for 5-weeks (Report Date/Number TT #96-068-0) were analyzed by

monitored by selected reaction monitored — The parent compound and 7 oxidative metabolites were measured to distinguish saturation of absorption of the parent compound versus enhanced metabolism of the parent compound yielding increased exposure to metabolites. Due to constraints in instrument performance and limited quantities of synthetic metabolite standards, complete calibration curves were not performed with each analytical run. Therefore, it was not possible to make precise quantitative measurements of metabolites. However, a sufficient number of standards were available for each run to ensure that detector responses were within the linear range of the instrument. Data are expressed as ratio of peak area of each metabolite to the internal standard. These intensity ratios were integrated over time to yield an AUC of intensity ratios. The systemic exposure to each metabolite was expressed by AUC of the — detector response of each metabolite relative to the internal standard over the measured period of time. The AUC in mice was integrated over the 2 to 10 hr time period. Due to large differences in detector response to each compound, these area ratios do not necessarily represent the relative proportions of each metabolite in the plasma. However, if a disproportionate increase in 1 or more metabolites were found, it should appear as a large increase in the intensity ratio AUC as a function of dose.

Results: For male mice, AUC values for L-754,030 and its metabolites, L-829,617,

L-829,615, L-809,771, and L-825,678, appeared to plateau between doses of 500 and 1000 mg/kg/day. Similarly for female mice, AUC values for L-754,030 and its metabolites, L-829,617 and L-829,615, appeared to plateau between doses of 500 and 1000 mg/kg/day. For male mice, AUC values for metabolites, L-829,674, L-755,446, and L-809,681, appeared to increase with ascending dose; however, increases were not proportional to dose. For female mice, the AUC values of L-755,446 increased with ascending dose; however, increases were not proportional to dose. Plateaus in plasma metabolite levels appear to generally parallel the plateau in plasma L-754,030 levels found at a dose of 500 mg/kg/day. Based upon this observation, doses ≥ 500 mg/kg/day should not provide increased systemic exposure to the parent compound or metabolites. This data appears to support the sponsor's contention that saturation of absorption of the parent compound limits systemic exposure. The sponsor has speculated that lack of absorption of parent compound at high doses is due to limited aqueous solubility, which constrains systemic exposure to the parent compound and its metabolites. It is not known quantitatively what fraction, the plasma AUC values for L-754,030 and these 7 metabolites, constitute of the total exposure; although, the sponsor has claimed that the parent compound and these metabolites form the majority of circulating drug-related substances in plasma.

Intensity Ratio AUCs of L-754,030 and its metabolites in male CD-1 mice following treatment with L-754,030 for 5 weeks by oral gavage.

Dose of L-754,030 mg/kg/day	L-829,617	L-829,615	L-754,030	L-809,771	L-829,674	L-825,678	L-755,446	L-809,861
125	0.155	0.325	51.2	0.0242	0.0684	0.290	5.45	0.206
500	0.219	0.455	72.9	0.0192	0.0694	0.397	6.45	0.204
1000	0.216	0.488	68.1	0.0174	0.103	0.329	7.79	0.283

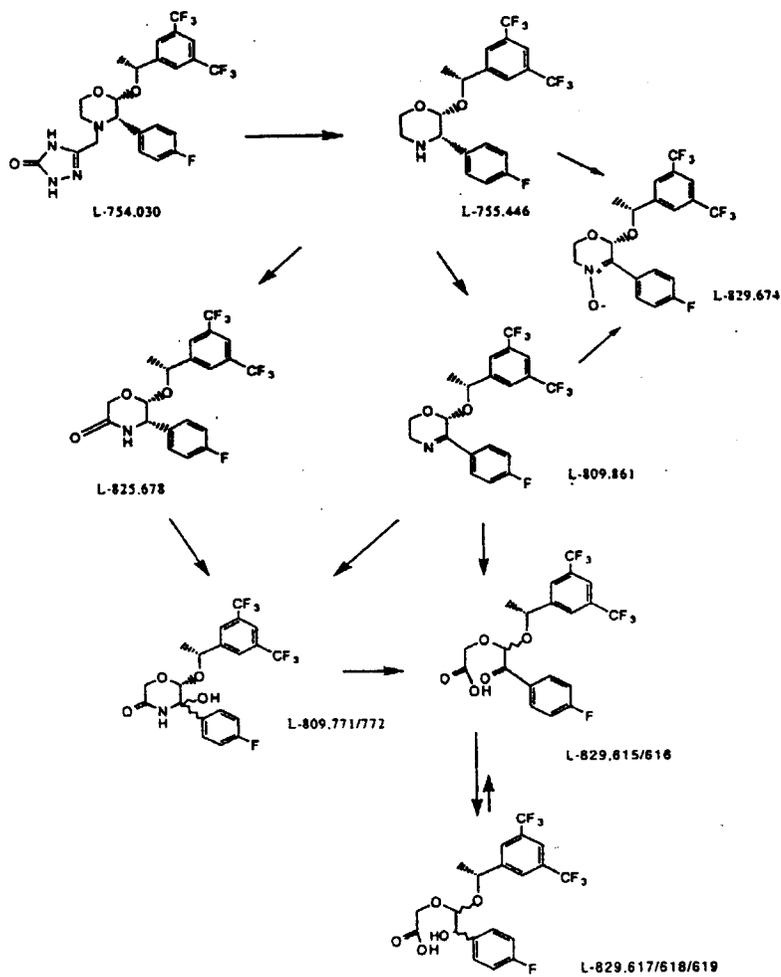
Intensity Ratios AUCs of L-754,030 and its metabolites in female CD-1 mice following treatment with L-754,030 for 5 weeks by oral gavage.

