

- **Food consumption:** Food consumption for dogs at doses  $\geq 25$  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/mm<sup>3</sup>), 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/mm<sup>3</sup>), 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 =	-	0	-	0	-	0	-	0	-	0	-	1	-	0	-	1
-reduced in size																
Prostate	-	4	-	4	-	4	-	4	-	4	-	4	-	4	-	4
n =	-	0	-	1	-	0	-	1	-	0	-	1	-	0	-	0
-reduced in size																
Thymus	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
n =	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
-reduced in size																
Ovaries	4	-	4	-	4	-	4	-	4	-	4	-	4	-	4	-
n =	0	-	0	-	0	-	1	-	2	-	2	-	1	-	1	-
-reduced in size																

- **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  $\geq 125$  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	-

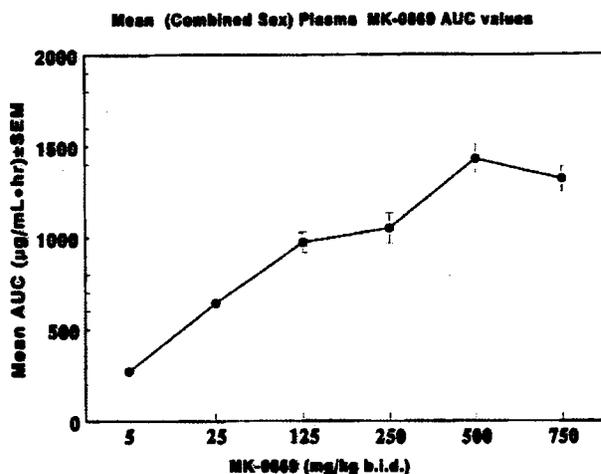
- **Toxicokinetics:** Plasma AUC values for MK-0869 increased with elevating doses at  $\leq 500$  mg/kg B.I.D.; however, observed increases were significantly less than proportional to dose. At plateau in AUC values for MK-0869 was evident for doses  $\geq 500$  mg/kg B.I.D. AUC values for the 12 metabolites 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  $\geq 500$  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 <sub>0-24 hr</sub> (µg·hr/mL)	281 ± 21.2	653 ± 57.9	1060 ± 54.3	1200 ± 86.8	1240 ± 53.1	1440 ± 52.8
C <sub>max</sub> (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 <sub>max</sub> (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 <sub>0-24 hr</sub> (µg·hr/mL)	259 ± 28.9	634 ± 40.0	887 ± 75.8	910 ± 100	1620 ± 66.2
C <sub>max</sub> (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 <sub>max</sub> (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 <sub>0-24 hr</sub> (µg·hr/mL)	270 ± 17.1	643 ± 32.8	973 ± 54.0	1050 ± 81.8	1430 ± 81.9
C <sub>max</sub> (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 <sub>max</sub> (hr)	9.5 ± 0.73	5.0 ± 1.1	10 ± 2.4	4.8 ± 1.3	5.8 ± 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

APPEARS THIS WAY  
ON ORIGINAL

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

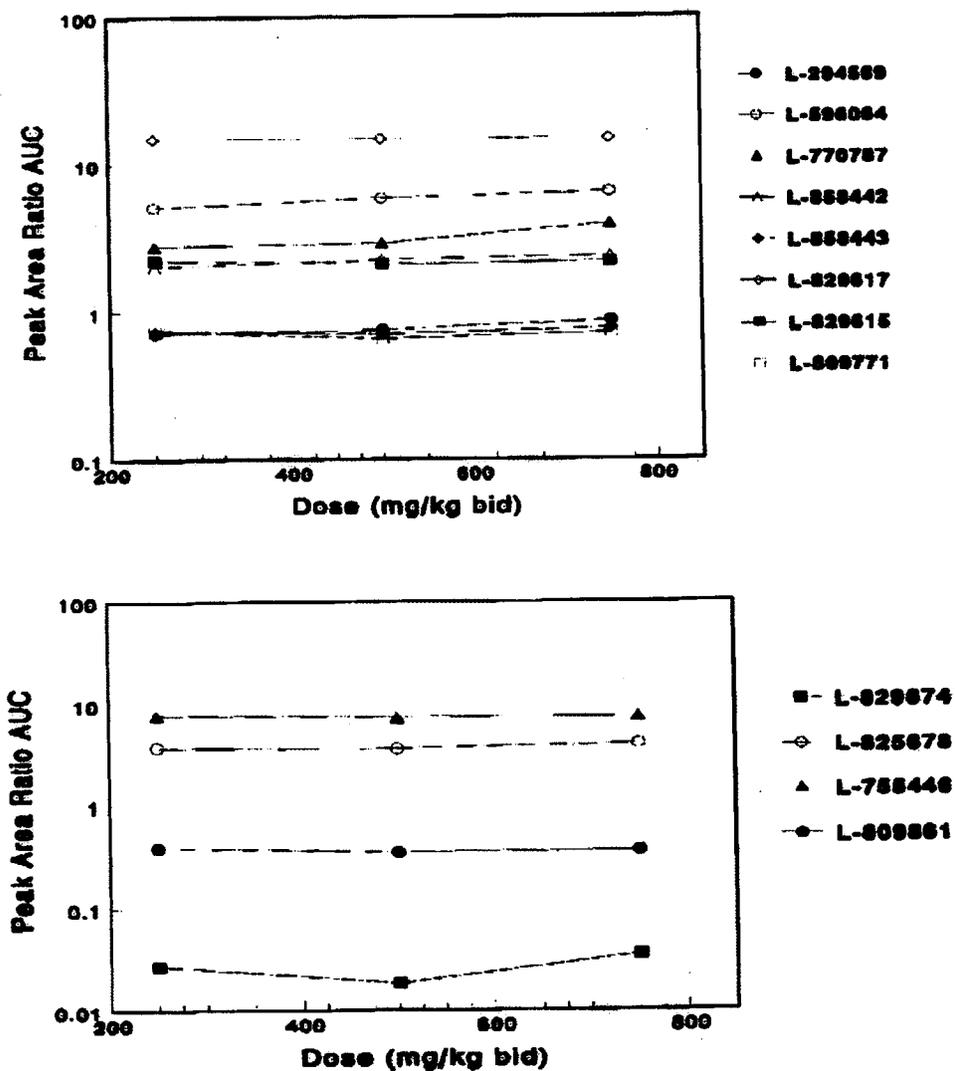
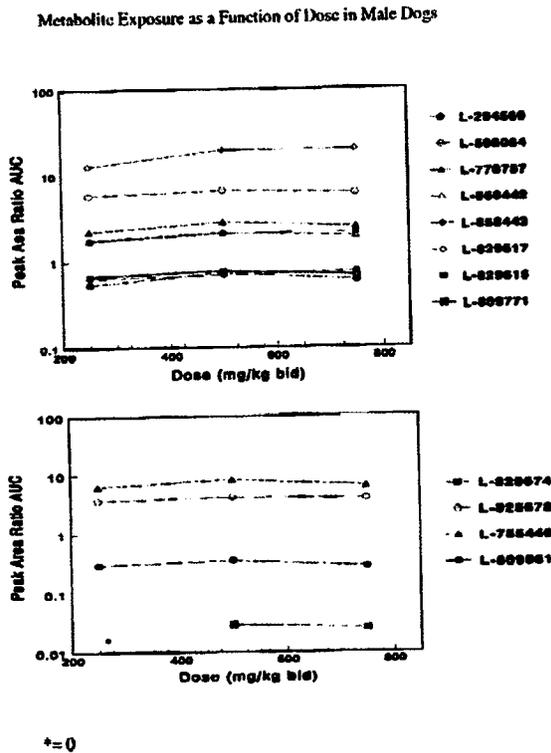


