

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

Toxicokinetic Parameters	Drug Day 1 - L- 754,030			
	Males		Females	
	Mean	S.E.M.	Mean	S.E.M.
AUC ($\mu\text{g}\cdot\text{hr}/\text{ml}$)	2.46	0.42	2.71	0.73
C _{max} ($\mu\text{g}/\text{ml}$)	0.63	0.05	0.72	0.14
T _{max} (hr)	0.03	0.00	0.03	0.00

Toxicokinetic Parameters	Drug Week 4 - L- 754,030			
	Males		Females	
	Mean	S.E.M.	Mean	S.E.M.
AUC ($\mu\text{g}\cdot\text{hr}/\text{ml}$)	3.49	0.63	4.50	0.50
C _{max} ($\mu\text{g}/\text{ml}$)	0.74	0.08	0.71	0.06
T _{max} (hr)	0.03	0.00	0.03	0.00

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

Drug Day 1 - L- 754,030				
Toxicokinetic Parameters	Males		Females	
	Mean	S.E.M.	Mean	S.E.M.
AUC ($\mu\text{g}\cdot\text{hr}/\text{ml}$)	17.4	3.46	18.36	3.26
C _{max} ($\mu\text{g}/\text{ml}$)	2.54	0.29	2.64	0.34
T _{max} (hr)	0.27	0.24	0.05	0.02

Drug Week 4 - L- 754,030				
Toxicokinetic Parameters	Males		Females	
	Mean	S.E.M.	Mean	S.E.M.
AUC ($\mu\text{g}\cdot\text{hr}/\text{ml}$)	31.81	8.93	24.78	4.44
C _{max} ($\mu\text{g}/\text{ml}$)	3.05	0.28	2.69	0.32
T _{max} (hr)	0.03	0.00	0.05	0.02

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

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

Date Started: October 25, 1999

Date Completed: August 3, 2000

GLP compliance: A statement of compliance with GLP regulations was included.

QA- Report Yes (X) No ()

Methods: In a 5-week oral dose range finding study, beagle dog received MK-0869 Formulation NB at doses of 5, 25, 125, 250, 500, and 750 mg/kg B.I.D. (total daily doses of 10, 50, 250, 500, 1000, and 1500 mg/kg/day, respectively). The treatment duration was 28 or 29 days. The MK-0869 average particle size in the colloidal dispersion was — Two control groups were included in this study. The first control group received deionized water.

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

Dosing:

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

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

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

Observations and times:

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

- **Organs weighed:** Absolute and relative (% of body weight and % of brain weight) organ weights were determined for the adrenal glands, brain, heart, kidneys, liver, thyroid (with parathyroid), ovaries, pituitary gland, prostate, spleen, and testes.

- **Histopathology:** A complete microscopic examination of paraffin-embedded, hematoxylin- and eosin-stained from all animals in the vehicle-control and 750 mg/kg B.I.D. groups was conducted. The thymus, ovaries (females), prostate and testes (male), and gross and ophthalmic changes from all animals in all groups were also submitted to microscopic examination. Tissues examined were as follows: salivary gland, stomach, small intestine, large intestine, liver, gallbladder, pancreas, adrenal glands, pituitary gland, thyroid gland, parathyroid, kidneys, urinary bladder, ovaries, skin, mammary gland (when present in skin section), lung, heart, spleen, lymph node, thymus, bone, bone marrow, skeletal muscle, brain, spinal cord, nerve - sciatic, eye (with optic nerve), uterus, testes (with epididymides), prostate, and esophagus.

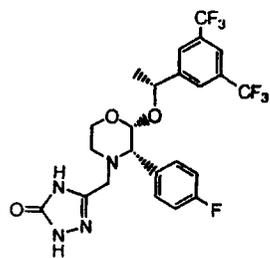
- **Toxicokinetics:** Blood samples for measurement plasma drug and metabolite concentrations were collected in week 4 at 2, 4, 6, 8, 12, 16, and 24 hr after the first daily dose. The second daily dose was administered immediately following the 6-hr time point. Plasma concentrations of MK-0869 were quantified using

Plasma samples from the 250, 500, and 750 mg/kg B.I.D. groups collected at the 4, 8, 16, and 24 hr time points were analyzed for 12 metabolites of MK-0869 by

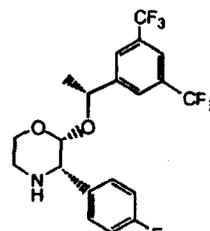
exposure to each metabolite was expressed as AUC_{0-24hr} of the response of each metabolite relative to internal standard. Metabolites were categorized into three groups based upon their similarities in molecular structure and polarity. Group 1 consisted of 3 very polar metabolites, L-770787, L-858442, and L-858443. Group 2 consisted of 2 polar metabolites, L-294569 and L-596064. Group 3 consisted of 5 nonpolar and 2 polar metabolites, L-809771, L-829674, L-825678, L-755446, L-809861, L-829617, and L-829615.

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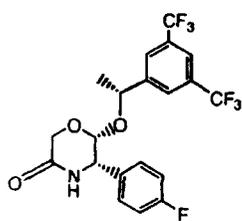
Structures of MK-0869 and 12 Plasma Metabolites Analyzed by



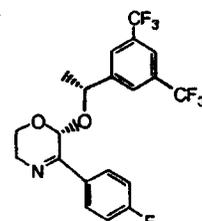
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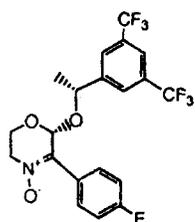
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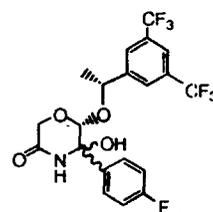
L-825678



L-809861

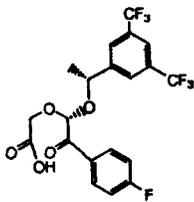


L-829674

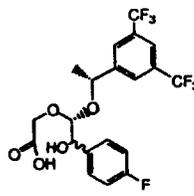


L-809771

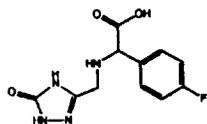
Structures of MK-0869 and 12 Plasma Metabolites Analyzed by



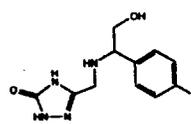
L-829615



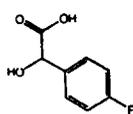
L-829617



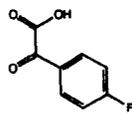
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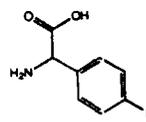
L-858443



L-596064



L-294569



L-770787

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

Results:

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

- **Mortality:** None.

- **Body weights:** Suppression of body weight gain was observed for dogs at 25 mg/kg B.I.D. and body weight loss was observed for dogs at doses ≥ 125 mg/kg B.I.D. Body weights for saline-control dogs at pretest -1 and week 4 were 3.0 and 8.2 kg, respectively, yielding a 2.50% increase of initial body weight. Body weights for vehicle-control dogs at pretest -1 and week 4 were 8.5 and 8.8 kg, respectively, yielding a 3.53% increase of initial body weight. Body weights for dogs at 5 mg/kg B.I.D. were increased by 4.8% over initial body weight. Body weights for dogs at 25 mg/kg B.I.D. were unchanged. Body weights for dogs at 125, 250, 500, and 750 mg/kg B.I.D. were decreased by 2.5, 6.9, 8.4, and 10.7% from initial body weights.

- **Food consumption:** Food consumption for dogs at doses ≥ 25 mg/kg B.I.D. was suppressed. Food consumption for dogs at 25, 125, 250, 500, and 750 mg/kg B.I.D. from weeks 1 through 4 was suppressed to 85.8, 84.2, 64.4, 68.5, and 55.6% of the saline-control group (289.4 g/day), respectively. Mean food consumption for saline-control and vehicle-control groups from weeks 1 to 4 was 289.4 and 294.5 g/day, respectively.

- **Ophthalmoscopy:** The sponsor reported that there were no treatment-related ophthalmic changes; however, no data was provided for independent verification.

- **Electrocardiography:** The sponsor reported that there were no treatment-related electrocardiographic changes; however, no data was provided for independent verification.

- **Hematology:** Lymphocyte counts for dogs at 125, 250, 500, and 750 mg/kg B.I.D. during week 2 were decreased to 84.6, 85.7, 85.4, and 81.2% of the control (2863 cells/mm³), respectively. Lymphocyte counts for dogs at 125, 250, 500, and 750 mg/kg B.I.D. during week 4 were decreased to 86, 95, 84.9, and 76.8% of the control (2635 cells/mm³), respectively.

- **Clinical chemistry:** Phosphorus levels for dogs at 25, 125, 250, 500, and 750 mg/kg B.I.D. during week 2 were decreased to 90.2, 86.9, 93.4, 90.2, and 88.5% of the control (6.1 mg/dL), respectively. Phosphorus levels for dogs at 25, 125, 250, 500, and 750 mg/kg B.I.D. during week 4 were decreased to 87.7, 84.2, 87.7, 87.5, and 82.1% of the control (5.6 mg/dL), respectively. Cholesterol levels for dogs at 25, 125, 250, 500, and 750 mg/kg B.I.D. during week 2 were increased to 114.5-128.3% of the control (152 mg/dL). Cholesterol levels for dogs at 25, 125, 250, 500, and 750 mg/kg B.I.D. during week 4 were increased to 122.7-132.5% of the control (154 mg/dL).

- **Urinalysis:** Changes of urinalysis parameters were observed; however, there were no histopathological findings in the kidneys. Urinary volume for dogs at 125, 250, 500, and 750 mg/kg B.I.D. during week 4/5 was decreased to 60.1, 41.2, 29.7, and 45.9% of the control (148 mL), respectively. An increased incidence of urinary leukocytes were observed for dogs at 125 mg/kg B.I.D. (2 at 0-5, 2 at 0-5/0-5, 1 at 0-5/6-10, 1 at 6-10, and 1 at 6-10/11-30), 250 mg/kg B.I.D. (3 at 0-5, 1 at 0-5/0-5, 1 at 6-10/6-10, 1 at 11-30/6-10, 1 at 11-30, and 1 at 11-30/11-30), 500 mg/kg B.I.D. (3 at 0-5, 2 at 0-5/0-5, 1 at 6-10, 1 at 6-10/6-10, and 1 at 11-30/11-30), and 750 mg/kg B.I.D. (1 at 0-5/0-5, 1 at 0-5/11-30, 4 at 6-10, 1 at 11-30/6-10, and 1 at 11-30/>30) as compared to the control (4 at 0-5, 3 at 0-5/0-5, and 1 at 0-5/>30). An increased incidence of tubular epithelial cells were observed for dogs at 25 mg/kg B.I.D. (4 at N-Occ, 1 at N-Occ/N-Occ, 1 at N-Occ/+1, and 2 at +1/+1), 125 mg/kg B.I.D. (3 at N-Occ, 1 at N-Occ/N-Occ, 1 at N-Occ/+1, and 2 at +1/+1), 250 mg/kg B.I.D. (4 at N-Occ, 3 at N-Occ/+1, and 1 at +2/+1), 500 mg/kg B.I.D. (4 at N-Occ, 2 at N-Occ/N-Occ, 1 at +1/N-Occ, and 1 at +1/+1), and 750 mg/kg B.I.D. (4 at N-Occ, 1 at N-Occ/N-Occ, 1 at +1/N-Occ, and 1 at +1/+2) as compared to the control (4 at N-Occ, 3 at N-Occ/N-Occ, and 1 at N-Occ/+1).