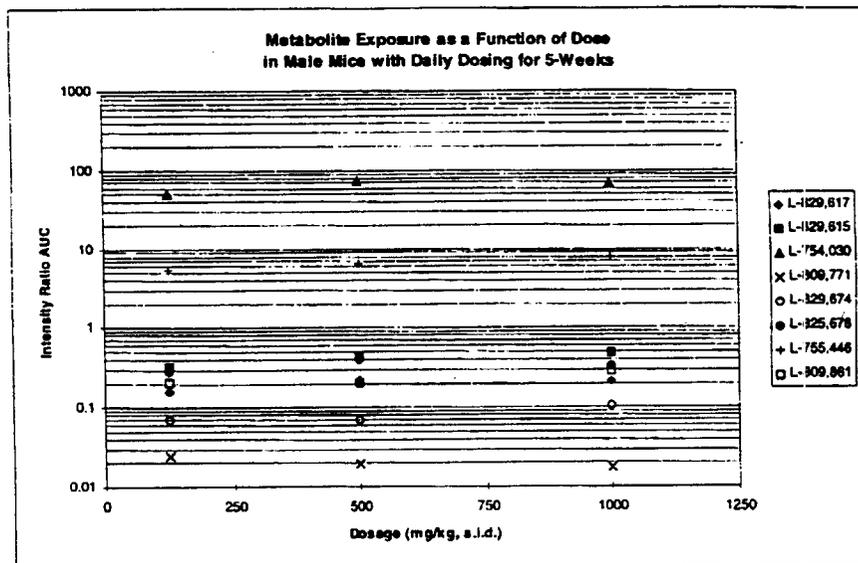
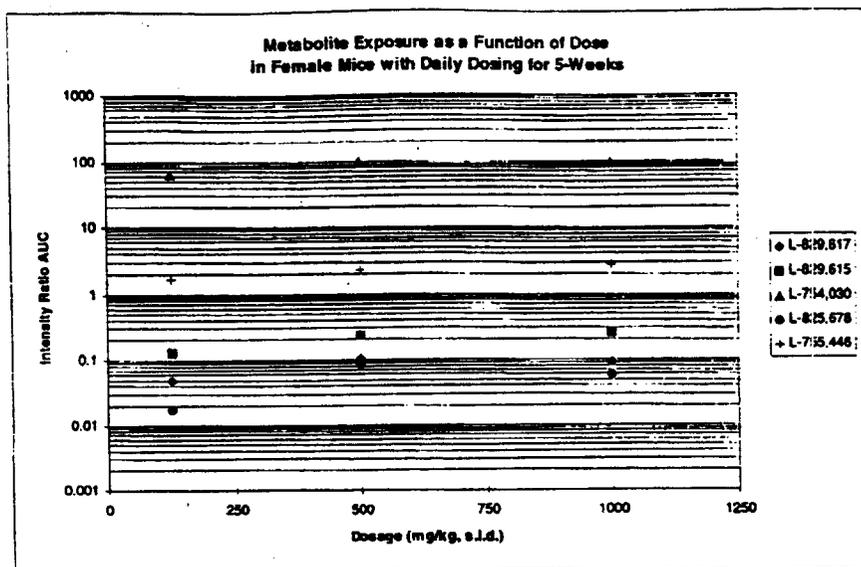
Dose of L-754,030 mg/kg/day	L-829,617	L-829,615	L-754,030	L-809,771	L-829,674	L-825,678	L-755,446	L-809,861
125	0.0492	0.133	63.3	ID	ID	ID	1.65	ID
500	0.106	0.237	98.1	ID	ID	0.0822	2.31	ID
1000	0.0930	0.253	95.6	ID	ID	ID	2.77	ID

I.D. = Insufficient data available for calculation.

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Metabolic Pathways of MK-0869





MK-0869: Exploratory 17-Day Oral Toxicokinetic Study in Mice (TT #99-046-0).

Methods: Plasma toxicokinetic parameters for 5 very polar metabolites of MK-0869 (i.e., L-294,569, L-596,064, L-770,787, L-858,422, and L-858,443) were assessed in CD-1 mice that received MK-0869 at oral doses of 500, 1000, 1250, and 1500 mg/kg/day for 16 days. Oxidative degradation of the morpholine ring of MK-0869 produced 5 very polar metabolites, which included L-294,569 (4-fluorophenylglyoxylic acid), L-596,064 (p-fluoromandelic acid), L-770,787 (4-fluorophenylglycine), L-858,442 (a triazolone-containing amino acid), and L-858,443 (a triazolone-containing amino alcohol) (structures are shown in Figure 2 below). The MK-0869 drug particle size used in these studies was — (i.e., Formulation M). There were 52 male and 28 female mice per treatment group. The vehicle was 0.5%

methylcellulose with 0.02% sodium lauryl sulfate in deionized water. Drug solutions were administered by oral gavage using a dose volume of 10 mL/kg. On the last day of drug treatment (day 16), 24 male mice/group received an oral dose of [morpholine-2-¹⁴C]-MK-0869. Remaining animals received unlabeled MK-0869. Blood samples for measurement of plasma metabolites levels were collected from 6 male mice/group/time point that received [¹⁴C]-MK-0869 at 2, 6, 10, and 24 hr after dosing. Plasma levels of polar metabolites were quantified by — Blood samples were collected from the remaining 4-mice/sex/group/time point that received unlabeled MK-0869 throughout the treatment period at 2, 4, 6, 8, 10, 16, and 24 hr after the last dose on day 16. |

Results: Plasma AUC levels for four very polar metabolites, L-294,569, L-596,064, L-770,787 and L-858,443, increased <1.6-fold as the dose of MK-0869 was increased 3-fold from 500 to 1500 mg/kg/day. The results appear to be consistent with a saturation absorption for MK-0869 as plasma levels of the parent drug and its metabolites plateaued at doses ≥500 mg/kg/day. Plasma levels of the very polar metabolite, L-858,442, were below the limit of detection.