Figure 3. MK-0869: Five-Week Oral Toxicity Study in Dogs. TT #99-082-0. -1



**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  $\sim$  nm. 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

b(4)

elevating doses at  $\leq 500$  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  $\geq 500$  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  $\geq 500$  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 50, 283 Amendment # 146 dated August 11, 2000 (pharmacologist's review of IND 50, 283 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: L-754030: Fourteen-Week Oral Toxicity Study in dogs**

**Study no.** TT#95-041-0

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

**Dates of study initiation & completion:** June 16, 1995 and May 01, 1996.

**GLP compliance:** Yes

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

**Drug, Lot #, radiolabel (if applicable), and % purity:** L-754030; Lot # L-754030-000Z008; purity approximately 100.2%.

**Formulation/vehicle:** L-754030 was suspended in deionized water containing 0.5% methylcellulose and 0.02% sodium lauryl sulfate.

**Methods:** L-754030 was administered orally (gavage) once a day to groups of beagle dogs (4 animals/sex/group) at dose levels of 2.0, 8.0 and 32.0 mg/kg/day. Control animals received the vehicle (0.5% methylcellulose and 0.02% sodium lauryl sulfate).

**Dosing:**

**Species/strain:** Beagle dogs

**Age:** 42 to 44 weeks

**Weight:** males – 8.3 to 13.5 kg; females- 7.9 to 11.6 kg.

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

**Satellite groups used for toxicokinetics or recovery:** N/A

**Doses in administered units:** L-754030 was administered at oral doses of 2, 8 and 32 mg/kg/day.

**Route, form, volume and infusion rate:** The drug was administered by oral gavage at a dosing volume of 5 ml/kg

**Times at which Observations were made:**

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

**Body weights-** Body weights were measured once a week.

**Food and water consumption-** Daily food consumption was measured 3-4 times per week.

**Ophthalmoscopy:** Ophthalmologic examinations were performed on all animals before initiation of dosing and in weeks 6 and 12 of the dosing period.

**Electrocardiography:** ECG recordings were performed on all animals before initiation of dosing and in weeks 4, 8 and 12.

**Hematology:** Blood samples for hematology were collected pre-dose and in weeks 4, 8 and 12 of the dosing period.

**Clinical chemistry-** Blood samples for clinical chemistry analyses were collected pre-dose and in weeks 4, 8 and 12 of the dosing period.

**Urinalysis-** Urine samples were collected from all animals before initiation of dosing and in weeks 8 and 12 of the dosing period.

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

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

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

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

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

**Toxicokinetics-** Blood samples for determination of plasma levels of L-754030 were collected on day 1 and week 13 at 0.5, 2, 4, 6, 8, 10 and 24 hours after administration of the dose. Plasma concentrations of the drug were measured by LC/MS/MS.

### **Results:**

**Mortality:** There were no mortalities in any group.

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

**Body weights:** The mean body weights of the control animals before initiation of dosing (day -1) and in week 13 were 10.3 and 10.7 kg, respectively. No treatment related changes in the body weights were observed in any group.

**Food consumption:** The mean food intake of the control animals in week 1 and week 13 were 339 and 350 g/day, respectively. No treatment related changes in food consumption were observed in any group.

**Ophthalmoscopy:** No treat related ophthalmologic changes were observed in any group.

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

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

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

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

**Gross Pathology:** No treatment-related gross pathological changes were observed in any group.

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

**Histopathology:** No treatment related histopathological changes were observed in any group.

**Toxicokinetics:** On **Drug Day 1**, the highest plasma levels of L-754030 was achieved in 2 to 6 hours after dosing, and the  $C_{max}$  values ranged from 0.302 to 0.531  $\mu\text{g/ml}$  in male dogs and from 0.533 to 1.06  $\mu\text{g/ml}$  in female dogs following administration of the 2 mg/kg dose. Plasma exposure levels ranged from 4.61 to 8.11  $\mu\text{g.h/ml}$  (mean, 6.45  $\mu\text{g.h/ml}$ ) for male dogs and from 5.16 to 13.36  $\mu\text{g.h/ml}$  (mean, 9.60  $\mu\text{g.h/ml}$ ) for female dogs.

In **Drug Week 13**, the plasma drug levels following administration of the 2 mg/kg dose were higher than that on Day 1. The  $C_{max}$  values ranged from 0.455 to 1.49  $\mu\text{g/ml}$  (mean 1.00  $\mu\text{g/ml}$ ) in males and 0.424 to 1.75  $\mu\text{g/ml}$  (mean 0.89  $\mu\text{g/ml}$ ) in females. Plasma exposure levels (AUC) ranged from 5.36 to 16.51  $\mu\text{g.h/ml}$  (mean 12.68  $\mu\text{g.h/ml}$ ) in males and 5.13 to 18.83  $\mu\text{g.h/ml}$  (mean 11.02  $\mu\text{g.h/ml}$ ) in females.

Following administration of the 8 mg/kg/day dose, the mean plasma  $C_{max}$  values were 1.15 and 0.94  $\mu\text{g/ml}$ , the mean AUC values were 17.15 and 14.04  $\mu\text{g}\cdot\text{h/ml}$  in male and female dogs, respectively on Day 1. In Week 4, the mean AUC values were 90.84 and 70.90  $\mu\text{g}\cdot\text{h/ml}$  in male and female dogs, respectively.

Following administration of the 32 mg/kg/day dose, the mean plasma  $C_{max}$  values were 1.71 and 1.83  $\mu\text{g/ml}$ , the mean AUC values were 28.62 and 30.46  $\mu\text{g}\cdot\text{h/ml}$  in male and female dogs, respectively on Day 1. In Week 4, the mean AUC values were 119.54 and 181.79  $\mu\text{g}\cdot\text{h/ml}$  in male and female dogs, respectively.

Thus, following oral administration of L-754030 to male and female dogs at 2, 8 and 32 mg/kg/day doses, the maximum plasma concentrations were reached in 2.5 to 5 hours. On both Day 1 and Week 13, the  $C_{max}$  and AUC values increased with increasing dose in a less than a dose-proportional manner. Repeated oral administration of L-754030 to dogs for about 13 weeks resulted in a 2 to 6-fold increase in AUC values, when compared to Drug Day 1, with the exception of the 2 mg/kg/day females which did not show any appreciable change in AUC values over 13 weeks. The toxicokinetic parameters of L-754030 in male and female dogs on Day 1 and Week 13 are summarized in the Tables below.