- **Organ Weights:** Alterations were observed in testes, prostate, ovaries, and thyroid gland weights that appear to correspond with observed histopathological changes.

Testes: Absolute testes weights for male dogs at 250, 500, and 750 mg/kg B.I.D. were decreased to 52.7, 59.9, and 63.8% of the control (14.59 g), respectively. Relative testes weights for male dogs at 250, 500, and 750 mg/kg B.I.D. were decreased to 53.9, 61.5, and 62.1% of the control (18.88% Br.W.), respectively.

Prostate: Absolute prostate weights for male dogs at 25, 125, 250, 500, and 750 mg/kg B.I.D. were decreased to 71.9-86.2% of the control (3.20 g). Relative prostate weights for male dogs at 25, 125, 250, 500, and 750 mg/kg B.I.D. were decreased to 70.7-85.7% of the control (4.21% Br.W.).

Ovaries: Absolute ovaries weights for female dogs at 750 mg/kg B.I.D. were decreased to 71% of the control value (0.62 g). Relative ovaries weights for female dogs at 25, 125, 250, 500, and 750 mg/kg B.I.D. were decreased to 68.8-88.3% of the control (0.8358% Br.W.).

Thyroid gland: Relative thyroid gland weights for dogs at 750 mg/kg B.I.D. were decreased to 83.7% of the control (0.8072% Br.W.).

- **Gross Pathology:** There were gross pathological findings for the testes, prostate, thymus, and ovaries, which corresponded with observed histopathological changes. Dose-response relationships were not evident for gross pathological findings.

Gross pathological changes for dogs that received MK-0869 Formulation NB at oral doses of 5, 25, 125, 250, 500, and 750 mg/kg B.I.D. There were two control groups, one group received deionized water and the other group received the vehicle.

Tissue	Water		Vehicle		5		25		125		250		500		750	
	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M
Testes	-	4	-	4	-	4	-	4	-	4	-	4	-	4	-	4
n =																
-reduced in size		0		0		0		0		0		1		0		1
Prostate	-	4	-	4	-	4	-	4	-	4	-	4	-	4	-	4
n =																
-reduced in size		0		1		0		1		0		1		0		0
Thymus	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
n =																
-reduced in size	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
Ovaries	4	-	4	-	4	-	4	-	4	-	4	-	4	-	4	-
n =																
-reduced in size	0		0		0		1		2		2		1		1	

- **Histopathology:** Target organs of toxicity were the testes, prostate, and thymus. Testicular degeneration was observed at 125 mg/kg B.I.D. (very slight), 250 mg/kg B.I.D. (moderate to marked), 500 mg/kg B.I.D. (very slight to marked), and 750 mg/kg B.I.D. (very slight to marked). In severe cases of testicular degeneration, the seminiferous tubules were lined by only Sertoli cells with an occasional immature germination cell in the lumen of the tubule or nestled within the Sertoli cells. With less severe changes, the normal developmental stages of the germinative cells lining the tubules were disturbed to varying degrees (i.e., normal numbers of mature forms were absent) with immature germ cells and/or multinucleated cells sometimes situated in the lumina of the tubules. An increased incidence

of prostatic atrophy was observed for male dogs at 125, 250, and 500 mg/kg B.I.D. The influence of ages of the dogs (i.e., 31-35 weeks at the start of treatment) on these findings in testes and prostate is unknown. An increased incidence of thymic atrophy was observed for dogs at doses ≥ 125 mg/kg B.I.D., although, a dose-response relationship was not evident. Cortices of the thymi that were atrophic were decreased in overall substance, although, in some cases, both the cortices and the medulla appeared to be decreased in size. A decreased number of ovarian follicles were observed for 1 female dog at 750 mg/kg B.I.D. Studies with MK-0869 Formulation M of 3, 6, and 12 months in durations with beagle dogs revealed no target organs of toxicity.

Histopathological changes for dogs that received MK-0869 Formulation NB at oral doses of 5, 25, 125, 250, 500, and 750 mg/kg B.I.D. There were two control groups, one group received deionized water and the other group received the vehicle.

Tissue	Water		Vehicle		5		25		125		250		500		750	
	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M
Testes n = -degeneration	-	4 0	-	4 0	-	4 0	-	4 0	-	4 2	-	4 4	-	4 4	-	4 4
Prostate n = -atrophy	-	4 0	-	4 1	-	4 0	-	4 0	-	4 3	-	4 4	-	4 4	-	4 4
Thymus n = -atrophy	4 0	4 1	4 1	4 0	4 0	4 1	4 0	4 1	4 3	4 3	4 1	4 1	4 3	4 3	4 3	4 3
Ovary n = -ovarian follicle, decreased number	4 0	-	4 0	-	4 0	-	4 0	-	4 0	-	4 0	-	4 0	-	4 1	-

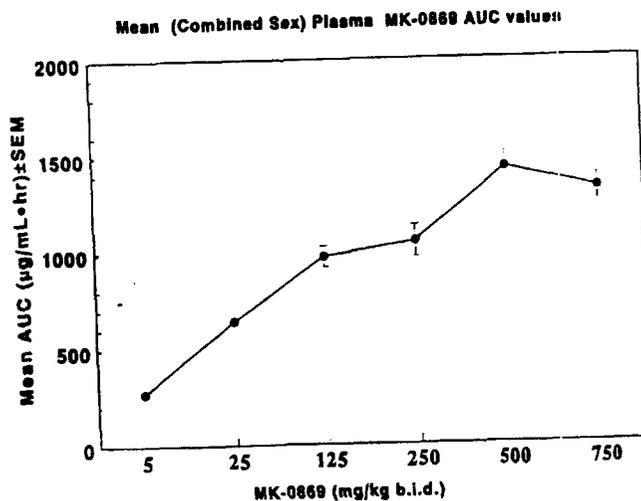
- **Toxicokinetics:** Plasma AUC values for MK-0869 increased with elevating doses at ≤ 500 mg/kg B.I.D.; however, observed increases were significantly less than proportional to dose. At plateau in AUC values for MK-0869 was evident for doses ≥ 500 mg/kg B.I.D. AUC values for the 12 metabolites of MK-0869 increased with elevating doses; however, increases were significantly less than proportional to dose. Plateaus in systemic exposure to 10 of these metabolites were generally evident at 250 or 500 mg/kg B.I.D. (i.e., L-294569, L-596064, L-858442, L-858443, L-829617, L-829615, L-809771, L-825678, L-755446, L-809861, L-294569, L-596064, L-770787, L-858442, L-858443, L-829617, L-829615, L-809771, L-829674, L-825678, L-755446, and L-809861). Slight increases in exposure were still evident for L-770787 and L-829674. In general, a plateau in systemic exposure to MK-0869 and its metabolites appeared to be evident at doses ≥ 500 mg/kg B.I.D.

Plasma MK-0869 Toxicokinetic Parameters - Drug Week 4

Parameters	MK-0869 (mg/kg B.I.D.)					
	Female (n=4/group)					
	5	25	125	250	500	750
AUC _{0-24 hr} (µg·hr/mL)	281 ± 21.2	653 ± 57.9	1060 ± 54.3	1200 ± 86.8	1240 ± 53.1	1440 ± 52.8
C _{max} (µg/mL)	13.8 ± 1.21	30.6 ± 2.58	50.0 ± 2.54	57.0 ± 4.46	59.9 ± 2.73	67.1 ± 3.40
T _{max} (hr)	9.0 ± 1.0	8.0 ± 0	16 ± 0	4.5 ± 1.3	6.5 ± 2.2	9.0 ± 1.9
Parameters	Male (n=4/group)					
	5	25	125	250	500	750
	AUC _{0-24 hr} (µg·hr/mL)	259 ± 28.9	634 ± 40.0	887 ± 75.8	910 ± 100	1620 ± 66.2
C _{max} (µg/mL)	12.3 ± 1.30	29.6 ± 1.93	46.0 ± 6.14	45.3 ± 5.30	77.9 ± 4.23	58.7 ± 4.44
T _{max} (hr)	10 ± 1.2	2.0 ± 0	4.0 ± 1.4	5.0 ± 2.4	5.0 ± 2.4	3.5 ± 1.5
Parameters	MK-0869 (mg/kg B.I.D.) (combined sexes)(n=8/group)					
	5	25	125	250	500	750
	AUC _{0-24 hr} (µg·hr/mL)	270 ± 17.1	643 ± 32.8	973 ± 54.0	1050 ± 81.8	1430 ± 81.9
C _{max} (µg/mL)	13.1 ± 0.872	30.1 ± 1.51	48.0 ± 3.17	51.1 ± 3.94	68.9 ± 4.13	62.9 ± 3.04
T _{max} (hr)	9.5 ± 0.73	5.0 ± 1.1	10 ± 2.4	4.8 ± 1.3	5.3 ± 1.5	6.3 ± 1.5

Values are the Mean ± Standard Error of the Mean.

Drug Week 4 Mean Plasma AUC (Combined Values From Both Sexes) in Dogs Treated Orally With Repeated B.I.D. Doses of MK-0869



Peak Area Ratio AUCs of MK-0869 Metabolites in Dogs

Females												
MK-0869 (mg/kg b.i.d.)	L-294569	L-596064	L-770787	L-858442	L-858443	L-829617	L-829615	L-809771	L-829674	L-825678	L-755446	L-809861
250	0.723	5.12	2.79	2.05	0.743	14.9	2.24	0.751	0.0224	3.76	7.70	0.383
500	0.761	5.88	2.92	2.26	0.711	14.8	2.13	0.659	0.0183	3.62	7.27	0.346
750	0.877	6.45	3.91	2.37	0.776	14.8	2.18	0.725	0.0350	3.99	7.46	0.356
Males												
MK-0869 (mg/kg b.i.d.)	L-294569	L-596064	L-770787	L-858442	L-858443	L-829617	L-829615	L-809771	L-829674	L-825678	L-755446	L-809861
250	0.536	5.77	2.24	1.77	0.631	12.9	1.73	0.664	0	3.67	6.36	0.298
500	0.732	6.59	2.84	2.15	0.687	19.7	2.11	0.768	0.0294	4.14	8.26	0.355
750	0.591	6.07	2.47	1.95	0.753	19.9	2.14	0.672	0.0253	3.94	6.33	0.278

Figure 2. MK-0869: Five-Week Oral Toxicity Study in Dogs. TT #99-082-0, -1
Metabolite Exposure as a Function of Dose in Female Dogs