Plasma AUC_{0-24hr} levels (μM·hr) of 5 very polar metabolites in male mice that received MK-0869 at oral doses of 500, 1000, 1250, and 1500 mg/kg/day for 16 days. On the last day of drug treatment (day 16), mice received an oral dose of [morpholine-2-¹⁴C] MK-0869.

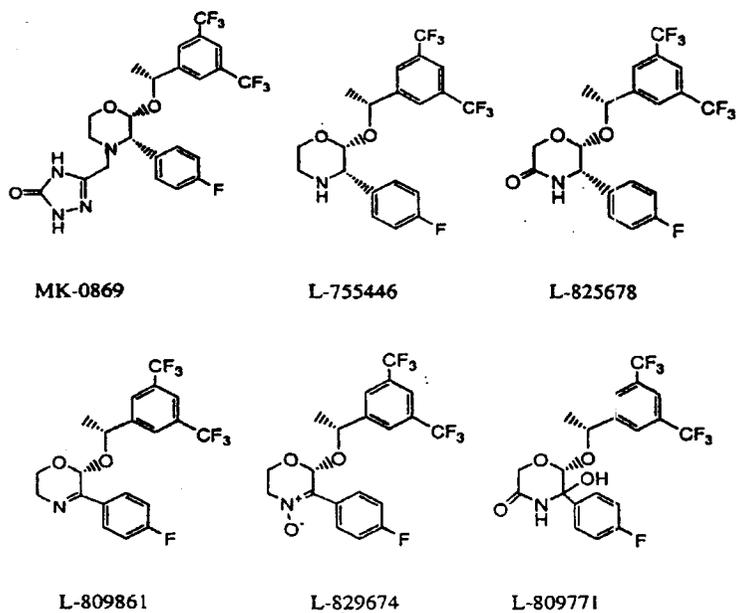
Metabolite	MK-0869 (mg/kg B.I.D.)			
	500	1000	1250	1500
L-294,569	33.02	38.59	37.54	39.20
L-596,064	3.45	4.02	4.22	4.04
L-770,787	5.12	7.87	7.25	7.12
L-858,442 ^a	-	-	-	-
L-858,443	0.83	1.00	1.36	0.89

a. No AUC values were calculated for L-858,442, as plasma levels at all time points were below the limit of quantitation.

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Figure 1
Structures of MK-0869 and its Nonpolar and Polar Metabolites

Nonpolar Metabolites



Polar Metabolites

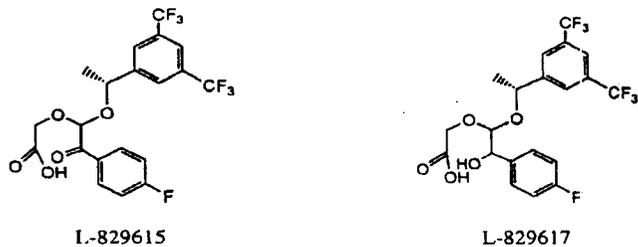
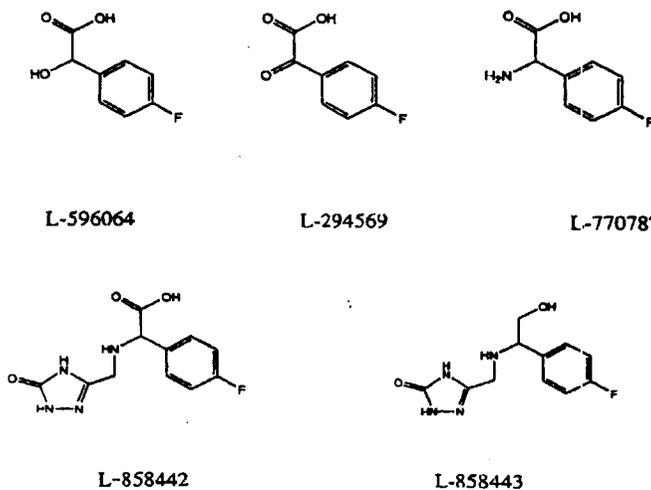


Figure 2

Structures of Five Very Polar Metabolites of MK-0869



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

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 _{0-24hr} 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, $\mu\text{g/mL}$	0.0820	0.790
Maternal plasma, $\mu\text{g/mL}$	0.238	1.40
Fetal/Maternal Ratio	0.345	0.564

Single-Dose Oral Toxicokinetic Study in Female Dogs (TT #96-107-0).

Methods: Plasma toxicokinetic parameters of L-754,030 (— drug particle size) were determined in female dogs following single oral doses of 32, 64, 128, 256, and 512 mg/kg. The vehicle was 0.5% aqueous methylcellulose with 0.02% sodium lauryl sulfate. There were 3 female dogs/group. Drug was administered by oral gavage using a dose volume was 5 mL/kg. Blood for determination of plasma levels of the parent compound was collected at 0.5, 2, 4, 8, 24, 36, and 48 hr after dosing. The parent compound was isolated from plasma by : — and analyzed by —

Results: Absorption of drug was slow and highly variable with T_{\max} values ranging from 8 to 28 hr. AUC values from 0 to 24 hr were less than one-half of values from 0 to 48 hr. Plasma C_{\max} and AUC values for L-754,030 obtained with doses ranging from 32 to 512 mg/kg displayed no relationship to dose. Drug exposure at 32 mg/kg was unusually high as it exceeded values obtained with greater doses.