MEAN  $C_{max}$ ,  $T_{max}$ , AND AUC VALUES ( $\pm$  S.E.M.) IN DRUG DAY OF L-754,030 IN DOGS FOLLOWING ORAL DOSING AT 2 TO 32 MG/KG/DAY

Parameters	Males		
	Dose (mg/kg/day)		
	2	8	32
$C_{max}$ ( $\mu\text{g/ml}$ )	0.44 $\pm$ 0.05	1.15 $\pm$ 0.11	1.71 $\pm$ 0.18
$T_{max}$ (hr)	2.50 $\pm$ 0.50	4.00 $\pm$ 1.41	4.50 $\pm$ 1.50
Mean AUC ( $\mu\text{g}\cdot\text{hr/ml}$ )	6.45 $\pm$ 0.91	17.15 $\pm$ 1.66	28.62 $\pm$ 4.25

Parameters	Females		
	Dose (mg/kg/day)		
	2	8	32
$C_{max}$ ( $\mu\text{g/ml}$ )	0.80 $\pm$ 0.11	0.94 $\pm$ 0.13	1.83 $\pm$ 0.23
$T_{max}$ (hr)	4.00 $\pm$ 1.15	2.50 $\pm$ 0.50	5.00 $\pm$ 0.58
Mean AUC ( $\mu\text{g}\cdot\text{hr/ml}$ )	9.60 $\pm$ 1.74	14.06 $\pm$ 2.22	30.46 $\pm$ 5.18

MEAN C<sub>max</sub>, T<sub>max</sub>, AND AUC VALUES (± S.E.M.) IN DRUG WEEK 13  
OF L-754,030 IN DOGS FOLLOWING ORAL DOSING AT  
2 TO 32 MG/KG/DAY

Parameters	Males		
	Dose (mg/kg/day)		
	2	8	32
C <sub>max</sub> (µg/ml)	1.00 ± 0.21	4.81 ± 1.28	7.27 ± 2.37
T <sub>max</sub> (hr)	4.50 ± 1.50	3.00 ± 0.58	2.13 ± 0.72
Mean AUC (µg•hr/ml)	12.68 ± 2.50	90.84 ± 24.02	119.54 ± 34.59

Parameters	Females		
	Dose (mg/kg/day)		
	2	8	32
C <sub>max</sub> (µg/ml)	0.89 ± 0.30	4.17 ± 1.09	9.41 ± 1.52
T <sub>max</sub> (hr)	4.00 ± 0.82	2.50 ± 0.50	8.00 ± 1.41
Mean AUC (µg•hr/ml)	11.02 ± 2.95	70.90 ± 23.78	181.79 ± 28.06

**Summary:** In the 14-week oral toxicity study with L-754030 in dogs, the drug was administered at doses of 2, 8 and 32 mg/kg/day. No treatment related effects were observed in any groups at doses up to 32 mg/kg, suggesting that L-754030 was not examined at sufficiently high doses to show any toxic effects. No target organ of toxicity was identified in this study.

**Study Title:** MK-0869: Fourteen-Week Oral Toxicity Study in dogs

**Study no.** TT#98-127-0

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

**Dates of study initiation & completion:** August 25, 1998 and November 25, 1998.

**GLP compliance:** Yes

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

**Drug, Lot #, radiolabel (if applicable), and % purity:** MK-0869 (also known as L-754030); Lot # L-754030-004H032; purity, 99.2%.

**Formulation/vehicle:** L-754030 was suspended in deionized water containing 0.5% methylcellulose and 0.02% sodium lauryl sulfate.

**Methods:** L-754030 was administered orally (gavage) to groups of beagle dogs (4 animals/sex/group) at dose levels of 4.0, 32.0 and 128.0 mg/kg b.i.d. (8, 64 and 256 mg/kg/day). Control animals received the vehicle (0.5% methylcellulose and 0.02% sodium lauryl sulfate in deionized water). The male animals received a total of 182 doses, and the female animals received a total of 184 doses. The doses were administered at an interval of at least 6 hours.

**Dosing:**

**Species/strain:** Beagle dogs

**Age:** 36 to 43 weeks

**Weight: males – 8.9 to 12.9 kg, females - 8.1 to 10.9 kg.**

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

**Satellite groups used for toxicokinetics or recovery:** N/A

**Doses in administered units:** L-754030 was administered to groups of animals at oral doses of 8, 64 and 256 mg/kg/day (4, 32 and 128 mg/kg, b.i.d).

**Route, form, volume and infusion rate:** The drug was administered by oral gavage at a dosing volume of 5 ml/kg

**Times at which Observations were made:**

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

**Body weights-** Body weights were measured once a week.

**Food consumption-** Daily food consumption was measured 2-4 times per week during the dosing period.

**Ophthalmoscopy:** Ophthalmologic examinations were performed on all animals before initiation of dosing and in weeks 6 and 12 of the dosing period.

**Electrocardiography:** ECG recordings were performed on all animals before initiation of dosing and in weeks 4, 8 and 12.

**Hematology:** Blood samples for hematology were collected pre-dose and in weeks 4, 8 and 12 of the dosing period.

**Clinical chemistry-** Blood samples for clinical chemistry analyses were collected pre-dose and in weeks 4, 8 and 12 of the dosing period.

**Urinalysis-** Urine samples were collected from all animals before initiation of dosing and in weeks 8 and 12 of the dosing period.

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

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

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

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

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

**Toxicokinetics-** Blood samples for determination of plasma levels of L-754030 were collected from all animals on day 1 and week 13 at 2, 4, 6, 8, 12, 16 and 24 hours after administration of the first dose. Plasma concentrations of the drug were measured by LC/MS/MS.

### Results:

**Mortality:** There were no mortalities in any group.

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

**Body weights:** The mean body weights of the control animals before initiation of dosing (day -1) and in week 13 were 9.9±1.5 and 10.4±1.9 kg, respectively. No treatment related changes in the body weights were observed in any group.

**Food consumption:** The mean food intake of the control animals in week 1 and week 13 were 251±90 and 276±105 g/day, respectively. No treatment related changes in food consumption were observed in any group.

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

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

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

**Clinical chemistry:** An increase in mean serum cholesterol levels (15% to 28% compared to concurrent controls) were observed in mid- and high- dose animals in Weeks 4, 8 and 12. Cholesterol levels for different groups of animals are shown in the Table below.

GROUP	Pre-test	Week 4	Week 8	Week 12
Control	145±20	154±29	155±33	156±22
4 mg/kg b.i.d.	138±16	150±17	159±35	158±28
32 mg/kg b.i.d.	142±25	190±70	188±57	180±46
128 mg/kg b.i.d.	146±22	182±28	198±60	193±44

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

**Gross Pathology:** No treatment-related gross pathological changes were observed in any group.

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

**Histopathology:** No treatment related histopathological changes were observed in any group.

**Toxicokinetics:** On **Drug Day 1**, the highest plasma concentrations of L-754030 were reached in 14 to 19 hours after dosing. The  $C_{max}$  and AUC values for L-754030 increased with increasing doses, but were not dose-proportional. In **Week 13**, the  $T_{max}$  ranged from 8.0 to 12 hours, and the  $C_{max}$  and AUC values for L-754030 increased with increasing doses in a non-dose-proportional manner. The  $C_{max}$  and AUC values for L-754030 were higher in Week 13 as compared to Day 1 (except at the 4 mg/kg b.i.d dose) suggesting that the steady state was not reached on Day 1. The toxicokinetic parameters for male and female dogs (combined) on Day 1 and Week 13 are shown in the Table below.