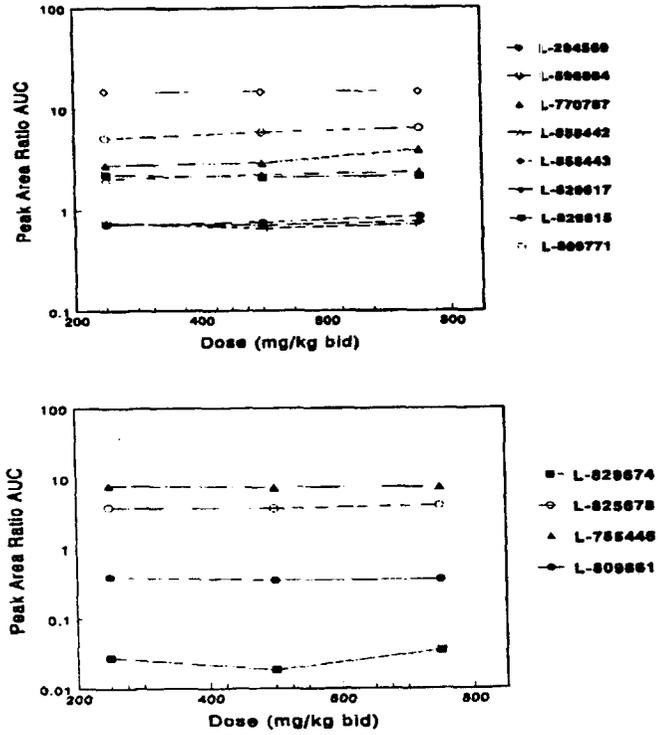
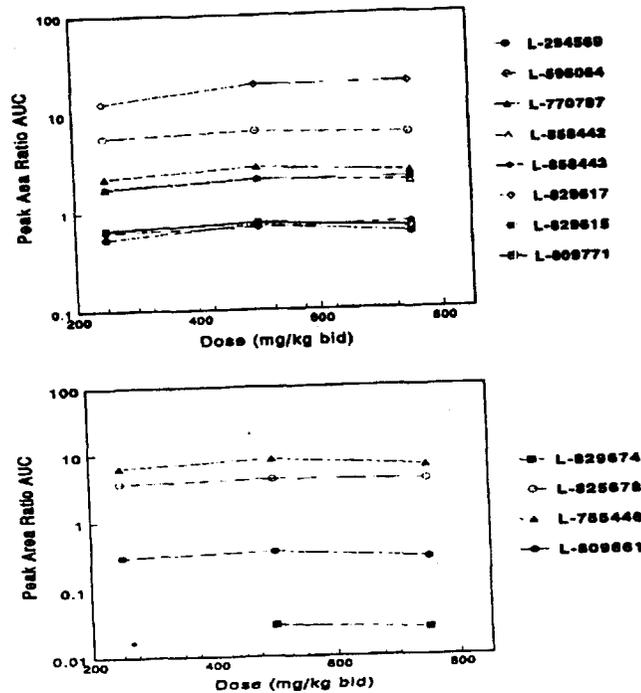


Figure 3. MK-0869: Five-Week Oral Toxicity Study in Dogs. TT #99-032-0, -1

Metabolic Exposure as a Function of Dose in Male Dogs



*=0

Key Study Findings: In a 5-week oral dose range finding study, beagle dog received MK-0869 Formulation NB at doses of 5, 25, 125, 250, 500, and 750 mg/kg B.I.D. (total daily doses of 10, 50, 250, 500, 1000, and 1500 mg/kg/day, respectively). The MK-0869 average particle size in the colloidal dispersion was ———. The treatment duration was 28 or 29 days. This study was intended a dose range finding study for a subsequent 1-year toxicology study with Formulation NB in dogs. Two control groups were included in the present study. The first control group received deionized water. The second control group received the vehicle, 3% hydroxypropyl cellulose, 15% sucrose, and 0.14% sodium lauryl sulfate. The no effect dose was approximately 125 mg/kg B.I.D. Target organs of toxicity were the testes, prostate, and thymus. Testicular degeneration was observed at 125 mg/kg B.I.D., 250 mg/kg B.I.D., 500 mg/kg B.I.D. (very slight to marked), and 750 mg/kg B.I.D. An increased incidence of prostatic atrophy was observed for male dogs at 125, 250, and 500 mg/kg B.I.D. The influence of ages of the dogs (i.e., 31-35 weeks at the start of treatment) on these findings in testes and prostate is unknown. An increased incidence of thymic atrophy was observed for dogs at doses \geq 125 mg/kg B.I.D., although, a dose-response relationship was not evident. A decreased number of ovarian follicles were observed for 1 female dog at 750 mg/kg B.I.D. Studies with MK-0869 Formulation M of 3, 6, and 12 months in durations with beagle dogs revealed no target organs of toxicity. Plasma AUC values for MK-0869 increased with

elevating doses at ≤ 500 mg/kg B.I.D.; however, observed increases were significantly less than proportional to dose. At plateau in AUC values for MK-0869 was evident for doses ≥ 500 mg/kg B.I.D. AUC values for the 12 metabolites of MK-0869 increased with elevating doses; however, increases were significantly less than proportional to dose. Plateaus in systemic exposure to 10 of these metabolites were generally evident at 250 or 500 mg/kg B.I.D. (i.e., L-294569, L-596064, L-858442, L-858443, L-829617, L-829615, L-809771, L-825678, L-755446, L-809861, L-294569, L-596064, L-770787, L-858442, L-858443, L-829617, L-829615, L-809771, L-829674, L-825678, L-755446, and L-809861). Slight increases in exposure were still evident for L-770787 and L-829674. In general, a plateau in systemic exposure to MK-0869 and its metabolites appeared to be evident at doses ≥ 500 mg/kg B.I.D.

Electrocardiographic data from the 5-week oral toxicity study in dogs (TT # 99-082-0, -1)

In a 5-week oral dose range finding study, beagle dogs were treated with MK-0869 at doses of 5, 25, 125, 250, 500, and 750 mg/kg B.I.D. (total daily doses of 10, 50, 250, 500, 1000, and 1500 mg/kg/day, respectively). This study was previously reviewed under IND [redacted] Amendment # 146 dated August 11, 2000 (pharmacologist's review of IND [redacted] Amendment # 146 dated November 8, 2000). Electrocardiograms were recorded from all dogs prior to the start of the treatment and during weeks 2 and 4. During weeks 2 and 4, ECGs were recorded approximately 2 to 4 hr after the second daily dose. ECGs were conducted in right lateral recumbency, and recordings were made from leads I, II, III, aVR, aVL, aVF, CV5RL, and V10. The heart rate and PR, QRS, and QT intervals were measured.

MK-0869 showed no effect on heart rate, PR, QRS and QT interval at the above-mentioned doses at week 2 and week 4 when compared to control or pretest values. In this report, the values of the above-mentioned ECG parameters were presented without any unit (second or millisecond).

Study title: Thirty Nine (39)-Week Oral Toxicity Study in Dogs.

Key study findings: In the 39-week oral toxicity study with MK-0869 in beagle dogs, groups of animals received 0, 5, 25, 125 and 500 mg/kg b.i.d. (0, 10, 50, 250 and 1000 mg/kg/day) doses of the drug. Suppression of body weight gains (15.4% to 69.2%) was observed at all doses. Increased plasma alkaline phosphatase and cholesterol levels were observed in all treatment group animals. Testicular degeneration and prostatic atrophy were observed in males receiving 25 mg/kg b.i.d. (50 mg/kg/day) and higher doses. The target organs of toxicity were the testis and prostate and the no effect dose was not established.

Study no: 00-103-0

Volume #, and page #: Vol #18, page # A6214

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

Date of study initiation: November 08, 2000

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, radiolabel, and % purity: Two batches of MK-0869 were used in the study. Batch #F0869OPP018C004 was prepared by Merck Research Laboratories and Batch #F0869OPP018C004 (also known as L-754030-016S002) and #F0869OPP018C004 (also known as L-754030-016S002) was obtained by blending 2 batches of MK-0869-coated beads.

Formulation/vehicle: Dosing formulations (100 mg/ml) were prepared daily by suspending MK-0869 from the coated beads in deionized water, Further dilutions were made in placebo control vehicle. The average particle size of MK-0869 in the dispersion was approximately

Methods: There were two control groups in the study, and the treatment groups received 5 mg/kg, 25 mg/kg, 125 mg/kg and 500 mg/kg b.i.d. doses of the drug. The daily second dose was administered approximately 6 hours after the first dose.

Dosing:

Species/strain: Beagle dogs.

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

Satellite groups used for toxicokinetics or recovery: None

Age: 28 to 36 weeks old at study initiation.

Weight: Males: 7.9 to 10 kg; Females: 9.3 to 12.4 kg.

Doses in administered units: Treatment group animals received the drug at 5, 25, 125 and 500 mg/kg b.i.d. (10, 50, 250 and 1000 mg/kg/day) doses.

Route, form, volume, and infusion rate: Dosing formulations were prepared by dispersing the drug-coated beads in deionized water and the doses were administered by oral gavage (5 ml/kg b.i.d).

Observations and times:

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

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

Food consumption: Food consumption was measured 4 times a week, except in Weeks 3, 7, 8, 15, 29 and 33 (2-3 time a week).

Ophthalmoscopy: Ophthalmoscopic examinations were performed of all control 1, control 2 and high dose animals during weeks 13, 26 and 37.

Electrocardiography: Electrocardiographic recordings from leads I, II, III, aVR, aVL, aVF, CV₅RL and V10 were made in Weeks 12, 26 and 37 from all dogs in all groups. The heart rate, PR, QRS and QT intervals were also measured.

Hematology: Blood samples for hematological examinations were collected in dosing weeks 4, 12, 25 and 38. The following hematological parameters were determined: erythrocytes, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, aPTT, PT, platelets, leukocytes, leukocytes differential count and cell morphology.

Clinical chemistry: Blood samples for clinical chemistry analyses were collected prior to initiation of dosing and in Drug Weeks 4, 12, 25 and 38.

Urinalysis: Urinalysis was performed on urine samples collected from all dogs in Dosing Weeks 12, 25 and 38.

Gross pathology: Complete necropsies of all animals were conducted at termination.

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

Histopathology: The following tissues from the control 2, high dose group animals were examined histologically.

Salivary gland, esophagus, stomach, small intestine (duodenum, jejunum, ileum), large intestine, liver, gall bladder, pancreas, adrenals, pituitary, thyroid, kidneys, urinary bladder, ovaries, uterus, testes and epididymides, prostate, skin, mammary gland, lung, heart, spleen, lymph nodes (mesenteric and retropharyngeal), thymus, bone marrow, bone, skeletal muscle, brain, spinal cord, peripheral nerve, eye, optic nerve.

Toxicokinetics: Not conducted.

Results:

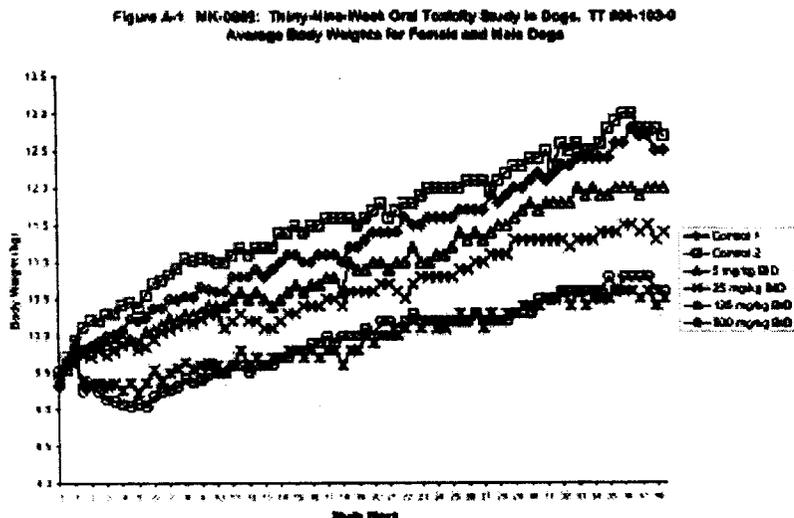
Mortality: There were no deaths in any group.