Plasma C_{\max} , T_{\max} , and AUC values for L-754,030 in female dogs that received L-754,030 at oral doses of 32, 64, 128, 256, and 512 mg/kg.

Parameters	Dose, mg/kg				
	32	64	128	256	512
C_{\max} , $\mu\text{g/mL}$	8.52	4.81	5.22	6.38	7.65
T_{\max} , hr	28.0	10.7	28.0	16.7	8.00
$\text{AUC}_{0-48\text{hr}}$, $\mu\text{g}\cdot\text{hr/mL}$	276	139	177	225	263
$\text{AUC}_{0-24\text{hr}}$, $\mu\text{g}\cdot\text{hr/mL}$	125	64.8	79.6	103	151

Oral Toxicokinetic Study in Dogs (TT #97-016-0).

Methods: Plasma toxicokinetic parameters for L-754,030 (— drug particle size) were determined in beagle dogs that received L-754,030 at oral doses of 32 mg/kg S.I.D. or 32 mg/kg B.I.D. in a cross-over design. Dogs in Group 1 received L-754,030 at 32 mg/kg S.I.D. on day 1 and 32 mg/kg B.I.D. on day 8. Dogs in Group 2 received L-754,030 at 32 mg/kg B.I.D. on day 1 and 32 mg/kg S.I.D. on day 8. For the B.I.D. regimen, the second dose was administered immediately following collection of blood at the 6 hr time point. The vehicle was 0.5% aqueous methylcellulose with 0.02% sodium lauryl sulfate. There were 4 dogs/sex/group. Drug was administered by oral gavage using a dose volume was 5 mL/kg. Blood for determination of plasma drug levels was collected on days 1 and 8 at 2,

4, 6, 8, 12, 24, and 48 hr after dosing. The parent compound was isolated from plasma by

Results: For Group 1, the B.I.D. regimen produced plasma C_{max} and AUC values for the parent compound that were 5 to 6 times greater than values observed for the S.I.D. regimen. However, for Group 2, plasma C_{max} and AUC values obtained with the B.I.D. regimen were only approximately 2 times greater than values observed with the S.I.D. regimen. T_{max} values obtained with the S.I.D. and B.I.D. regimens were highly variable as the range extended from 2.50 to 16.5 hr. T_{max} values suggest that drug absorption could be highly variable and potentially prolonged.

Plasma toxicokinetic parameters of L-754,030 in dogs that received L-754,030 by oral gavage at 32 mg/kg S.I.D. and B.I.D. Dosing Regimens.

Parameters	Group 1				Group 2			
	S.I.D.		B.I.D.		B.I.D.		S.I.D.	
	Males	Females	Males	Females	Males	Females	Males	Females
C_{max} , $\mu\text{g/mL}$	1.66	1.57	9.30	8.42	7.20	7.24	3.04	4.03
T_{max} , hr	16.5	2.50	15.0	13.0	13.0	12.0	5.00	5.00
AUC_{0-24hr} , $\mu\text{g}\cdot\text{hr/mL}$	25.6	23.1	150	134	116	117	50.0	74.8

Oral Toxicokinetic Study in Dogs (TT #97-072-0).

Methods: Plasma toxicokinetic parameters for L-754,030 (— drug particle size) were determined in beagle dogs that received L-754,030 by the oral route at doses of 32, 64, 128, and 256 mg/kg B.I.D. Each dog received 2 doses (i.e., B.I.D.) of L-754,030 with the second dose administered immediately following the 6-hr blood collection time point. There were 4 dogs/sex/group. The vehicle was 0.5% aqueous methylcellulose with 0.02% sodium lauryl sulfate. Drug was administered by oral gavage using a dose volume was 5 mL/kg. Blood for determination of plasma levels of the parent compound was collected at 2, 4, 6, 8, 12, 24, and 48 hr after dosing. The parent compound was isolated from plasma by

Results: With the B.I.D. regimen, T_{max} values ranged from 11.0 to 24.0 suggesting that drug absorption was slow and prolonged. With the exception of male dogs at 32 mg/kg B.I.D., plasma C_{max} and AUC values increased with elevating dose from 32 to 128 mg/kg B.I.D. in an approximate dose proportional manner. There appeared to be a plateau in drug exposure at 128 mg/kg B.I.D. as AUC values at doses of 128 and 256 mg/kg B.I.D. were equivalent. Similarly, C_{max} values at doses of 128 and 256 mg/kg B.I.D. were approximately equivalent.

Plasma toxicokinetic parameters of L-754,030 in male and female dogs that received L-754,030 by oral gavage at doses of 32, 64, 128, and 256 mg/kg B.I.D.

Parameter	32 mg/kg B.I.D.		64 mg/kg B.I.D.		128 mg/kg B.I.D.		256 mg/kg B.I.D.	
	Male	Female	Male	Female	Male	Female	Male	Female
C_{max} , $\mu\text{g/mL}$	9.43	4.72	8.57	8.39	17.3	13.7	18.1	13.0
T_{max} , hr	21.0	11.0	15.0	20.0	20.0	15.0	24.0	15.0
AUC_{0-24hr} , $\mu\text{g}\cdot\text{hr/mL}$	145	78.8	135	133	250	207	255	201

Single Dose Toxicokinetic Study in Dogs (TT #97-034-0).