Plasma MK-0869 Toxicokinetic Parameters - Drug Day 1  
Mean of Values From Both Sexes

Parameters	MK-0869 (mg/kg b.i.d.)		
	4	32	128
AUC <sub>0-24 hr</sub> (μg•hr/mL)	66.2 ± 5.13	97.9 ± 22.6	179 ± 15.1
$C_{max}$ (μg/mL)	4.11 ± 0.357	6.90 ± 1.29	11.1 ± 1.26
$T_{max}$ (hr)	14 ± 0.756	19 ± 1.68	15 ± 1.05

Values are the Mean ± Standard of the Mean; n = 8.

Plasma MK-0869 Toxicokinetic Parameters - Drug Week 13  
Mean of Values From Both Sexes

Parameters	MK-0869 (mg/kg b.i.d.)		
	4	32	128
AUC <sub>0-24 hr</sub> (ng•hr/mL)	70.4 ± 9.53	272 ± 38.6	479 ± 59.3
$C_{max}$ (ng/mL)	3.68 ± 0.499	13.0 ± 1.72	22.4 ± 2.91
$T_{max}$ (hr)	8.3 ± 1.33	9.5 ± 1.50	12 ± 1.07

Values are the Mean ± Standard of the Mean; n = 8.

**Summary:** In the 14-week oral toxicity study with MK-0869 in dogs, the drug was administered at 4, 32 and 128 mg/kg b.i.d (8, 64 and 256 mg/kg/day) doses. No treatment related toxic effects were observed in any groups at doses up to 128 mg/kg b.i.d, suggesting that L-754030 was not examined at sufficiently high doses to show any toxic effects. No target organ of toxicity was identified in this study.

**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

APPEARS THIS WAY  
ON ORIGINAL

**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- \_\_\_\_\_

**Formulation/vehicle:** Dosing formulations (100 mg/ml) were prepared daily by suspending MK-0869 from the \_\_\_\_\_ in deionized water, Further dilutions were made in placebo control vehicle. The average particle size of MK-0869 in the dispersion was approximately \_\_\_\_\_ nm. b(4)

**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 \_\_\_\_\_ in deionized water and the doses were administered by oral gavage (5 ml/kg b.i.d). b(4)

**Observations and times:**

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

**Body weights:** Body weights were recorded once prior to initiation of dosing and 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, CV<sub>5</sub>RL 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:

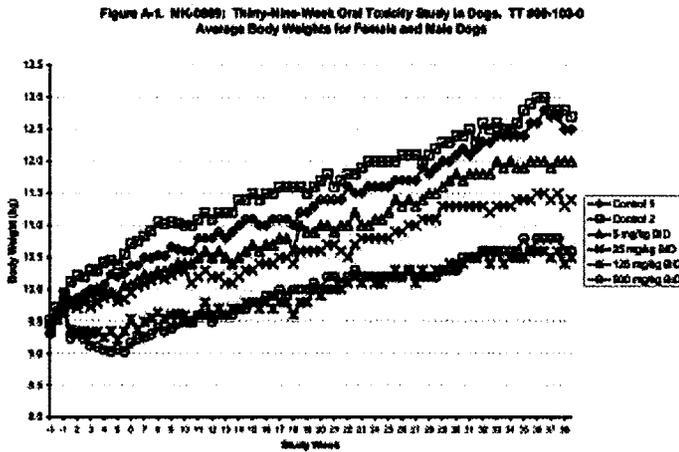
**Mortality:** There were no deaths in any group.

**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.
<b>Week -1</b>						
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%
<b>Week 1</b>						
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%
<b>Week 7</b>						
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%
<b>Week 21</b>						
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%
<b>Week 38</b>						
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.



**Food consumption:** The mean food consumption of the control animals in Week-1 was  $323 \pm 53$  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	$1.1 \pm 0.1$	-9	-9	-9	-18
	Week-25	$1.2 \pm 0.2$	-17	-17	-8	-17
	Week-38	$1.4 \pm 0.3$	-14	-21	-14	-21
Alkaline Phosphatase (U/L)	Week-12	$88 \pm 35$	+32	+64	+78	+119
	Week-25	$80 \pm 42$	+57	+124	+119	+160
	Week-38	$58 \pm 15$	+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. b(4)

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

**Methods:** Beagle dogs received L-754,030 (n 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, CV<sub>5</sub>RL, and V<sub>10</sub>. 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, b(4)

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

b(4)

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.

b(4)

### 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 \_\_\_\_\_ 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.

b(4)

**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 µg/kg/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, \_\_\_\_\_ mg/mL NaCl, and \_\_\_\_\_ ng/mL NaOH, in water with a pH of \_\_\_\_\_ and an osmolality of \_\_\_\_\_ mOsm) at a MK-0869 concentration of 20 µg/mL. MK-0869 at 20 µg/mL was the maximum feasible concentration in this vehicle solution. The intravenous dosing volumes for the 0, 80, 160, and 240 µg/kg/day groups were 12, 4, 8, and 12 mL/kg, respectively. Control animals received the vehicle, designated as L-931,175, which consisted of 5.0 mg/mL ethyl alcohol (190 proof), 1.92 mg/mL citric acid (anhydrous), \_\_\_\_\_ g/mL polysorbate 80, 8.20 mg/mL NaCl, \_\_\_\_\_ µg/mL NaOH, and \_\_\_\_\_ /mL 1 N HCl in water with a pH of \_\_\_\_\_ and an osmolality of \_\_\_\_\_ 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

b(4)

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/mL 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.

b(4)

**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:** \_\_\_\_\_ The composition (per ml) of the vehicle is as follows: Polysorbate 80 ( \_\_\_\_\_ mg; D-lactose-; \_\_\_\_\_ g; meglumine- \_\_\_\_\_ mg \_\_\_\_\_ 1

**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.

#### 2.6.6.1 Overall toxicology summary

Acute toxicity studies of fosaprepitant (L-758, 298) were conducted in mice and rats following i.v. administration of 200 and 500 mg/kg doses, and an oral dose of 500 mg/kg. The minimal lethal dose (MLD) by the i.v. route was 500 mg/kg in both rats and mice. There were no deaths of rats and mice at the 500 mg/kg oral dose. The clinical signs observed in mice after i.v. dosing included gasping, convulsions, bradypnea and loss of righting reflex, which disappeared within 3 hours. In rats, the clinical signs included gasping and bradypnea.