Clinical signs: White-colored stools, attributed to the presence of drug, were occasionally observed in 1 female and all males receiving the high dose. One male and one female receiving the 500 mg/kg b.i.d. dose had mild alopecia in multiple areas.

Body weights: Decreases in the body weights and body weight gains, as compared to controls, were observed at 125 mg/kg b.i.d. and higher doses. At the 125 mg/kg and 500 mg/kg b.i.d. doses, there were more than 10% decreases in the body weights. There were decreases in the body weight gains (15.4% to 69.2%) at all doses. The changes in body weight and body weight gains of the control and treatment group dogs before initiation of treatment and treatment weeks 1, 7, 21 and 38 are shown in the Table below.

Weeks	Control 1	Control 2	5 mg/kg b.i.d.	25 mg/kg b.i.d.	125 mg/kg b.i.d.	500 mg/kg b.i.d.
Week - 1						
Body Weight (kg)	9.8	9.9	9.8	9.9	9.6	9.7
% of Control	100.0%	101.0%	100.0%	101.0%	97.9%	99.0%
Week 1						
Body Weight (kg)	9.8	10.1	9.8	9.7	9.4	9.2
% of Control	100.0%	103.1%	100.0%	99.0%	95.9%	93.9%
Week 7						
Body Weight (kg)	10.5	10.8	10.2	10.1	9.5	9.3
% of Control	100.0%	102.8%	97.1%	96.2%	90.5%	88.6%
Week 21						
Body Weight (kg)	11.4	11.6	10.9	10.7	10.0	10.2
% of Control	100.0%	101.8%	95.6%	93.9%	87.7%	89.5%
Week 38						
Body Weight (kg)	12.5	12.7	12.0	11.4	10.5	10.6
% of Control	100.0%	101.6%	96.0%	91.2%	84.0%	84.8%
Body wt. Gain (kg)	2.6	2.8	2.2	1.4	0.8	1.0
% of Control	100.0%	107.6%	84.6%	53.9%	30.8%	38.5%

The body weights (kg) of the control and treatment groups dogs during the entire dosing period are shown in the sponsor's Figure below.



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Food consumption: The mean food consumption of the control animals in Week-1 was 323 ± 53 g/day. There were decreases in the food consumption in animals receiving 25 mg/kg, 125 mg/kg and 500 mg/kg doses during the entire dosing period. The largest decreases in the food consumption was observed in the first week of dosing, and during this time, there were 20%, 32% and 35% decreases in low, mid and high dose groups.

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

Electrocardiography: There were no treatment-related electrocardiographic changes in any group.

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

Clinical chemistry: Animals receiving MK-0869 had decreased albumin to globulin (A/G) ratios as compared with that of controls. The treatment group animals had higher alkaline phosphatase levels and animals receiving the 25 mg/kg b.i.d. and higher doses had higher cholesterol levels. The decrease in A/G ratio and increase in cholesterol levels were not dose-dependent effects. The changes in the clinical chemistry parameters in the MK-0869-treated animals are summarized in the Table below.

Parameter	Treatment Week	Control values	Percent changes from control			
			5 mg/kg	25mg/kg	125mg/kg	500 mg/kg
A/G Ratio	Week-12	1.1 ± 0.1	-9	-9	-9	-18
	Week-25	1.2 ± 0.2	-17	-17	-8	-17
	Week-38	1.4 ± 0.3	-14	-21	-14	-21
Alkaline Phosphatase (U/L)	Week-12	88 ± 35	+32	+64	+78	+119
	Week-25	80 ± 42	+57	+124	+119	+160
	Week-38	58 ± 15	+27	+105	+147	+149

Cholesterol (mg/dL)	Week-12	169 ± 43	-	+29	+30	+31
	Week-25	181 ± 44	-	+43	+37	+43
	Week-38	193 ± 43	-	+39	+35	+32

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

Organ weights: An increase in the liver weight (both absolute and relative) was observed in all treatment group animals. In addition, decreased prostate weights (absolute and relative) were observed in all males receiving the drug. The mean liver and prostate weights (in grams) and the changes in their absolute and relative weights in different treatment groups are summarized in the Table below.

Organs	Control Weights	Percent Changes From Control			
		5 mg/kg b.i.d	25 mg/kg b.i.d	125 mg/kg b.i.d	500 mg/kg b.i.d
Liver (absolute)	270.5 g	+7.6%	+11.4%	+10.0%	+16.0%
Liver (relative)	2.22	+14.7%	+26.2%	+34.2%	+39.6%
Prostate (absolute)	10.84 g	-18.0%	-19.7%	-30.1%	-40.5%
Prostate (relative)	0.09	-16.7%	-16.7%	-16.7%	-33.3%

Gross pathology: No treatment-related gross pathological changes in any group were reported.

Histopathology: Very slight to moderate testicular degeneration and prostatic atrophy were observed in males receiving 25 mg/kg b.i.d (50 mg/kg/day) and higher doses. The most severe cases of testicular degeneration were characterized by vacuolation and the presence of degenerate cells within the lumina, and the least severe cases were characterized primarily by vacuolation. One (of 4) high dose animal had stromal focal hyperplasia of the prostate. No treatment-related histopathological changes were observed in the female dogs. Histopathological changes observed in the testis and prostate of the male animals summarized in the Table below:

Organ	Control 2	5 mg/kg b.i.d.	25 mg/kg b.i.d.	125 mg/kg b.i.d.	500 mg/kg b.i.d.
Testes					
Seminiferous tubule degeneration	0/4	0/4	2/4	1/4	4/4
Prostate					
Atrophy	0/4	0/4	2/4	1/4	4/4
Stromal focal hyperplasia	0/4	0/4	0/4	0/4	1/4

Toxicokinetics: Not conducted.

Summary: In the 39-week oral toxicity study MK-0869 in beagle dogs, groups of animals received 5, 25, 125 and 500 mg/kg b.i.d. (10, 50, 250 and 1000 mg/kg/day) doses of the drug. Suppression of body weight gains (15.4% to 69.2%) was observed at all doses. Increased plasma alkaline phosphatase and cholesterol levels were observed in all treatment group animals. Testicular degeneration and prostatic atrophy were observed in males receiving 25 mg/kg b.i.d. (50 mg/kg/day) and higher doses. The target organs of toxicity were the testis and prostate, and the no effect dose was not established.

53-Week Oral Toxicity Study in Dogs with a 27-Week Interim Necropsy (TT #97-614-0).

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

Date Started: August 4, 1997

Date Completed: October 29, 1998

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

Animals: Beagle dogs were obtained from _____ At the start of treatment, animals were 31 to 35 weeks of age and had a body weight range of 7.0 to 9.6 kg for female dogs and 7.9 to 11.9 kg for male dogs.

Drug Batch: L-754,030-004H, Lot #21 (— drug particle size)

Methods: Beagle dogs received L-754,030 (— drug particle size) by the oral route of administration at doses of 0, 4, 16, or 32 mg/kg/day for periods of 27 or 53 weeks. Animals in the control group received the vehicle, 0.5% methylcellulose/0.2% sodium lauryl sulfate in water. There were 8 dogs/sex/group at the start of the study. Drug was administered by oral gavage with a pliable gavage tube using a dose volume of 5 mL/kg. Animals were given physical examinations on a daily basis. Body weights were measured on a weekly basis. All dogs were fed approximately 350 g diet per day except numbers 97-0061, 97-0074, 97-0090, and 97-0086 which were given 450 g diet per day from weeks 9, 11, 26, and 34 onwards, respectively, when their body weights had reached -10% of pretest values. Food consumption was measured on a weekly basis up to week 13 (except during weeks 4 and 12 due to fasting before scheduled bleedings), and then every 4 weeks thereafter, based on a 3 to 4 day consumption period. Animals given an increased ration of diet were excluded from calculations of food consumption. Ophthalmic examinations were performed during drug weeks 12, 26, 38, and 51. Blood for determination of hematological and serum biochemistry parameters was collected during weeks 4, 12, 24, 39, and 52. Urine for determination of urinalysis parameters was collected during weeks 12, 24, 39, and 52. Blood for determinations of plasma drug levels was collected on day 1 and during weeks 13, 26, and 48 at 2, 4, 6, 8, 12, 16, and 24 after dosing. Electrocardiograms were recorded from all animals prior to the start of treatment and during weeks 11, 25, 38, and 52. Recording were obtained from leads I, II, III, aVL, aVR, aVF, CV₅RL, and V₁₀. Heart rate and PR, QRS, and QT intervals were measured from tracings. During weeks 27 (182-184 doses) and 53 (354-366 doses), 4 dogs/sex/group were sacrificed and submitted to complete necropsy. Absolute and relative organ weights were determined for the brain, pituitary, spleen, heart, kidneys, liver, adrenal glands, thyroid glands, testes/ovaries, and prostate. For each necropsy, samples of most tissues and all gross changes for each dog were preserved. Organs and tissues from the control and 32 mg/kg/day groups were processed, stained with hematoxylin and eosin, and submitted to microscopic examination as follows: salivary gland, esophagus, stomach, small intestine, large intestine, liver, gallbladder, pancreas, adrenal glands, thyroid glands, parathyroids, pituitary glands, kidneys, urinary bladder, ovaries/testes (to include epididymides), uterus/prostate, skin, mammary gland, lung, heart,

spleen, lymph nodes, thymus, skeletal muscle, bone, bone marrow, brain (to include cerebral cortex, and subcortical white matter, thalamus and hypothalamus, mid-brain, cerebellum and pons, and medulla), cervical spinal cord, nerve (sciatic), eye (to include optic nerve). Bone marrow smears from animals in the control and 32 mg/kg/day groups were prepared and stained with May-Grunwald-Giemsa stain were also examined. Data for male and female dogs was combined.

Results:

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

2. Mortality: None.

3. Body Weight and Food Consumption: Final body weights for dogs at 16 and 32 mg/kg/day during week 52 were suppressed >10%. Body weights of controls at pretest, week 26, week 27, and week 52 were 9.2 (n=8), 10.7 (n=8), 10.5 (n=4), and 12.3 (n=4) kg, respectively. Body weight gains, expressed as a percentage of pretest body weight, for dogs at 4, 16, and 32 mg/kg/day from weeks 0 to 26 were 16.3, 12, 10, and 6.6%, respectively, as compared to 16.3% for the control. Final body weights of dogs at 4, 16, and 32 mg/kg/day during week 26 were 96.3, 92.5, and 90.7% of the control, respectively. Body weight gains, expressed as a percentage of pretest body weight, for dogs at 4, 16, and 32 mg/kg/day from weeks 27 to 52 were 6.5, 11.2, and 12.5%, respectively, as compared to 17.1% for the control. Body weight gains, expressed as a percentage of pretest body weight, for dogs at 4, 16, and 32 mg/kg/day from weeks 0 to 52 were 23.9, 21.1, and 18.7%, respectively, as compared to 33.7% for the control. Final body weights of dogs at 4, 16, and 32 mg/kg/day during week 52 were 92.7, 88.6, and 87.8% of the control, respectively.

4. Hematology: No treatment-related changes.

5. Blood Biochemistry and Urinalysis: There were slight changes of the serum biochemical parameters, albumin, triglycerides, and alkaline phosphatase, although, their biological significance was questionable.