Methods: Plasma toxicokinetic parameters of L-754,030 were determined in dogs that received single oral doses of two L-754,030 formulations of different particle sizes (——— particle size formulations). The mean L-754,030 particle size of Formulation M was ——— which was prepared in a vehicle of 0.5% aqueous methylcellulose with 0.02% sodium lauryl sulfate. The mean L-754,030 particle size of Formulation N was ——— which was prepared in a 5-to-1 ratio of L-754,030 to hydroxypropylcellulose SL. L-754,030, Formulation M or N, was administered by oral gavage at doses of 2, 32, or 128 mg/kg. There were 4 dogs/sex/group. The dose volume was 5 mL/kg. Blood for determination of plasma levels of the parent compound was collected at 0.5, 2, 4, 6, 8, 10, and 24 hr after dosing. The parent compound was isolated from plasma by ———

Results: At doses of 2 and 32 mg/kg, Formulation N produced plasma C_{max} and AUC values for L-754,030 that were at least 2 times greater than corresponding values obtained with Formulation M. At a dose of 128 mg/kg, plasma C_{max} and AUC values obtained with Formulation N were greater than those observed with Formulation M; although, there was significant variability in C_{max} and AUC values between male and female dogs that received Formulation N. For both Formulations M and N, plasma C_{max} and AUC values for L-754,030 increased with elevating dose; although, increases were less than proportional to dose.

Plasma toxicokinetic parameters of L-754,030 in dogs that received L-754,030, Formulation M or N, as a single oral dose of 2, 32, or 128 mg/kg. The mean L-754,030 particle size of Formulation M was ——— The mean L-754,030 particle size of Formulation N was ———

Parameter	2 mg/kg				32 mg/kg				128 mg/kg			
	Formulation M		Formulation N		Formulation M		Formulation N		Formulation M		Formulation N	
	M	F	M	F	M	F	M	F	M	F	M	F
C _{max} , µg/mL	0.262	0.635	1.32	1.51	1.44	1.82	6.41	4.96	3.52	4.98	5.44	12.5
T _{max} , µg/mL/hr	2.00	3.50	2.00	2.00	5.50	8.00	4.00	2.50	10.5	4.50	9.00	7.50
AUC _{0-24hr} , µg*hr/mL	3.06	9.46	19.1	21.5	25.6	26.3	103	83.2	61.0	73.1	95.0	219

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Investigation of MK-0869 as a Substrate and Inhibitor of P-Glycoprotein.

Methods: MK-0869 was examined as a potential P-glycoprotein (Pgp) substrate and inhibitor in vitro using four cell lines: Caco-2, a human colon adenocarcinoma cell line; KB-VI, a human epidermoid carcinoma cell line with a high level of MDR1 Pgp; L-MDR1, a human MDR1 transfected porcine renal epithelial cell line; and L-mdr 1a, a mouse mdr 1a transfected porcine renal epithelial cell line. Vinblastine, a known Pgp substrate, and cyclosporin A (CsA) and verapamil, two known Pgp inhibitors, were used as positive controls.

Transport experiments in cultured Caco-2 cells were carried out by the addition of aliquots of transport medium containing [³H]MK-0869 (20 μM) to either the apical (A to B) or basolateral (B to A) compartment, respectively, in the presence or absence of CsA (10 μM) or verapamil (100 μM). The corresponding receiver solutions, without the radiolabeled compound were added in the opposite compartment. After specific time points, aliquots were collected from the receiver compartment and replaced with an equal volume of fresh transport medium. Samples from the donor compartment were collected at the end of the 2 hr period. The radioactivity in the samples was measured and the percentage of radioactivity appearing in the opposite compartment was determined. A to B or B to A permeability ratio was calculated and this qualitatively represents the significance of Pgp-mediated transport across the cell monolayer membranes. To examine the potential for MK-0869 as an Pgp inhibitor, similar transport experiments were conducted using [³H]vinblastine as a substrate and unlabeled MK-0869 was used in both the donor and receiver compartments. Similar transport experiments in KB-3-1, KB-VI, MDR1 and mdr 1a cell monolayers were conducted using the method described by Yamazaki et al (J Pharmacol Exp Ther 2001; 296: 723-735).

Results: In Caco-2 cells, MK-0869 was a weak substrate for P-glycoprotein (Pgp), as compared to vinblastine, a known Pgp substrate. At 20 μM [³H]MK-0869, the permeability (P_{app}) value for B to A transport (BA) was 2.3-fold higher than the permeability for A to B (AB). For vinblastine, the P_{app} value for B to A was about 7-fold higher than that for A to B. The difference between A to B and B to A transport of [³H]MK-0869 was eliminated (BA/AB ratio decreased from 2.3 to 1.07) in the presence of 10 μM CsA (a strong Pgp inhibitor) and was reduced (BA/AB ratio 1.5) in the presence of 100 μM verapamil (a weak inhibitor). The A to B and B to A transport values for MK-0869 and vinblastine in the absence and presence of the Pgp inhibitors, verapamil and CsA are shown in the sponsor's Table below.

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Apparent Permeability Coefficient of [³H]MK-0869 and [³H]Vinblastine from Apical-to-Basolateral (AB) and Basolateral-to-Apical (BA) Directions in Caco-2 Cells With and Without Verapamil or Cyclosporin A (CsA)

Substrate	Inhibitor	$P_{app} \times 10^3$ (cm/sec)		
		A→B	B→A	(B→A)/(A→B) Ratio
[³ H]MK-0869 (20 μM)	Control	2.6 ± 0.6	6.0 ± 0.4	2.3
	+Verapamil (100 μM)	2.5 ± 0.4	3.8 ± 0.1	1.5
	+CsA (10 μM)	4.5 ± 0.2	4.8 ± 1.0	1.07
[³ H]Vinblastine (5 μM)	Control	1.8 ± 1.0	12.7 ± 2.4	7.1
	+Verapamil (100 μM)	4.0 ± 0.9	7.2 ± 1.1	1.8
	+CsA (10 μM)	5.2 ± 1.5	5.4 ± 0.6	1.04

*Mean ± SD (n=3).