Repeat dose toxicity studies with aprepitant (MK-0869) or fosaprepitant were conducted in rats, mice, dogs and monkeys. Seventeen-day i.v. toxicity studies with the lyophilized i.v. formulation of fosaprepitant were conducted in rats and dogs. In rats, fosaprepitant doses of 2.5, 5.0 and 7.5 mg/kg/day were used, and injection site changes (cellular proliferation of venous intima, venous necrosis or thrombosis, skin necrosis, subcutaneous edema, cellular infiltration and degeneration of muscle fibers) were observed in all groups. In the 17-day i.v. toxicity study in dogs, the target organ of toxicity was also the injection site (venous thrombosis, fibroplasia and necrosis), and the 2.0 mg/kg/day dose was the no effect dose. Thus, in both rats and dogs, i.v. administration of fosaprepitant was not associated with any toxic effects on any organs other than the injection sites. In repeat dose oral toxicity studies with aprepitant in rats, the target organs of toxicity were the liver (centrilobular hepatocellular hypertrophy) and thyroid (follicular cell hyperplasia), and these effects may be related to induction of hepatic drug metabolizing enzymes. In the mouse liver microsomes, aprepitant caused an induction of several drug metabolizing enzymes. 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 aprepitant in dogs, the target organs of toxicity were the testes (tubular degeneration) and prostate (atrophy). Testicular degeneration and an atrophy of the prostate and thymus were also observed in a 5-week oral toxicity study in dogs. However, in a 53-week oral toxicity study with a 27-week interim sacrifice, no target organ of toxicity was identified. In monkeys, intravenous 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.

#### 2.6.6.4 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.

**Study Title:** L-754,030- Microbial Mutagenesis Assay.

**Sponsor's ID:** TT #95-8043 and TT # 95-8045

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

**Date of Study Initiation/completion:** May 23, 1995/August 01, 1995

**GLP Compliance:** Yes

**Drug Lot Number:** L-754,030, Lot # 000Z008; purity, 99.4%.

**Study Endpoint:** Mutagenesis

#### **METHODOLOGY:**

**Strains/Species/Cell line:** *Salmonella typhimurium* strains TA97, TA98, TA100 and TA1535,  
and *Escherichia coli* strain WP2uvrA

**Dose Selection Criteria:** Dose selection was based on a previous exploratory assay in which precipitate interfered with scoring of the plates at a concentration of 10,000 µg/plate. For the genotoxicity studies, 6,000 µg/plate was selected as the highest dose.

**Test Agent Stability Considerations:** The sponsor determined the concentration of L-754,030 in the solution, and it was stated that the stability of the solution in DMSO were within acceptable limits.

**Metabolic Activation System:** Rat liver microsomal S9 fraction prepared from male Sprague-Dawley rats treated with Phenobarbital/Beta-Naphthoflavone was used as the metabolic activation system.

**Controls:** Dimethylsulfoxide (DMSO) was used as a negative control. The following agents were used as positive controls in the absence of metabolic activation: 2-aminoanthracene, sodium azide, daunomycin and methyl methanesulfonate.

**Exposure conditions:** L-754,030 was used at final concentrations of 30, 100, 300, 1000, 3000 and 6000 µg/plate. The tests were performed using the plate incorporation method with or without S9 mix. The bacterial suspensions (with the test substance or controls in absence or presence of metabolic activation) were added to agar plates and incubated for 48 hours at 37°C.

**Analysis:** Triplicate samples were used for each concentration. The number of revertant colonies were counted manually. The mean of the number of revertant colonies at each concentration was determined and compared with the negative controls.

**Criteria for Positive Results:** The study was considered positive if the number of revertant colonies induced was at least 2-fold higher than the negative control, and there was an evidence of dose-related increase.

## RESULTS

**Study Validity:** The study was valid, as the mean number of revertant colonies in the negative controls fell within historical control ranges from the conducting laboratory and the positive controls induced a clear increase in the number of revertant colonies.

**Study Outcome:** No inhibition of bacterial lawn growth was observed at the highest concentration (6,000 µg/plate) of L-754,030. L-754,030, at the highest concentration tested, did not produce a 2-fold or greater increase in the number of revertants relative to the solvent control, either in the absence or presence of metabolic activation. The positive controls caused a significant increase in the number revertant colonies for all strains examined. Thus, L-754,030 was not mutagenic under the conditions of the assay. A second assay was also negative. The number of mean revertant colonies in the main study for different strains in the absence or presence of metabolic activation is shown in the Table below.

APPEARS THIS WAY ON ORIGINAL

Microbial Mutagenesis Assay (1)

TT #95-8045 Compound: L-754,030-000Z008

Solvent: DMSO

Salmonella His<sup>+</sup> Revertants Per Plate

Conc. (µg/plate)	TA100 07-JUN-95				TA1535 07-JUN-95				TA97a 07-JUN-95				TA98 07-JUN-95			
	Without S9		With S9		Without S9		With S9		Without S9		With S9		Without S9		With S9	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0 (±S.D.)	122.9	12.0	131.4	11.6	11.6	3.6	15.7	3.0	108.3	17.1	139.7	15.9	26.7	3.4	38.2	5.2
30									94.0	1.7	139.0	8.5				
100	112.3	21.6	136.7	6.1	14.3	2.5	17.7	4.9	96.3	3.8	121.3	2.1	30.7	3.2	42.7	2.9
300	116.7*	7.0	123.7*	11.0	13.0*	1.0	10.0*	3.6	92.0*	8.9	81.7*	3.2	23.7*	3.5	36.0*	6.9
1000	121.7*	14.2	116.7*	6.8	14.7*	2.5	7.0*	3.0	108.7*	7.8	74.3*	14.0	27.7*	2.5	30.3*	3.5
3000	123.3*	9.2	128.7*	9.1	10.3*	1.5	7.0*	3.0	104.7*	13.4	104.3*	12.0	32.0*	4.6	32.7*	3.8
6000	124.3*	10.4	130.0*	10.4	4.7*	0.6	8.7*	0.6	94.7*	13.1	106.3*	8.0	27.3*	4.5	29.7*	4.0

Salmonella typhimurium genotypes:

TA1535 his G46 (base substitution) uvrB rfa  
 TA97a his D6610 (frameshift) uvrB rfa pKM101 (R factor)  
 TA98 his D3052 (frameshift) uvrB rfa pKM101 (R factor)  
 TA100 his G46 (base subst) uvrB rfa pKM101 (R factor)  
 1 = D.M. Maron and B.N. Ames, Mutation Res. 113:173-215, 1983

+S9 = Metabolic activation mixture  
 -S9 = Saline-buffer control mixture  
 SD = Standard deviation

\* = precipitate; did not interfere with scoring

Microbial Mutagenesis Assay (1)