Week 4: Alkaline phosphatase activities of treatment groups were increased to 107.6-114.5% of the control (145 U/L).

Week 12: Albumin levels of treatment groups were decreased to 91.7-94.4% of the control (3.6 g/100 mL). Alkaline phosphatase activities of treatment groups were increased to 113.3-149.5% of the control (105 U/L).

Week 24: Albumin levels at 16 and 32 mg/kg/day were both decreased to 91.4% of the control (3.5 g/100 mL). Triglyceride levels of treatment groups were decreased to 87.5-89.6% of the control (48 mg/100 mL), respectively. Alkaline phosphatase activities of treatment groups were increased to 126.5-173.5% of the control (83 U/L). Urinary leukocytes content (400x) at 16 (0-5, 8; 6-10, 2; 11-30, 3; and >30, 3) and 32 (0-5, 10; 11-30, 2; and >30, 4) mg/kg/day were increased as compared to the control (0-5, 11; 6-10, 2; 11-30, 2; and >30, 1).

Week 39: Albumin levels of treatment groups were decreased to 91.2-97% of the control (3.4 g/100 mL), respectively.

Week 52: Albumin levels of treatment groups were decreased to 89.2-91.9% of the control (3.7 g/100mL). Urinary volume of treatment groups were increased to 119.5-128.3% of the control (159 mL).

6. Ophthalmic and Electrocardiographic Examinations: The sponsor reported that there were no treatment-related ophthalmic or electrocardiographic changes, although, no data was provided.

7. Organ Weights: Observed organ weight changes did not correlate with any histopathological changes.

Interim Sacrifice (Week 27): Changes in liver, ovary, and prostate weights were observed; however, there were no corresponding histopathological changes.

Liver: Absolute liver weights at 4, 16, and 32 mg/kg/day were increased to 112.6, 130.8, and 118.1% of the control (235.62 g), respectively. Relative liver at 4, 16, and 32 mg/kg/day were increased to 121.3, 141.3, and 132.9% of the control (2.25%), respectively.

Prostate: Absolute prostate weights at 4, 16, and 32 mg/kg/day were decreased to 70.6, 58.1, and 58.7% of the control (10.56 g), respectively. Relative prostate weights at 4, 16, and 32 mg/kg/day were decreased to 72.3, 63.8, and 61.7% of the control (0.094%), respectively.

Ovaries: Absolute ovary weights at 4, 16, and 32 mg/kg/day were increased to 110.5, 108.1, and 115% of the control (0.86 g), respectively. Relative ovary weights at 4, 16, and 32 mg/kg/day were increased to 122.2, 111.1, and 133.3% of the control (0.009%), respectively.

Final Sacrifice (Week 53): Changes in liver, ovary, and heart weights were observed; however, there were no corresponding histopathological changes.

Liver: Absolute liver weights at 4, 16, and 32 mg/kg/day were increased to 115.2, 114.5, and 116.9% of the control (254.93 g), respectively. Relative liver weights at 4, 16, and 32 mg/kg/day were increased to 126.1, 131.4, and 135.3% of the control (2.07%), respectively.

Ovaries: Absolute and relative ovary weights at 32 mg/kg/day were increased to 132.3 and 187.5% of the control (0.99 g and 0.008% BW), respectively.

Heart: Relative heart weights at 4, 16, and 32 mg/kg/day were increased to 114.7, 117.7, and 125% of the control (0.68% BW), respectively.

8. Gross Pathology: There were no treatment-related gross pathological findings at either the interim or final sacrifices.

9. Histopathology: There were no treatment-related histopathological findings at either the interim or final sacrifices.

10. Plasma Drug Levels: Plasma drug levels were not reported in the present study report.

Beagle dogs received L-754,030 (— drug particle size) by the oral route of administration at doses of 0, 4, 16, or 32 mg/kg/day for 27 or 53 weeks. The no effect dose was 32 mg/kg/day. A target organ of toxicity was not identified. Selection of doses in this study appears to be inadequate as no limiting toxicity was observed. Due to lack of toxicity of toxicity demonstrated in the present study, the sponsor apparently initiated one or more dose range finding studies using the — drug particle size to assist in dose selection for a second 1-year study in dogs. In a review of Amendment #099 (Document Room Date: August 2, 1999), it was communicated to the sponsor that any further toxicology studies with — drug particle size would appear to have little or no value given the change in drug particle size for the clinical formulation. Further, it was recommended to the sponsor that if they plan to conduct a 1-year toxicology study in beagle dogs, use of the — drug particle

size would have the greatest value in characterizing the potential toxicity of this compound. Based on these communications, the sponsor reported in an informational amendment #115 submitted on January 7, 2000 that they have postponed initiation of a second 1-year toxicology study in dogs pending results from a 5-week oral toxicity/toxicokinetic study in dogs using the — drug particle size formulation.

Monkey

MK-0869: 17-Day Intravenous Toxicity Study in Monkeys (TT #98-162-0).

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

Date Started: December 30, 1998

Date Completed: June 11, 1999

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

Animals: Rhesus monkeys were obtained from [REDACTED] At the start of treatment, animals were approximately 1 to 3 years of age and had body weight ranges of 2.5 to 3.8 kg for male monkeys and 3.0 to 4.0 kg for female monkeys.

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

Methods: In a 17-day intravenous toxicity study, monkeys received MK-0869 (— drug particle size) at doses of 0, 80, 160, and 240 $\mu\text{g}/\text{kg}/\text{day}$. There were 4 monkeys/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 $\mu\text{g}/\text{mL}$. MK-0869 at 20 $\mu\text{g}/\text{mL}$ was the maximum feasible concentration in this vehicle solution. The intravenous dosing volumes for the 0, 80, 160, and 240 $\mu\text{g}/\text{kg}/\text{day}$ 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. Animals were observed for clinical signs of toxicity and mortality on a daily basis. Body weight was measured once prior to the start of treatment and on a weekly basis during the treatment period. Food consumption was estimated 5 days/week for all animals. Ophthalmic examinations were conducted in all animals prior to the start of treatment and during week 2. Blood for determination of hematology and serum biochemistry parameters was collected during week 2. Urine samples were collected overnight from all animals prior to the start of treatment and during week 2. At scheduled termination, animals were sacrificed and submitted to a complete necropsy. The absolute and relative organ weights of the adrenal glands, brain, heart, ovaries, kidneys, liver, pituitary, prostate, spleen, testes, and thyroid gland. The testes and epididymides from all male

monkeys were fixed in Bouin's solution. All remaining tissues from all animals were fixed in 10% neutral buffered formalin. Tissue sections from the control and 320 µg/kg/day groups were prepared using standard techniques, stained with hematoxylin and eosin, and submitted to microscopic examination as follows: salivary gland, esophagus, stomach, small intestine (duodenum, jejunum, and ileum), liver, pancreas, 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), adrenal glands, parathyroids, pituitary, thyroid gland, kidneys, urinary bladder, ovaries, uterus, testes and epididymides, prostate, bone (rib), skeletal muscle, brain (including cerebral cortex and subcortical white matter, cerebellum, and pons), spinal cord (cervical), peripheral nerve (sciatic), optic nerve, eye, injection sites, and gall bladder. All gross abnormalities were processed, stained, and submitted to microscopic examination. Data for male and female monkeys was combined by the sponsor.

Results:

1. **Observed Effects:** Red discoloration on the back of the legs near injection sites was observed in both control and treatment groups.

2. **Mortality:** There was no treatment-related mortality.

3. **Body Weight and Food Consumption:** There were no treatment-related effects on body weight gain or food consumption. Mean body weights of controls at weeks -1 and 3 were identical at 3.2 kg. Thus, there was no change in body weight for controls during the treatment period. Body weight gain, expressed as a percentage of body weight at week -1, during the treatment period for animals at 80, 160, and 240 µg/kg/day was 2.9, 3.1, and 3.1%, respectively.

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

5. **Serum Biochemistry and Urinalysis:** Increases of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities as well as total cholesterol levels were observed primarily at 240 µg/kg/day; however, these changes appeared to have little or no biological significance as there were no corresponding histopathological changes in the liver. AST activity for animals at 240 µg/kg/day was increased to 116.7% (range of 21 to 69 U/L with 1 animal at 69 U/L) of the control (mean = 30 U/L with a range of 23 to 39 U/L). ALT activity for animals at 240 µg/kg/day was increased to 123.3% (range of 29 to 55 U/L with 1 animal each at 53 and 55 U/L) of the control (mean = 30 U/L with a range of 26 to 36 U/L). Total cholesterol levels for animals at 160 and 240 µg/kg/day were both increased to 111.5% of the control (156 mg/dL). There were no treatment-related changes of urinalysis parameters.

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

7. **Organ Weights:** Changes in organ weights were observed for adrenal glands, pituitary gland, thyroid gland, prostate, and ovaries; however, with the possible exception of the adrenal glands, there were no corresponding histopathological changes.

Adrenal gland: Absolute adrenal gland weights at 80, 160, and 240 µg/kg/day were decreased to 90.9, 84.4, and 77.9% of the control (0.77 g), respectively. Relative adrenal gland weights at 80,

160, and 240 µg/kg/day were decreased to 84, 83.7, and 78.8% of the control (0.0245%B.W.), respectively.

Pituitary gland: Absolute and relative pituitary gland weights at 240 µg/kg/day were decreased to 84.5 and 88.9% of control values (0.0581 g and 0.18% B.W.), respectively.

Thyroid gland: Absolute and relative thyroid gland weights for MK-0869 treatment groups were decreased to 84.7-88.1% and 78.4-84.7% of control values (0.59 g and 0.0190% B.W.), respectively.

Prostate: Absolute and relative prostate weights for MK-0869 treatment groups were increased to 133.3-157% and 131.8-163.6% of control values (0.21 g and 0.22% Br.W.), respectively.

Ovaries: Relative and absolute ovary weights at 160 and 240 µg/kg/day were decreased to 69.6-73.9% and 75.4-79.7% of control values (0.23 g and 0.0069% B.W.), respectively.

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

9. Histopathology: For the small intestine, hemosiderosis was observed for 1 of 4 male monkeys at 240 µg/kg/day. For the adrenal gland, 1 of 4 male monkeys at 240 µg/kg/day was observed with a cyst in the zona fasciculata and 1 of 4 female monkeys at 240 µg/kg/day was observed with nodular hyperplasia in the zona glomerulosa.

In a 17-day intravenous toxicity study, rhesus monkeys received MK-0869 (→ drug particle size) at doses of 0, 80, 160, and 240 µg/kg/day. The no effect dose was 240 µg/kg/day. Solubility of MK-0869 in the vehicle limited the amount of drug that could be administered by the intravenous route. Therefore, doses used appeared to be inadequate to assess the toxicity of MK-0869 when administered by the intravenous route. A target organ of toxicity was not identified. For the adrenal gland, 1 of 4 female monkeys at 240 µg/kg/day was observed with nodular hyperplasia in the zona glomerulosa.

Study title: 5-Week intravenous toxicity study in monkeys (TT#97-605-0)

Study no: TT#97-605-0

Conducting laboratory and location: Merck Sharp & Dohme-Chibret,
Center de Recherche, France

Date of study initiation: February 4, 1997

Date of study report: July 22, 1997

GLP compliance: A statement of GLP compliance was included.