The accumulation of [³H]MK-0869 was lower in the Pgp overexpressed KB-VI cells (337.8 pmol/well) as compared to that in the parental KB-3-1 cells (536.9 pmol/well; KB-3-1/KB-VI ratio, 1.59). In the presence of CsA, the accumulation of [³H]MK-0869 in KB-VI cells increased to 656.7 pmol/well. Thus, the efflux of [³H]MK-0869 was inhibited by CsA suggesting that MK-0869 may be a substrate for Pgp. The accumulation of [³H]vinblastine to Pgp overexpressed KB-VI cells (18.66 pmol/well) was lower than that in the parental KB-3-1 cells (182.1 pmol/well), and the KB-3-1 KB-VI ratio was 9.76. In the presence of 10 μM CsA, the KB-3-1/KB-VI ratio was much smaller (1.96). In human MDR1 and mouse mdr 1a transfected cell lines, the B to A versus A to B transport ratios for [³H]vinblastine (1 μM) were 11 and 19 respectively. These values were much higher than those observed in Caco-2 and KB cells. The BA/AB ratios for [³H]MK-0869 were also high in both systems. In human MDR1 and mouse mdr 1a transfected cell lines, the BA/AB ratios for [³H]MK-0869 were 7.4 and 13, respectively. In the presence of 10 μM CsA, the ratios were reduced to close to 1. The transport of MK-0869 and vinblastine in human MDR1 and mouse mdr 1a transfected cell lines and its inhibition by CsA is shown in the sponsor's Table below.

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Transport of [³H]MK-0869 and [³H]Vinblastine in Human MDR1 and Mouse mdrla Transfected Cells Lines

		Transport within 3 hr (pmol/cm ²) ^a		(B→A) to (A→B) Ratio
		A→B	B→A	
Human MDR1				
[³ H]Vinblastine (1 μM)	Control	5.6 ± 1.7	58.6 ± 4.1	11
[³ H]MK-0869 (1 μM)	Control	11.8 ± 1.8	87.5 ± 10.4	7.4
	+ CsA (10 μM)	55.7 ± 5.3	51.4 ± 4.3	0.92
Mouse mdrla				
[³ H]Vinblastine (1 μM)	Control	3.4 ± 1.4	65.9 ± 3.8	19
[³ H]MK-0869 (1 μM)	Control	6.7 ± 0.3	84.4 ± 12.9	13
	+ CsA (10 μM)	50.0 ± 1.5	47.3 ± 5.7	0.95

^aMean ± SD (n=4)

In Caco-2 cells, MK-0869 (10 μM) had a weak inhibitory activity on the net transport of [³H]vinblastine (1 μM) as compared to CsA and verapamil (both at 10 μM). There were 36%, 87% and 60% inhibitions of Pgp-mediated transport of [³H]vinblastine by MK0869, CsA and verapamil, respectively. Similar results were obtained when a higher concentration (5 μM) of [³H]vinblastine was used as a substrate. In MDR1 overexpressed KV-VI cells, MK-0869 (2 and 10 μM) and verapamil (10 μM) had no substantial effect on [³H]vinblastine accumulation. CsA, on the other hand, caused an increase in the vinblastine accumulation, thus reducing the KB-3-1/KB-VI ratio from 9.76 (control) to 1.96(CsA, 10 μM). The permeability coefficient of [³H]vinblastine in the presence of MK-0869, CsA and verapamil in Caco-2 cells are shown in the sponsor's Table below.

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Apparent Permeability Coefficient of [³H]Vinblastine in the Presence of MK-0869, Cyclosporin A (CsA), and Verapamil in Caco-2 Cells

Substrate	Inhibitor	$P_{app} \times 10^6$ (cm/sec) ^a		Ratio (B→A)/ (A→B)	Inhibition % ^b
		A→B	B→A		
[³ H]Vinblastine (1 μM)	Control	1.99 ± 0.21	12.6 ± 0.93	6.3	.
	+ CsA (10 μM)	3.86 ± 0.92	6.45 ± 0.49	1.7	87
	+ MK-0869 (10 μM)	2.93 ± 0.47	12.8 ± 1.52	4.4	36
	+ Verapamil (10 μM)	3.32 ± 1.24	11.0 ± 0.93	3.1	60

^aMean ± SD (n=3).

^b% of Inhibition was calculated as: [(Control ratio - 1) - (Ratio in the presence of inhibitor - 1)] / (Control ratio - 1) × 100%.

Thus, MK-0869 was found to be a substrate of Pgp in Caco-2 and Pgp-overexpressed (KV-VI, MDR1 or mdrla transfected cells). However, it was a weaker substrate than the known Pgp substrate, vinblastine.

PK/TK summary:

Pharmacokinetics/toxicokinetics studies were conducted with both the pro-drug, L-758, 298 and the active drug, L-758, 030 (MK-0869), following i.v. and oral administration to rats, mice, dogs and ferrets. The absorption of MK-0869 was rapid after oral dosing, and the maximum plasma concentration was reached in between 2-4 hours. The oral bioavailability of MK-0869 was 43%, 42.4% and 45.4% in rats, mice and ferrets. Following i.v. administration of a 5 mg/kg dose of MK-0869 to rats, the AUC, clearance (Cl), the volume of distribution (Vd_{ss}) and t_{1/2} values were 6377 ng.hr/ml, 13.4 ml/kg/min, 2.5 L/kg and 2.4 hr, respectively. Following an i.v. dose of 2 mg/kg in mice, the plasma t_{1/2} was 2.6 hours, total plasma clearance (Cl) was 4.9 ml/min/kg and the volume of distribution (Vd_{ss}) was 1.2 L/kg. Following a 10 mg/kg oral dose to male mice, the C_{max} and AUC values were 2175.7 ng/ml and 14328.1 ng.hr/ml, respectively. In dogs, the t_{1/2} value ranged from 5.7 – 7.3 hours and the AUC value increased with increasing doses. After an i.v. dose of 0.5 mg/kg to dogs, the Cl and Vd_{ss} values were 2.3 ml/min/kg and 1.1 L/kg, respectively. Following i.v. dosing of a 0.5 mg/kg dose of MK-0869 in ferrets, the half life was 10 hours, the total plasma clearance was 1.5 ml/kg/min, and the Vd_{ss} was 1.3 L/kg. Thus, the clearance of MK-0869 was lower in ferrets as compared with rats and mice. There was evidence of saturation of absorption of MK-0869 in mice and rats during 5-week oral b.i.d dosing of the NB formulation (127 nm particle size). MK-0869 is a weak substrate and inhibitor of P-glycoprotein (Pgp). MK-0869 is highly bound to plasma proteins from rat, dog and human (>98%). It can penetrate the blood brain barrier, as shown in rats and ferrets; following i.v. or oral administration, unchanged drug was detected in the brain of both species. MK-

0869 can cross the placental barrier, and the placental transfer of the drug in pregnant rats ranged from 9% to 26.5%. It was excreted in the milk of lactating rats after oral dosing; the milk to maternal plasma concentration ratio was 0.90. N-dealkylation is the major metabolism pathway of MK-0869. Following *in vitro* incubation of [4-fluorophenyl-3-³H]L-754, 030 with cultured rat hepatocytes, four metabolites were identified, among which L-755, 446 (N-dealkylated derivative of L-754, 030) was the major metabolite. Other metabolites included, the acetic acid derivative, the acetamide derivative and the methyl ester of acetic acid derivative of L-755, 446. After oral administration of [¹⁴C]MK-0869 to rats, several nonpolar, polar and very polar metabolites were identified in the plasma. In dog plasma, these metabolites and several additional nonpolar and polar metabolites were identified. After i.v. dosing of a 100 mg dose of [¹⁴C]L-758298 to humans, several, polar and very polar metabolites were identified in the plasma, which are similar to those found in rats. The metabolic profiles of MK-0869 by rat and human liver microsomes were also similar; the major nonpolar metabolite was L-755446 that underwent further metabolism to yield several polar metabolites. In the human liver microsomes, CYP3A4 was identified as the main CYP450 isozyme involved in the metabolism of MK-0869. Two other CYP450 isozymes, CYP1A2 and CYP2C19, were also involved in the metabolism of the compound. Fecal excretion is the predominant excretory pathway in rats. In dogs, fecal excretion was similar to that of urinary excretion; after a 2 mg/kg i.v. dose, 39.1% and 37.7% of dose was excreted in feces and urine, respectively, in 168 hours.

PK/TK conclusions:

Following oral administration, MK-0869 (L-754, 030) is absorbed rapidly. The $t_{1/2}$ is variable in different animal species, the shortest value was observed in mice and longest value in ferrets. The drug is metabolized by the CYP450 enzyme system, and CYP3A4 has been identified as the major CYP450 isozyme involved in its metabolism. Thus, there is a potential for drug-drug interactions with drugs that are metabolized by this enzyme system. In rats, MK-0869 has been found to cross the placental barrier to fetal circulation. The drug is also excreted in the milk of lactating rats. Thus, a consideration should be made for the risks of its use during pregnancy and lactation.

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IV. GENERAL TOXICOLOGY:

ACUTE TOXICITY:

Methods: Female (F) (mean body weight of 24.5 g; 6-7 weeks age) ICR mice were intravenously administered L-758,298 (200 and 500 mg/kg); vehicle was 0.9% saline solution. There were 3 mice per group; observation period was 14 days.

F (mean body weight of 24.0 g; 6-7 weeks age) ICR mice were orally administered L-758,298 (500 mg/kg); vehicle was 0.9% saline solution. There were 3 mice in the group; observation period was 14 days.

F (mean body weight of 148 g; 6-7 weeks age) Sprague-Dawley rats were intravenously administered L-758,298 (200 and 500 mg/kg); vehicle was 0.9% saline solution. There were 3 rats in the 200 mg/kg group and 1 rat in the 500 mg/kg group; observation period was 14 days.

F (mean body weight of 120 g; 6-7 weeks age) Sprague-Dawley rats were orally administered L-758,298 (500 mg/kg); vehicle was 0.9% saline solution. There were 3 rats in the group; observation period was 14 days.

Results: The results of the acute toxicity studies are summarized in the following table. Intravenously administered L-758,298 in mice produced gasping, convulsions, bradypnea and loss of righting reflex; all surviving mice appeared normal within 3 hr. Intravenously administered L-758,298 in rats produced gasping and bradypnea followed rapidly by death. Orally administered L-758,298 did not produce any clinical signs of toxicity.

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Summary of acute toxicity data for L-758,298 in the mouse and rat

Species	Route of Adm.	LD ₅₀ (mg/kg)	Time until death	Minimum lethal dose (mg/kg)
Mouse	i.v.	>500 mg/kg	12 min	500 mg/kg
Mouse	oral	Not determined; >500 mg/kg	No deaths occurred	Not determined; >500 mg/kg
Rat	i.v.	>200 mg/kg	8 min	500 mg/kg
Rat	oral	Not determined; >500 mg/kg	No deaths occurred	Not determined; >500 mg/kg

Study title: Acute Oral Toxicity Study in Mice with L-755446 (a metabolite of MK-0869 and a process intermediate).