TT #95-8045 Compound: L-754,030-000Z008

Solvent: DMSO

Salmonella His<sup>+</sup> Revertants Per Plate

Conc. (µg/plate)	TA100 07-JUN-95				TA1535 07-JUN-95				TA97a 07-JUN-95				TA98 07-JUN-95			
	Without S9		With S9		Without S9		With S9		Without S9		With S9		Without S9		With S9	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0 (±S.D.)	122.9	12.0	131.4	11.6	11.6	3.6	15.7	3.0	108.3	17.1	139.7	15.9	26.7	3.4	38.2	5.2
30									94.0	1.7	139.0	8.5				
100	112.3	21.6	136.7	6.1	14.3	2.5	17.7	4.9	96.3	3.8	121.3	2.1	30.7	3.2	42.7	2.9
300	116.7*	7.0	123.7*	11.0	13.0*	1.0	10.0*	3.6	92.0*	8.9	81.7*	3.2	23.7*	3.5	36.0*	6.9
1000	121.7*	14.2	116.7*	6.8	14.7*	2.5	7.0*	3.0	108.7*	7.8	74.3*	14.0	27.7*	2.5	30.3*	3.5
3000	123.3*	9.2	128.7*	9.1	10.3*	1.5	7.0*	3.0	104.7*	13.4	104.3*	12.0	32.0*	4.6	32.7*	3.8
6000	124.3*	10.4	130.0*	10.4	4.7*	0.6	8.7*	0.6	94.7*	13.1	106.3*	8.0	27.3*	4.5	29.7*	4.0

Salmonella typhimurium genotypes:

TA1535 his G46 (base substitution) uvrB rfa  
 TA97a his D6610 (frameshift) uvrB rfa pKM101 (R factor)  
 TA98 his D3052 (frameshift) uvrB rfa pKM101 (R factor)  
 TA100 his G46 (base subst) uvrB rfa pKM101 (R factor)  
 1 = D.M. Maron and B.N. Ames, Mutation Res. 113:173-215, 1983

+S9 = Metabolic activation mixture  
 -S9 = Saline-buffer control mixture  
 SD = Standard deviation

\* = precipitate; did not interfere with scoring

**SUMMARY:**

L-754,030 did not cause a 2-fold or higher increase in the number of revertant colonies for any of the tester strains in the absence or presence of metabolic activation. Thus, the compound was not mutagenic in the test systems under the experimental conditions.

## 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.  $2.2 \times 10^6$  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% CO<sub>2</sub> 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 B<sub>1</sub> (final concentration of 1 µM); vehicle for aflatoxin B<sub>1</sub> was DMSO. For a radiation positive control, untreated cell suspensions were irradiated with 3 Gy of gamma 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 5 ml aliquot of the cell suspension was loaded on a 2.0 µ pore polycarbonate 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 non-fluorescent 0.2 µ pore polycarbonate filters and the amount of DNA on each filter was measured as the fluorescent 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 (subclone of WBL) were used.  $1.2 \times 10^6$  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% CO<sub>2</sub>.

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

APPEARS THIS WAY ON ORIGINAL

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  $\mu\text{M}$  were studied. Positive control with S-9 metabolic activation was cyclophosphamide (2.5 and 5.0  $\mu\text{M}$ ). Positive control without S-9 metabolic activation was mitomycin C (0.35 and 0.75  $\mu\text{M}$ ). 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  $\mu\text{M}$ ; at doses higher than 80  $\mu\text{M}$ , 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.

**Study Title: L-754,030: Assay of Chromosome Aberrations *In Vitro* in Chinese Hamster Ovary Cells.**

**Study Report No.** TT#95-8669 and TT#95-8672.

**Conducting Laboratory:** Merck Sharp & Dohme Research Laboratories, Merck & Co., Inc., West Point, PA.

**Date of Study Initiation/completion:** July 07, 1995/September 21, 1995

**GLP Compliance:** Yes

**Drug Lot Number:** L-754,030; Lot # L-754,030-000Z008; purity, 99.4%.

**Study Endpoint:** Chromosomal aberration.

**METHODOLOGY:**

**Strains/Species/Cell line:** Chinese hamster ovary (CHO) cells.

**Dose Selection Criteria:** In the chromosomal aberration assay, the selection of the highest dose was based on the solubility of the compound in DMSO, and this dose should not produce more than 50% reduction of the cell growth. In the range finding study, cells were incubated with increasing concentrations (5, 10, 20, 40, 60, 80, 100, 120 and 150  $\mu\text{M}$ , in the absence or presence of metabolic activation) of the test agent for 7 and 24 hours, and total number of cells and mitotic index determined.

**Test Agent Stability Considerations:** The sponsor stated that determination of the concentration of L-754,030 solution in DMSO showed that it was within acceptable limits. The stability of the solution was also within acceptable limits.

**Metabolic Activation System:** Rat liver microsomal S9 fraction from beta-naphthoflavone and Phenobarbital treated animals was used as a metabolic activator.

**Controls:** Dimethylsulfoxide (DMSO) was used as a negative control. Cyclophosphamide was used a positive control in the presence of metabolic activation, and Mitomycin C was used as a positive control in the absence of metabolic activation.

**Exposure conditions:** Cultures of CHO cells were treated with L-754,030 and positive or negative controls for 3 hours, washed twice, incubated in complete medium at 37°C for 17 hours and harvested. All flasks were treated with a final concentration of 0.1  $\mu\text{g}/\text{ml}$  of colcemid two to three hours before harvest to induce mitotic arrest. Twenty hours from beginning of treatment, the cultures were harvested by trypsinization. An aliquot of cells from each flask was counted and the total number of cells in each flask was calculated. The remaining cell suspension was fixed and slides were prepared and stained with Giemsa for analysis of chromosomal aberrations.

**Analysis:** At least two hundred metaphase spreads per dose were scored under code, from a minimum of 3 doses of the test compound, negative and/or solvent controls. For positive controls, a minimum of 50 cells were scored from each treatment.

**Criteria for Positive Results:** The results were considered positive if there was a significant increase (Fisher's Exact test,  $p < 0.05$ ) in the proportion of cells with structural aberrations at two separate concentrations of the test article over that of concurrent controls.

## RESULTS

**Study Validity:** The study was valid, as the proportion of cells with structural aberrations fell within the historical control range, and the positive controls induced statistically significant increases in the number of cells with structural chromosome aberrations.

**Study Outcome:** Precipitates were observed in the culture medium at L-754,030 concentrations of  $\geq 90 \mu\text{M}$ . In the range finding study, all cells in the cultures were killed by the 3 hr treatment at 120  $\mu\text{M}$  and above with S9, and 100  $\mu\text{M}$  and above without S9 activation. At 24 hours with S9 activation, cell

counts were reduced to 61% of controls at 60  $\mu$ M. At 24 hours without S9 activation, cell counts were reduced to 47% of controls at 60  $\mu$ M. The results of the cytotoxicity assay are shown in the Table below.