QA report: yes (X) no ()

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

Formulation/vehicle: Tween-sodium citrate diluent (TSCD), pH 7.5. The composition (per ml) of the vehicle is as follows: Polysorbate 80 (Tween 80)-5.0 mg; D-lactose-10.0 mg; mannitol-10.0 mg; meglumine-1.24 mg; sodium citrate (dihydrate)-5.88 mg; citric acid (anhydrous)-0.38 mg; sodium chloride-9.0 mg.

Methods:

Dosing:

Species/strain: Rhesus monkeys (*Macaca mulatta*)

#/sex/group or time point (main study): 4/sex/group

Satellite groups used for toxicokinetics or recovery: None

Age: Approximately 2 years old

Weight: Males: 2.7 kg to 3.9 kg; Females: 2.2 kg to 3.2 kg

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

Route, form, volume, and infusion rate: Intravenous via saphenous veins, 12 ml/min.

The following table presents the study design:

Group No.	Treatment	Dose (mg/kg/day)	Concentration (mg/ml)	Dose Volume (ml/kg)	No. of Males	No. of Females
1	Saline	0	0	5	4	4
2	Vehicle	0	0	5	4	4
3	L758, 298	2	0.4	5	4	4
4	L758, 298	5	1	5	4	4
5	L758, 298	10	2	5	4	4

Observations and times:

Clinical signs: Daily

Body weights: Weekly

Food consumption: Daily

Ophthalmoscopy: During week 4

Hematology: Pretest and Week 2 and 4

Clinical chemistry: Pretest and Week 2 and 4

Urinalysis: Pretest and Week 2 and 4

Gross pathology: At necropsy

Organs weighed: At necropsy. The following organs were weighed: adrenals, brain, heart, kidneys, liver, ovaries, pituitary, prostate, spleen, testes (with epididymides) and thyroids.

Histopathology: At the scheduled necropsies. The following organs and tissues were used for histopathology: salivary gland, esophagus, stomach, small intestine, large intestine, liver, gallbladder, pancreas, adrenal, thyroid, parathyroid (when present in thyroidal sections), pituitary, kidney, urinary bladder, ovary/testis (to include epididymis), uterus/prostate, skin from mammary region, mammary gland (when present in skin section), lung, heart, spleen, lymph node, thymus, skeletal muscle, bone, bone marrow, brain (to include cerebral cortex and subcortical white matter, thalamus and hypothalamus, mid-brain, cerebellum and pons, and medulla), cervical spinal cord, nerve (sciatic), eye, optic nerve and injection sites.

Toxicokinetics: Blood samples were taken at 4 and 8 minutes, 1, 2, 5, 7, 10 and 24 hours after dosing for toxicokinetic analysis.

Results:

1. **Clinical Signs:** No treatment-related clinical signs were observed.
2. **Mortality:** None
3. **Body Weight:** The mean initial and final body weights of control 2 (vehicle-treated males and females) were 2.8 kg and 3.0 kg, respectively. No treatment-related changes were observed.
4. **Food Consumption:** No quantitative data available. However, the sponsor mentioned that there were no treatment-related changes.
5. **Hematology:** There were no significant treatment-related findings.
6. **Blood Chemistry:** No treatment-related changes were observed.
7. **Urinalysis:** There were no drug-related urinary changes.
8. **Ophthalmology:** No treatment-related ocular changes were observed.
9. **Organ Weights:** No treatment-related changes were observed.
10. **Gross Pathology:** No drug-related changes were observed.

11. **Histopathology:** There were no significant treatment-related findings.
12. **Toxicokinetics:** The sponsor stated that the results of the toxicokinetic analysis would be submitted later.

In 5-week intravenous toxicity study in monkeys, animals were administered L-758, 298 at 2, 5 and 10 mg/kg/day. The NOEL was considered as 10 mg/kg/day. The tested doses did not allow the identification of any target organ of toxicity.

Toxicology Summary:

Acute toxicity studies with L-758, 298 (a prodrug of MK-0869) were conducted in mice and rats after i.v. administration of 200 and 500 mg/kg doses and oral administration of a 500 mg/kg dose. The minimal lethal dose (MLD) by the i.v. route was 500 mg/kg in both mice and rats. There were no deaths of mice or rats receiving the 500 mg/kg oral dose; thus, the MLD by the oral route was not known. The clinical signs observed in mice after i.v. dosing included gasping, convulsions, bradypnea and loss of righting reflex that disappeared within 3 hours. In rats, the clinical signs included gasping and bradypnea.

Subacute/subchronic/chronic toxicity studies with MK-0869 were conducted in rats, mice and dogs. In a 5-week oral dose range-finding study in rats, the target organs of toxicity were identified as the liver (hepatocellular hypertrophy), thyroid gland (follicular cell hyperplasia) and the pituitary gland (vacuolation), and the no effect dose was not established. In a 5-week oral toxicity study with the particle-size formulation (Formulation NB) in rats, the target organs of toxicity were also identified as the liver and the thyroid gland. A plateau of plasma drug level was observed in both males and females receiving b.i.d doses of formulation NB. In a 14-week study with MK-0869 in rats, the target organs of toxicity were the liver, thyroid gland and the pituitary gland, and the no effect dose was not established. Similarly, in the 27-week and 53-week oral toxicity studies in rats, the target organs of toxicity were also the liver and the thyroid gland. In the 5-week oral dose range-finding study in mice, 0, 500 and 1000 mg/kg b.i.d doses were used. The no effect dose was identified as 1000 mg/kg/day (500 mg/kg b.i.d), and a target organ of toxicity was not identified. In the 5-week oral toxicity study with MK-0869 in mice, groups of animals received formulation M (particle size) at 0 and 500 mg/kg/day and formulation NB at 0, 25, 500, 1000, 1250 and 1500 mg/kg/day S.I.D, and 0, 12.5, 250, 500, 625 and 750 mg/kg b.i.d doses. Centrilobular hypertrophy of the liver was observed in both males and females receiving the drug. The no effect dose in males was not established, and in females, it was 25 mg/kg/day. Plasma exposure levels of the parent drug after administration of formulation NB was ≤ 2 times higher than that achieved with formulation M. Centrilobular hepatocellular hypertrophy was observed in both male and female mice, and hydropic degeneration of tubules of the kidney was observed in female mice during 14-week oral administration of MK-0869.

In a 5-week oral dose range finding study in dogs, groups of animals received 0, 5, 25, 125, 250, 500 and 750 mg/kg b.i.d doses of formulation NB (particle size). A plateau in the plasma exposure levels was observed at the 500 mg/kg b.i.d dose. The no effect dose was approximately 125 mg/kg b.i.d, and the target organs of toxicity were the testes (testicular degeneration), prostate (atrophy) and the thymus (atrophy). In a 5-week i.v. toxicity study with L-758, 298 in dogs, the no effect dose was 2 mg/kg/day, and no target organs of toxicity were identified. In a 39-week oral toxicity study with Formulation NB (particle size) in dogs, 0, 5, 25, 125 and 500 mg/kg b.i.d

(0, 10, 50, 250 and 1000 mg/kg/day) doses were used. Similar to the 5-week study, the target organs of toxicity were the testes (tubular degeneration) and prostate (atrophy) in this study. In a 53-week oral toxicity study, with L-754, 030 in dogs, with a 27-week interim sacrifice, the no effect dose was 32 mg/kg/day and no target organs of toxicity were identified. In monkeys, i.v. dosing of L-758, 298 for up to 240 mg/kg/day for 17 days, and up to 10 mg/kg/day for 5 weeks was not associated with any adverse effects, and no target organs of toxicity were identified.

Toxicology conclusions:

The sponsor has conducted acute, subacute/subchronic and chronic toxicity studies with MK-0869 (or its pro-drug, L-758, 298) in different animal species. In oral acute toxicity studies in rats and mice, the minimal lethal dose was 500 mg/kg. In the subacute/subchronic and chronic toxicity studies in rats, the target organs of toxicity were the pituitary (vacuolation), liver and the thyroid gland. Hepatocellular hypertrophy and thyroid follicular cell hyperplasia were observed in both males and females in the 5-week, 14-week, 27-week and 52-week oral toxicity studies in rats. In a 5-week oral toxicity study with MK-0869 in rats, benign parafollicular cell adenoma was observed in two animals receiving the drug (one each at 125 mg/kg/day and 250 mg/kg/day doses). Hepatocellular hypertrophy was also observed in the 5-week and 14-week oral toxicity studies in mice. The changes in the thyroid gland and the liver may be related to the induction of CYP 450 enzymes in the liver. In dogs, tubular degeneration of the testes and prostatic atrophy were observed at high doses (<125 mg/kg/day in the 5-week toxicity study and >10 mg/kg/day in the 39-week toxicity study. In the 5-week i.v. toxicity study in the monkey, no target organ of toxicity was identified at doses up to 10 mg/kg/day. Thus, from the results of the toxicology studies with MK-0869, it appears to have low toxicity profiles in the rodent and non-rodent animals.

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V. GENETIC TOXICOLOGY:**1. Ames test in *Salmonella typhimurium* and *Escherichia coli*
(Study TT #95-8018 and TT #95-8019)**

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

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

Date Study Started: March 15, 1995

Date Study Completed: May 11, 1995

Bacterial strains:

S. typhimurium: TA1535, TA97a, TA98 and TA100
E. coli: WP2, WP2 uvrA and WP2 uvrA pKM101

Methods: 0.5 ml of the S-9 metabolic activation mixture or buffered saline was added to the various concentrations of L-758,298 or controls, followed by the addition of 2 ml of soft agar containing 0.1 ml of the bacterial suspensions.

L-758,298 doses of 30, 100, 300, 1,000, 3,000 and 10,000 µg/plate were studied. The highest dose of L-758,298 studied (10,000 µg/plate) produced precipitation, inhibition of background lawn and/or inhibition of revertants; thus, the sponsor suggests that the study is valid.

Plates were incubated at 37° C for 48 hrs. Revertant colonies were counted. The experiment was done in triplicate. Revertant colony counts were averaged.

Positive controls were 2-aminoanthracene (2 and 5 µg/plate or 10 and 15 µg/plate) for all *S. typhimurium* strains and *E. coli* strains WP2 uvrA and Wp2 uvrA pKM10s with and without S-9 metabolic activation, and hydrazine sulfate (500 and 1000 µg/plate) for *E. coli* strain WP2 with S-9 metabolic activation.

Diagnostic mutagens were sodium azide (1.5 µg/plate), methyl methanesulfonate (2 µg/plate), daunomycin (5 µg/plate) and ICR-191 (5 µg/plate) for *S. typhimurium* strains, and methyl methanesulfonate (2 µg/plate) for *E. coli* strains.

Solvents for positive controls and diagnostic mutagens were DMSO or water.

Criteria for positive mutagenic effect were a two-fold increase in number of revertant colonies compared to solvent negative control and evidence of a dose-related increase revertant colonies.

Results: L-758,298 did not produce any significant increases in number of revertant colony counts. Positive controls and diagnostic mutagens did produce significant increases in number of revertant colony counts. Thus, L-758,298 was not mutagenic in the Ames test.