Key study findings: In the acute oral toxicity studies with L-755, 446 (a metabolite of MK-0869) in female mice, groups of animals received 500 or 2000 mg/kg (1st and second study), and 800 and 2000 mg/kg (3rd study) doses. In the third study, all animals receiving the 2000 mg/kg dose died and there were no deaths at the 800 mg/kg dose. Thus, the minimal lethal dose (MLD) was between 800 and 2000 mg/kg. Clinical signs observed were decreased activity, bradypnea, lateral recumbency, ptosis, clonic and rolling convulsions, and sternal recumbency.

Study no: #96-2552, #99-2502, #00-2546.

Volume #, and page #: Volume #48, page #H-164, #H-245, #H-323.

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

Date of study initiation: #96-2552, March 14, 1996; #99-2502, January 12, 1999; #00-2546, March 16, 2000.

GLP compliance: No

QA report: yes () no (X)

Drug lot #, radiolabel, and % purity: L-755, 446; Lot # L-755446-001E001; L-755, 446-004L (Lot #1), and L-755, 446-002G (Lot #5); Purity, 98.1% to 99.1%.

Methods: Three acute oral toxicity studies were conducted in mice with L-755, 446 following oral administration of single doses of L-755, 446, a metabolite and processing intermediate of MK-0869.

Dosing:

Species/strain: Cr1:CD-1 (ICR) BR mice.

Doses in administered units: In the first study, 3 female mice received an oral dose of 500 mg/kg/day and in the second study, 3 females received an oral dose of 2000 mg/kg/day. In the third study, 2 groups of female mice (3/group) received single oral doses of 800 and 2000 mg/kg/day of L-755, 446.

Route, form, volume, and infusion rate: The drug was suspended in 0.5% methylcellulose in deionized water and administered by oral gavage at a dosing volume of 2.5 ml/kg.

Observations and times:

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

Body weights: Body weights were measured before initiation of treatment and on Day 1.

On Day 7, the animals were sacrificed and discarded without necropsy.

Results:

Mortality: There was no mortality in the first 2 studies. In the third study (Study #00-2546), all 3 animals receiving the 2000 mg/kg dose died during the 7-day observation period. Two animals died on Day 4 and the third animal died on Day 5.

Clinical signs: No treatment-related clinical sign was observed in animals receiving a single oral dose of 500 mg/kg L-755, 446. In the second study, animals receiving the 2000 mg/kg dose had signs of decreased activity (20 minutes after dosing), ataxia (60 minutes after dosing) and sternal recumbency (90 minutes after dosing). Ataxia and sternal recumbency lasted for 2 days, while decreased activity lasted for 3 days.

In study # 00-2546, in which two groups of animals received single doses of 800 and 2000 mg/kg of L-755, 446, animals receiving the 2000 mg/kg dose had signs of decreased activity and bradypnea (within 5 minutes), lateral recumbency, ptosis, clonic and rolling convulsions (within 1.5 hrs), and sternal recumbency (within 3.5 hrs). Ptosis was also observed in animals of the 800 mg/kg dose group within 2 hours of dosing.

Body weights: There were slight decreases in the body weights of animals on Day 7, as compared with the pretreatment values (2-9%).

Summary: In acute oral toxicity studies with L-755, 446 (a metabolite and processing intermediate of MK-0869) in mice, groups of female animals received single oral doses of 500 mg/kg (study #96-2552), 2000 mg/kg (study #99-2502), and 800 and 2000 mg/kg (study #00-2546) of the drug. In study #00-2546, all 3 animals receiving the 2000 mg/kg dose died during the 7-day observation period. There were no deaths in any other groups. Thus, the minimal lethal dose (MLD) was between 800 and 2000 mg/kg. The clinical signs observed, included decreased activity, bradypnea, lateral recumbency, ptosis, clonic and rolling convulsions, and sternal recumbency.

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 [CrI:CD[®](SD)IGS 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.

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

Methods: 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. There were 15 rats/sex/group. Sterile dosing solutions were supplied pre-formulated in vehicle (5.0 mg/mL ethyl alcohol (190 proof), 1.92 mg/mL citric acid (anhydrous), 2.50 mg/mL polysorbate 80, 8.20 mg/mL NaCl, and 0.95 mg/mL NaOH, in water with a pH of 5.45 and an osmolality of 386.0 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 5.0 mg/mL ethyl alcohol (190 proof), 1.92 mg/mL citric acid (anhydrous), 2.50 mg/mL polysorbate 80, 8.20 mg/mL NaCl, 1.22 mg/mL NaOH, and 3.87 mg/mL 1 N HCl in water with a pH of 5.90 and an osmolality of 392.0 mOsm. Male and female rats received — Certified Rodent Diet 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

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 $\mu\text{g}/\text{kg}/\text{day}$ 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 $\mu\text{g}/\text{kg}/\text{day}$ 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 $\mu\text{g}/\text{kg}/\text{day}$ 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 or 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 $\mu\text{g}/\text{kg}/\text{day}$. The no effect dose appeared to be 240 $\mu\text{g}/\text{kg}/\text{day}$. 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 $\mu\text{g}/\text{kg}/\text{day}$ were impaired by >10%; however, there were no effects on corresponding male treatment groups. A target organ of toxicity was not identified.

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.

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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.

5-Week intravenous toxicity study in rats (TT#95-607-0)

This study has previously been reviewed under IND [redacted] Original submission dated September 28, 1995 (pharmacologist's review of IND [redacted] 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 0.25, 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 [redacted] method. Maximum mean plasma concentrations of the active drug, L-754, 030, were attained very rapidly (within 4 minutes postdose). The mean plasma C_{max} increased in a less than dose-proportional manner. This could be due to saturation of the dephosphorylation pathway. The systemic exposure or AUC_{0-24h} 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.