### Cytotoxicity Result Summary

<u>Treatment</u>	<u>7 Hour Cytotoxicity</u>			<u>24 Hour Cytotoxicity</u>		
	<u>Cell Counts (% Control)</u>	<u>Mitotic Index<sup>a</sup></u>	<u>Morphology /Comments<sup>b</sup></u>	<u>Cell Counts (% Control)</u>	<u>Mitotic Index<sup>a</sup></u>	<u>Morphology /Comments<sup>b</sup></u>
<u>With S-9</u>						
Medium		9.6	Normal		5.8	Normal
DMSO		10.4	Normal		6.2	Normal
Cyclophosphamide						
40 $\mu$ M	103	3.2	Normal	55	2.4	Large cells
<u>L-754,030</u>						
20 $\mu$ M	101	8.8	Normal	96	7.6	Normal
40 $\mu$ M	95	8.8	Normal	88	6.2	Normal
60 $\mu$ M	83	0.4	Normal	61	9.8	Rounding
80 $\mu$ M $\downarrow$	62	0.0	Some dead, Rounding	75	8.4	Rounding
100 $\mu$ M $\downarrow$	14	NS	Many dead	6	NS	All dead
120 $\mu$ M $\downarrow$	ND	ND	Dead, discarded <sup>c</sup>	ND	ND	Dead, discarded <sup>c</sup>
<u>Without S-9</u>						
Medium		12.8	Normal		7.3	Normal
DMSO		18.6	Normal		7.2	Normal
Mitomycin C						
10 $\mu$ M	91	7.6	Normal	68	10.4	Large cells
<u>L-754,030</u>						
10 $\mu$ M	100	21.4	Normal	98	8.2	Normal
20 $\mu$ M	92	18.2	Normal	94	6.6	Normal
40 $\mu$ M	83	22.0	Normal	83	6.8	Normal
60 $\mu$ M	74	0.6	Rounding, some dead	47	3.4	Rounding
80 $\mu$ M $\downarrow$	33	0.4	Rounding, many dead	9	NS	All dead
100 $\mu$ M $\downarrow$	ND	ND	Dead, discarded <sup>c</sup>	ND	ND	Dead, discarded <sup>c</sup>

ND = not done, doses greater than 100  $\mu$ M and less than 20  $\mu$ M with S-9 and greater than 80  $\mu$ M and less than 10  $\mu$ M without S-9 were discarded before harvest. NS = not scored due to excessive toxicity.

DMSO = dimethylsulfoxide.

$\downarrow$  = precipitate during treatment.

<sup>a</sup>Percentages of mitotic cells, based on 500 to 1000 cells.

<sup>b</sup>Monolayer morphology.

<sup>c</sup>Precipitate at the end of the 3 hour treatment.

In the chromosomal aberration assay, L-754,030 doses selected were 20, 30, 40, 50, 60, 80 and 100  $\mu\text{M}$  with S9 and 20, 30, 40, 50, 60, 70 and 80  $\mu\text{M}$  without S9. There were no increases in the proportion of cells with chromosomal aberration either in the absence or presence of metabolic activation. Thus, L-754030 was negative in the CHO cell chromosomal aberration assay under the condition of the assay.

The number of cells with chromosomal aberrations in the absence and presence of metabolic activation are shown in the Tables below.

**Table 2. L-754,030: Assay for Chromosomal Aberrations *In Vitro*, in Chinese Hamster Ovary Cells. TT #95-8672.**

**20 Hour Cytotoxicity and Aberration Report Summary**

<u>Treatment</u>	<u>Cell Counts (% Controls)</u>	<u>% Aberrant Cells</u>	<u>Frequency Abs per 100 Cells</u>
<b><u>With S-9 Activation</u></b>			
Medium		2.0	3.5
DMSO		1.0	1.0
CP 2.5 $\mu\text{M}$	84	11.0**	13.0
CP 5.0 $\mu\text{M}^a$	72	25.0**	28.0
<b><u>L-754,030</u></b>			
20 $\mu\text{M}$	99	Not scored	
30 $\mu\text{M}$	91	Not scored	
40 $\mu\text{M}$	86	2.5	3.5
50 $\mu\text{M}$	79	1.0	1.0
60 $\mu\text{M}$	59	2.0	2.0
70 $\mu\text{M}\downarrow$	32	Not scored: toxic	
80 $\mu\text{M}\downarrow$	Dead	Discarded	
100 $\mu\text{M}\downarrow$	Dead	Discarded	
<b><u>Without S-9 Activation</u></b>			
Medium		2.0	2.0
DMSO		0.5	0.5
MMC 0.35 $\mu\text{M}$	83	8.0**	9.0
MMC 0.75 $\mu\text{M}^a$	77	22.0**	27.0
<b><u>L-754,030</u></b>			
20 $\mu\text{M}$	99	Not scored	
30 $\mu\text{M}$	83	Not scored	
40 $\mu\text{M}$	73	1.5	1.5
50 $\mu\text{M}$	67	2.5	3.0
60 $\mu\text{M}$	47	1.0	1.0
70 $\mu\text{M}\downarrow$	44	Not scored: toxic	
80 $\mu\text{M}\downarrow$	5	Not scored: toxic	

DMSO = Dimethylsulfoxide; Cyclophosphamide (CP) and Mitomycin C (MMC) are positive controls.  
% Aberrant cells = The percentage of cells with aberrations, 200 cells scored per point except for high dose positive control: <sup>a</sup> 100 cells scored;  $\downarrow$  = precipitate during treatment.

Frequency Abs/100 cells = The total number of aberrations observed expressed as a frequency per 100 cells; this number reflects the occurrences of more than one aberration in a cell.

\*  $P \leq 0.05$ ; \*\*  $P \leq 0.01$  compared to the relevant control group using a one-sided Fisher's Exact Test. Since several comparisons with a common control were made, an adjustment procedure of Dunnett was used to assess the overall significance of each comparison for doses of test compound.

**SUMMARY:** L-754,030 was not mutagenic in the CHO cell chromosomal aberration assay in the absence or presence of metabolic activation.

**Study Title: L-758,298 – Assay for Micronucleus Induction in Mouse Bone Marrow**

**Sponsor's ID #** 98-8600

**Descriptive Title:** *In vivo* bone marrow micronucleus assay in mice.

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

**Date of Study Initiation/completion:** January 06, 1998/ February 03, 1998

**GLP Compliance:** Yes

**Drug Lot Number:** L-758,298, Lot no. L-758,298-003C013

**Study Endpoint:** Micronuclei in polychromatic erythrocytes.

**METHODOLOGY:**

b(4)

**Strains/Species/Cell line:** \_\_\_\_\_ BR mice

**Dose Selection Criteria:** The high dose of 200 mg/kg for the bone marrow micronucleus assay was selected on the basis of an acute i.v. toxicity study in male and female mice, in which clinical signs (decreased activity, bradypnea and kicking convulsions) were observed at the 200 mg/kg dose. The mid and low doses were selected as 100 and 50 mg/kg, respectively.

**Test Agent Stability Considerations:** The sponsor stated that the concentrations of L-758,298 in the solution, and stability of the solutions were within acceptable limits.

**Metabolic Activation System:** N/A.

**Controls:** Sodium chloride (0.9%) was used as a negative control. Mitomycin C (2.0 and 0.35 mg/kg), dissolved in physiological saline, was used a positive control.

**Exposure conditions:** Groups of mice (about 4 weeks old; 5 to 7 animals/sex/group) were administered L-758,298 by a single bolus i.v. injection at doses of 50, 100 and 200 mg/kg. Control animals were administered normal saline. The positive control was administered at a single i.p. dose of 2.0 and 0.35 mg/kg. The positive control animals were sacrificed at 24 hours after dosing, and the negative control and treatment group animals were sacrificed at 24 and 48 hours after dosing. Bone marrow samples were collected from the femurs, spread on slides and fixed in methanol before staining with Acridine orange.