2. DNA Damage in Rat Hepatocytes (Study TT #95-8413)

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

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

Date Study Started: May 8, 1995

Date Study Completed: August 16, 1995

Methods: Primary hepatocytes were obtained from male Sprague-Dawley rats. 2.2×10^6 cells were added to 11 ml of Leibovitz's medium per plate. Various concentrations of L-758,298, and positive and negative controls were added. Cell cultures were incubated at 37° C in 5% CO₂ in air for 3 hrs.

In a dose-ranging study (TT #95-8412), L-758,298 did not induce significant toxicity in cultured primary rat hepatocytes at concentrations up to and including 67 µM. L-758,298 was insoluble in the culture medium at the two highest concentrations studied (60 and 67 µM). Thus, L-758,298 concentrations of 10, 32, 40, 50, 60 and 67 µM were studied. Negative control was water. Positive control was aflatoxin B₁ (final concentration of 1 µM); vehicle for aflatoxin B₁ was DMSO. For a radiation positive control, untreated cell suspensions were irradiated with 3 Gy of — radiation.

After 3 hr of incubation, cell cultures were harvested by gentle scraping with a cell scraper and suspended in the original medium. Cytotoxicity was estimated by trypan blue exclusion assays and by assessing ATP content.

Induction of DNA strand breaks was determined by the alkaline elution assay. A ml aliquot of the cell suspension was loaded on a filter and lysed. A solution of tetrapropyl ammonium hydroxide (pH 12.1) was added to the DNA held on the filter and elutions were collected. DNA from each of three fractions was trapped on filters and the amount of DNA on each filter was measured as the product of 3,5-diaminobenzoic acid and deoxyribose.

Criteria for defining significant DNA damage were that soluble doses of a compound would produce an elution slope of 0.034 or greater that was not associated with significant cytotoxicity.

Results: L-758,298 did not produce significant cytotoxicity and did not produce elution slopes that were greater than 0.034. The positive control produced an elution slope of 0.111, but did not produce significant cytotoxicity. The radiation positive control produced an elution slope of 0.119. Thus, L-758,298 was not clastogenic in the rat hepatocyte assay.

3. Chromosomal Aberrations in Chinese Hamster Ovary Cells (Study TT #95-8637)

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

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

Date Study Started: March 27, 1995

Date Study Completed: July 14, 1995

Methods: Chinese hamster ovary (CHO) cells were used. 1.2×10^6 cells were seeded in 10 of McCoy's 5A medium supplemented with fetal bovine serum, L-glutamine and penicillin-streptomycin for 24 hr. Various concentrations of L-758,298, and positive and negative controls were added for 3 hrs with and without S-9 metabolic activation. Cell cultures were incubated at 37° C in 5% CO₂.

In a dose range-finding study (TT #95-8636), precipitate was clearly observed at a L-758,298 concentration of 125 μM and above with S-9 metabolic activation and at a concentration of 75 μM above without S-9 metabolic activation. There was no significant

dose-related suppression of growth. Therefore, higher concentrations were limited by solubility. Thus, L-758,298 concentrations of 10, 20, 40, 60, 80, 100 and 125 μM were studied. Positive control with S-9 metabolic activation was cyclophosphamide (2.5 and 5.0 μM). Positive control without S-9 metabolic activation was mitomycin C (0.35 and 0.75 μM). Solvent control was distilled water. Negative control was Leibovitz's L-15 medium.

After 3 hr of treatment, cell cultures were washed, placed in fresh medium and incubated for 17 more hrs. Cells were harvested by trypsinization. Aliquots of cells were counted by a Coulter counter. The remainder of each cell culture was fixed on slides and stained with Giemsa solution. Aberrations were scored microscopically.

Criteria for defining significant DNA damage were that there must be a significant increase in percentages of cells with chromosomal aberrations compared to concurrent controls, with less than 50% cytotoxicity. Statistical significance was determined by Fisher's Exact test.

Results: L-758,298 did not produce significant increases in chromosomal aberrations at concentrations up to 80 μM ; at doses higher than 80 μM , precipitation was observed. Positive controls produced significant increases in chromosomal aberrations at concentrations that did not produce significant cytotoxicity. Thus, L-758,298 was not clastogenic in Chinese hamster ovary cells.

Assay for Micronucleus Induction in Mouse Bone Marrow (Report Date/Number TT #98-8608).

Testing Laboratory: Department of Safety Assessment
Merck Research Laboratories
Merck & Co., Inc.
West Point, PA 19486

Date Started: January 27, 1998

Date Completed: September 16, 1998

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

Animals: CrI:CD-1[®](ICR)BR mice were used in this study. At the start of treatment, animals were approximately 4 weeks old and had a body weight range of 17.9-27.7 g for male mice and 18.2-23.4 g for female mice.

Drug Batch: L-754,030-004H030

Methods: The genotoxic potential of L-754,030 was evaluated in CD-1 mice using the assay for micronucleus induction in bone marrow. Mice received L-754,030 by oral gavage at doses of 0, 125, 250, and 500 mg/kg. Mice in the control group received the vehicle, 0.5% aqueous methylcellulose with 0.02% sodium lauryl sulfate. There were 10 mice/ sex/group at doses of 0, 125, and 250 mg/kg and 7 mice/sex/group at the dose of 500 mg/ kg. The dose volume was 10 mL/kg. Dose selection was based upon 5-week oral dose range finding and toxicokinetic studies in CD-1 mice described above (TT #96-068-0 and TT #97-018-0). These studies suggested that systemic exposure was saturated in both male and female mice at doses ≥ 500 mg/kg/day. Mice in positive control groups received mitomycin C at 0.350 or 2 mg/kg. There were 5 mice/sex/dose in positive control groups. For mice receiving L-754,030 at 0, 125, or 250 mg/kg, 5 animals/sex/group were sacrificed at 24 and 48 hr after dosing and both femurs of each animal were removed for harvesting bone marrow cells. For mice receiving L-754,030 at 500 mg/kg/day, 7 animals/sex/group were sacrificed at 24 and 48 hr after dosing for harvesting bone marrow. Mice in positive control groups were sacrificed at 24 hr after dosing for harvesting bone marrow. Bone marrow cells were placed on slides and stained with acridine orange. Two thousand polychromatic erythrocytes (PCE) were scored per mouse for micronuclei, and the frequencies of PCE and normochromatic erythrocytes (NCE) were determined based on 1000 erythrocytes per animal. The assay would be considered positive if a significant increase in the frequency of micronucleated PCE ($p \leq 0.05$) occurred at a minimum of 2 doses when compared with the concurrent negative control mean: either 2 doses at a given sacrifice time or 1 dose at each of 2 sacrifice times.

Results: There were no statistically significant increases in micronucleated PCE at L-754,030 doses ≤ 500 mg/kg. The positive control group was observed with expected increases in micronucleated PCE.

L-754,030 at oral doses ≤ 500 mg/kg was negative in the assay for micronucleus induction in mouse bone marrow.

Study title: TK6 Human Cell Mutagenesis Assay with L-758, 298.

Key findings: L-758, 298 was not mutagenic in the *in vitro* mammalian cell gene mutation assay using TK6 human lymphoblastoid cells.

Study no: #98-8308

Study type: In vitro mammalian cell gene mutation assay.

Date of study initiation: March 02, 1998

GLP compliance: Yes

QA reports: yes (X) no ()

Drug lot #, radiolabel, and % purity: L 758, 298-004H030, purity 99.8%.

Formulation/vehicle: L-758, 298 was dissolved in distilled water.

Methods: The study was conducted to determine the mutagenicity potential of L-758, 298, measured as resistance to trifluorothymidine (TFT), at the TK locus in TK6 human lymphoblastoid cells.

Strains/species/cell line: TK6 human lymphoblastoid cells.

Dose selection criteria: Dose selection for the TK6 mutagenesis assay was based either on the toxicity or on the solubility of the compound. The selection of high dose was based on the survival of the cells between 10 and 50%. If there was no limit of solubility or toxicity, the high dose selected was 10 mM.

Basis for dose selection: The dose selection for the mutagenesis assay was determined on the basis of a dose range-finding assay (TT #98-8304) in which the relative survival of the cells was determined in the presence or absence of metabolic activation. As this study was not sufficient to determine the appropriate dose ranges, a second dose-ranging study (TT #8305) was conducted

Range finding studies: In the first range-finding study (TT #98-8304), L-758, 298 concentrations of 0.05 mM to 0.24 mM were used both in the absence and presence of metabolic activation. The relative survival in the absence of S-9 ranged from 102% to 73%, and in the presence of S-9, it ranged from 78% to 1%. Since these data were insufficient to determine appropriate dose ranges for the mutation assay, a second dose-ranging study (TT #98-8305) was conducted. In this study, L-758, 298 concentrations of 0.15 mM to 0.30 mM were used in the absence of metabolic activation and 0.10 mM to 0.26 mM concentrations were used in the presence of metabolic activation. The relative survival in the absence of metabolic activation ranged from 88% to 21% and in the presence of metabolic activation, it was 108% to 0%. Based on these findings, 0.100 to 0.300 mM concentrations were used in the absence and 0.100 to 0.230 mM concentrations were used in the presence of metabolic activation.

Test agent stability: The sponsor stated that the stability of MK-0869 in DMSO solution was within acceptable limits (No data provided).

Metabolic activation system: Phenobarbital/ β -naphthoflavone-induced rat liver S-9 fraction was used as the metabolic activation system ( Lot # 0690).

Controls:

Vehicle: The drug was dissolved in distilled water.

Negative controls: Distilled water was used as the negative control.

Positive controls: N-nitroso-N-ethylurea (ENU, 10 μ M) was used as the positive control in the absence of metabolic activation and 3-methylchloroanthrene (3MC, 1 μ M) was used in the presence of metabolic activation. Both agents were dissolved in DMSO.

Exposure conditions:

Incubation and sampling times: The cells were incubated at 37^oC for 3 hours with the test agent, positive and negative controls in the presence or absence of metabolic activation. After termination of treatment, the cells were subcultured during an expression period of 4 days. The plating efficiency was measured by dispensing the diluted subcultures at 1 cell/well/0.2 ml in 96 well titer plates. For measurement of mutation, trifluorothymidine (TFT) was added to a final concentration of 0.4 μ g/ml and an aliquot of this suspension containing an average of 40,000 cells/well, was dispensed into microtiter plates. The plates were removed from the incubator approximately 14 to 18 days after seeding and counted.

Doses used in definitive study: In the absence of S-9, 0.10, 0.20, 0.26 and 0.30 mM, and in the presence of S-9, 0.10, 0.20, 0.215 and 0.230 mM concentrations were used.

Study design: The design of the study and the dose selection are appropriate. The number of cells per treatment group and the cell survival are acceptable. The doses were selected on the basis of a dose-ranging study in which the relative survival of the cells ranged from 88% to 21% in the absence and 108% to 0% in the presence of S-9 mix. The study was considered valid, if there were significant increases in the mutant fraction (MF) in the positive control groups, both in the absence and presence of metabolic activation and there were four analyzable concentrations both in the absence and presence of metabolic activation.

Analysis:

No. of replicates: Triplicate cultures were used at each dose level, in the absence or presence of metabolic activation.

Counting method: The colonies were counted using a Colony Counter.

Criteria for positive results: The assay was considered positive if there was a statistically significant increase (t-test with Dunnett's correction for multiple comparisons) in the mutant fraction (MF) relative to the combined negative control (2 or more concentrations) and at least one of these induced mutant fractions (IMF) was 2.5×10^{-6} or greater.

Summary:

Study validity: The study was valid, as there were significant increases in the mutant fraction (MF) in the positive control groups, both in the absence and presence of metabolic activation and there were four analyzable concentrations both in the absence and presence of metabolic activation.

Study outcome: There were no significant increases in the MF at any concentration either in the presence or absence of metabolic activation. However, there were significant increases in the MF in the positive controls, both in the absence and presence of metabolic activation. Thus, L-758, 298 was not mutagenic in the *in vitro* mammalian cell gene mutation assay using TK6 human lymphoblastoid cells. The mutagenesis assay data is summarized in the sponsor's Table below.

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Table 5. L-758,298: TK6 Human Cell Mutagenesis Assay. TT #98-8308

Summary of Induced Mutant Fraction

Treatment	S-9 Metabolic Activation	Precipitate at Dosing	Mean Mutant Fraction ($\times 10^{-4}$)	Induced Mutant Fraction ($\times 10^{-4}$) ^a	Statistical Significance (p Value)
Negative Control - 1% Water	-	-	3.26		
Positive Control, ethylnitrosourea, 10.0 μ M	-	-	8.53	5.29	<0.0001
Positive Control, 3-methylcholanthrene, 1.0 μ M	+	-	32.75	29.48	<0.0001
L-758,298					
0.100 mM	-	-	4.84	1.58	0.2575
0.200 mM	-	-	4.68	1.41	0.3112
0.260 mM	-	-	4.15	0.89	0.8153
0.300 mM	-	-	2.99	0.00	0.9994
0.400 mM	+	-	3.81	0.55	0.9827
0.200 mM	+	-	4.65	1.39	0.3667
0.215 mM	+	-	4.53	1.27	0.4939
0.230 mM	+	-	4.24	0.97	0.6887

a) Mean mutant fraction of treated minus mean mutant fraction of control (negative numbers are shown as zero).

Study title: TK6 Human Cell Mutagenesis Assay with MK-0869.

Key findings: MK-0869 was not mutagenic in the *in vitro* mammalian cell gene mutation assay using TK6 human lymphoblastoid cells.

Study no: # 98-8309 and # 98-8313

Study type: In vitro mammalian cell gene mutation assay.

Date of study initiation: March 12, 1998

GLP compliance: Yes

QA reports: yes (X) no ()

Drug lot #, radiolabel, and % purity: L 754,030-004H030, purity 99.8%.

Formulation/vehicle: MK-0869 was dissolved in dimethyl sulfoxide (DMSO).

Methods:

Strains/species/cell line: TK6 human lymphoblastoid cells.

Dose selection criteria: Dose selection for TK6 mutagenesis assay was based either on the toxicity or on the solubility of the compound.

Basis for dose selection: The dose selection for the mutagenesis assay was based on a range-finding assay (TT #98-8309), in which the doses were set on the basis of solubility seen in an

exploratory solubility test and the cytotoxicity seen in a previous chromosome aberration assay (TT #8672).

Range finding studies: In the range-finding study TT #98-8309, MK-0869 concentrations 0.01 mM to 0.11 mM were used in the absence of metabolic activation and 0.02 mM to 0.12 mM concentrations were used in the presence of metabolic activation. There was no precipitation of the compound in the presence or absence of metabolic activation. The relative survival in the absence of S-9 ranged from 87% to 15%, and in the presence of S-9, it ranged from 112% to 28%.

Test agent stability: The sponsor stated that the stability of MK-0869 in DMSO solution was within acceptable limits (No data provided).

Metabolic activation system: Phenobarbital/ β -naphthoflavone-induced rat liver S-9 fraction was used as the metabolic activation system (Lot # 0690).

Controls:

Vehicle: The drug was dissolved in dimethyl sulfoxide (DMSO).

Negative controls: DMSO was used as the negative control.

Positive controls: N-nitroso-N-ethylurea (ENU, 10 μ M) was used as the positive control in the absence of metabolic activation and 3-methylchloroanthrene (3MC, 1 μ M) was used in the presence of metabolic activation. Both agents were dissolved in DMSO.

Exposure conditions:

Incubation and sampling times: The cells were incubated at 37°C for 3 hours with the test agent and positive or negative controls in the presence or absence of metabolic activation. After termination of treatment, the cells were subcultured during an expression period of 4 days. The plating efficiency was measured by dispensing the diluted subcultures in 96 well titer plates. For measurement of mutation, trifluorothymidine (TFT) was added to a final concentration of 0.4 μ g/ml and an aliquot of this suspension containing an average of 40,000 cells/well was dispensed into microtiter plates. The plates were removed from the incubator approximately 14 to 18 days after seeding, and counted.

Doses used in definitive study: 0.01, 0.05, 0.09 and 0.11 mM concentrations were used in the absence and 0.02, 0.05, 0.10 and 0.12 mM concentrations were used in the presence of metabolic activation.

Study design: The design of the study and the dose selection are appropriate. The number of cells per treatment group and the cell survival are acceptable. The doses were selected on the basis of a dose-ranging study in which the relative survival of the cells ranged from 87% to 15% in the absence of S-9 mix, and from 112% to 28% in the presence of S-9 mix.

Analysis:

No. of replicates: Triplicate cultures were used at each dose level, in the absence or presence of metabolic activation.

Counting method: The colonies were counted using a Colony Counter.

Criteria for positive results: The assay was considered positive if there was a statistically significant increase (t-test with Dunnett's correction for multiple comparisons) in the mutant fraction (MF) relative to the combined negative control (2 or more concentrations) and at least one of these induced mutant fractions (IMF) was 2.5×10^{-6} or greater.

Summary:

Study validity: The study was valid, as there were significant increases in the mutant fraction (MF) in the positive control groups, both in the absence and presence of metabolic activation and there were at least four analyzable concentrations for the test agent.

Study outcome: There were no significant increases in the MF at any concentration either in the presence or absence of metabolic activation. However, there were significant increases in the MF for the positive control groups, both in the absence and presence of metabolic activation. Thus, MK-0869 was not mutagenic in the *in vitro* mammalian cell gene mutation assay using TK6 human lymphoblastoid cells, under the conditions of the assay. The data is summarized in the sponsor's Table below.

Summary of Induced Mutant Fraction - TT #08-K313

Treatment	S-9 Metabolic Activation	Precipitate at Dosing	Mean Mutant Fraction (x 10 ⁶) ^a	Induced Mutant Fraction (x 10 ⁶) ^a	Statistical Significance (p Value) ^b
Negative Control - 1% DMSO	-	-	5.72		
	+	-			
Positive Control, ethylnitrosourea, 10.0 uM	-	-	13.22	7.50	<0.0001
Positive Control, 3-methylcholanthrene, 1.0 uM	+	-	31.06	25.35	<0.0001
MK-0869					
0.01 mM	-	-	4.64	0.00	N/A
0.05 mM	-	-	3.87	0.00	N/A
0.09 mM	-	-	4.53	0.00	N/A
0.11 mM	-	-	4.34	0.00	N/A
0.02 mM	+	-	3.30	0.00	N/A
0.05 mM	+	-	3.27	0.00	N/A
0.10 mM	+	-	3.93	0.00	N/A
0.12 mM	+	-	3.45	0.00	N/A

a) Mean mutant fraction of treated minus mean mutant fraction of control (negative numbers are shown as zero).
b) N/A = Not applicable since MF is less than negative control.

Genotoxic toxicology summary:

The genotoxic potential for L-758, 298 (a pro-drug of MK-0869) was by the bacterial reverse mutation assay (Ames assay), the rat hepatocyte DNA damage assay, the mutagenesis assay in TK6 human lymphoblastoid cells and the chromosomal aberrations assay in the Chinese hamster ovary (CHO) cells. It was not found to be genotoxic in any of the assays, either in the absence or presence of metabolic activation.

The genotoxic potential for L-758, 030 (MK-0869) was examined by the *in vivo* mouse bone marrow micronucleus assay and the mutagenesis assay in TK6 human lymphoblastoid cells. It was not found to have any genotoxic potential in these assays.

Genetic toxicology conclusions:

MK-0869 or its pro-drug, L-758, 298 was not found to be genotoxic in the standard battery of genotoxicity assays. However, the sponsor did not examine the genotoxic potential of MK-0869 by the Ames assay.

Labeling recommendations: Aprepitant was not genotoxic in a battery of *in vitro* and *in vivo* genotoxicity assays.

VI. CARCINOGENICITY:**RAT CARCINOGENICITY STUDY** (multiple studies? Std1; Std2 etc.):

RAT STUDY DURATION (weeks): 106

STUDY STARTING DATE: December 15, 1997

STUDY ENDING DATE: July 18, 2002

RAT STRAIN: Cr1:CD@ (SD)IGS BR

ROUTE: Oral gavage

DOSING COMMENTS: The doses were administered twice a day at 0, 0.05, 0.25 and 1.0 mg/kg (0.10, 0.50 and 2.0 mg/kg/day). The sponsor stated that the daily doses were at least 6 hours apart from each other.

NUMBER OF RATS:

- Control 1 (C1): 50/sex
- Control 2 (C2): 50/sex
- Low Dose (LD): 50/sex
- Middle Dose (MD): 50/sex
- High Dose (HD): 50/sex

RAT DOSE LEVELS (mg/kg/day):

- Low Dose: 0.10 mg/kg/day
 - Middle Dose: 0.50 mg/kg/day
 - High Dose: 2.0 mg/kg/day
- (*Dose adjusted during study): Yes

BASIS FOR DOSES SELECTED (MTD; AUC ratio; saturation; maximum feasible): The basis for dose selection for the 105-week carcinogenicity study in rats was not stated.

RAT CARCINOGENICITY (negative; positive; MF; M; F): negative in M and F

PRIOR FDA DOSE CONCURRENCE (Div./CAC)? (y/n; Date): No.

RAT CARCINOGENICITY (conclusion: negative; positive; MF; M; F): Oral administration of MK-0869 to Sprague Dawley rats for 106 weeks was associated with an increase in the incidence of skin papilloma in the male rats ($p=0.042$). However, on the basis of CDER statistical standard, the incidence of this tumor was not significant as the P-value (0.042) exceeded the required P-value of 0.005 for common tumors (incidences $>1\%$), and the incidences of skin papilloma were within the spontaneous incidences reported for this strain of rat. Thus, MK-0869 was not carcinogenic in male and female SD rats in the 106-week carcinogenicity study at oral doses up to 2 mg/kg/day.

RAT TUMOR FINDINGS: Treatment with MK-0869 was associated with increased incidences of papilloma of the skin (Control 1, 0/50 [0%], control 2, 1/50 [2%]; low dose, 1/50 [2%]; mid dose, 0/50 [0%]; high dose, 3/50 [6%]; $P=0.042$, Trend test) in the male animals. However, the incidence of skin papilloma was within the spontaneous incidences reported for this tumor in this strain of rat (0.87% to 6.0%). On the basis of CDER statistical standard, the incidence of this

tumor was not significant as the P-value (0.042) exceeded the required P-value of 0.005 for common tumors (incidences >1%).

RAT STUDY COMMENTS: The sponsor did not mention on what basis the doses were selected for the 106-week carcinogenicity study in rats. The doses were not in concurrence with the CDER Executive CAC. The 2.0 mg/kg/day high dose seems to be too low as no changes in the body weights or clinical signs were observed in the treated animals.

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