**Analysis:** The number of micronucleated erythrocytes, polychromatic erythrocytes (PCE) and polychromatic and normochromatic erythrocytes (NCE) were counted. The proportions of polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE) were determined by counting at least 2000

PCEs. The ratio of PCE/NCE for each animal and the mean for each group was calculated. The individual and group mean frequency of micronucleated PCE/1000 cells was also determined. The numbers of micronucleated PCE in each treatment group were then compared with the numbers in the vehicle control groups.

**Criteria for Positive Results:** The assay was considered positive if there was a significant increase in the frequency of micronucleated PCE (ANOVA;  $p < 0.05$ ) in the treatment groups as compared to the negative control, at a minimum of two dose points.

## RESULTS

**Study Validity:** The study was valid, because the frequencies of micronucleated PCEs in the negative control group fell within the historical control range from the conducting laboratory, and the positive control induced a statistically significant increase in the frequency of micronucleated PCEs.

**Study Outcome:** Clinical signs of toxicity were observed in animals administered 100 and 200 mg/kg doses, which included decreased activity and kicking convulsions. One female mice receiving the 200 mg/kg dose died within 15 minutes of dosing.

There was no significant increase in the frequency of micronucleated PCE in any treatment groups as compared with the negative control group. Groups of mice treated with L-758,298 exhibited PCE/NCE ratios that were similar to the vehicle control group. There was a significant increase in micronucleated PCEs in animals treated with the positive control. The group mean frequencies of micronucleated PCE/1000 cells in male and female mice are shown in the Table below.

APPEARS THIS WAY ON ORIGINAL

Summary of Treatment Group Data

Treatment Dose	Sacrifice Hour	Males				Females			
		Total PCE	Total MN-PCE	MN-PCE per 1000 PCE <sup>a</sup>	% PCE	Total PCE	Total MN-PCE	MN-PCE per 1000 PCE <sup>a</sup>	% PCE
0.9% Saline 10 mL/kg	24	10000	18	1.8 ± 0.4	53.4	10000	25	2.5 ± 0.8	60.4
L-758,298 50 mg/kg	24	10000	14	1.4 ± 0.4	55.7	10000	20	2.0 ± 0.3	50.8
L-758,298 100 mg/kg	24	10000	19	1.9 ± 0.2	60.2	10000	31	3.1 ± 0.7	62.8
L-758,298 200 mg/kg	24	10000	23	2.3 ± 0.3	51.5	10000	24	2.4 ± 0.9	63.4
Mitomycin C 0.35 mg/kg	24	10000	95	9.5 ± 1.3	51.1	10000	72	7.2 ± 1.4	66.6
Mitomycin C 2.0 mg/kg	24	10000	561	56.1 ± 22.4	57.9	10000	919	91.9 ± 5.2	59.0
0.9% Saline 10 mL/kg	48	10000	23	2.3 ± 0.3	57.9	10000	39	3.9 ± 0.8	59.7
L-758,298 50 mg/kg	48	10000	17	1.7 ± 0.5	58.6	10000	29	2.9 ± 0.7	56.0
L-758,298 100 mg/kg	48	10000	19	1.9 ± 0.4	61.6	10000	28	2.8 ± 0.4	64.2
L-758,298 200 mg/kg	48	10000	18	1.8 ± 0.4	46.9	10000	33	3.3 ± 0.7	57.4

PCE = Polychromatic erythrocytes.  
 MN-PCE = micronucleated PCE.  
<sup>a</sup> group mean ± standard error.

**Summary:** Thus, L-758,298 was not positive in the mouse bone marrow micronucleus assay at i.v. doses up to 200 mg/kg/day under the experimental conditions.

APPEARS THIS WAY ON ORIGINAL

**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:** 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.

b(4)

**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  $\geq 500$  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  $\leq 500$  mg/kg. The positive control group was observed with expected increases in micronucleated PCE.

L-754,030 at oral doses  $\leq 500$  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.

APPEARS THIS WAY ON ORIGINAL

**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/ $\beta$ -naphthoflavone-induced rat liver S-9 fraction was used as the metabolic activation system ( ).

b(4)

**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  $\mu$ M) was used as the positive control in the absence of metabolic activation and 3-methylchloroanthrene (3MC, 1  $\mu$ 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, 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  $\mu$ 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 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

APPEARS THIS WAY ON ORIGINAL

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/ $\beta$ -naphthoflavone-induced rat liver S-9 fraction was used as the metabolic activation system: \_\_\_\_\_

b(4)

**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  $\mu$ M) was used as the positive control in the absence of metabolic activation and 3-methylchloroanthrene (3MC, 1  $\mu$ 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  $\mu$ 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 \_\_\_\_\_

**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  $2.5 \times 10^{-6}$  or greater.

b(4)

**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 #98-8313

Treatment	S-9		Mean Mutant Fraction (x 10 <sup>-6</sup> )	Induced Mutant Fraction (x 10 <sup>-6</sup> ) <sup>a</sup>	Statistical Significance (p Value) <sup>b</sup>
	Metabolic Activation	Precipitate at Dosing			
Negative Control - 1% DMSO	-	-	3.72		
	+	-			
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
<b>MK-0869</b>					
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.

### Genetic toxicology summary:

The genotoxic potential for L-758, 298 (fosaprepitant) was examined in the bacterial reverse mutation assay (Ames assay), the rat hepatocyte DNA damage assay, the mutagenicity assay in TK6 human lymphoblastoid cells, the chromosomal aberrations assay in the Chinese hamster ovary cells, and the *in vivo* mouse bone marrow micronucleus assay. L-758, 298 was not genotoxic in any of the assays.

The genotoxic potential for L-754, 030 (aprepitant; MK-0869) was examined in the bacterial reverse mutation assay (Ames assay), the *in vivo* mouse bone marrow micronucleus assay and the mutagenesis assay in TK6 human lymphoblastoid cells. It had no genotoxic potential in any of these assays.

### Genetic toxicology conclusions:

Fosaprepitant (L-758, 298) or its active metabolite, aprepitant was not genotoxic in a standard battery of genotoxicity assays.

### 2.6.6.5 Carcinogenicity

The sponsor conducted three 106-week oral carcinogenicity studies in Sprague-Dawley rats and two 105-week oral carcinogenicity studies in mice with MK-0869. The studies were reviewed earlier, and summarized below.

#### **Rat Carcinogenicity Studies:**

In the first oral carcinogenicity study with MK-0869 in rats, oral doses of 0.10, 0.50 and 2.0 mg/kg/day (0.05, 0.25 and 1.0 mg/kg b.i.d.) were used, and the exposure levels of the compound at the high dose were lower than that in humans at the proposed clinical dose. In this 106-week carcinogenicity study in rats, treatment with MK-0869 was not associated with any tumors in male and female rats.

In the second 106-week oral carcinogenicity study with MK-0869 in rats, groups of animals were administered 10, 50 and 250 mg/kg/day (5, 25 and 125 mg/kg b.i.d.) doses of the drug. The doses were selected on the basis of saturation of absorption at the high dose. However, in the dose-ranging study, the exposure levels of only the parent compound were determined, and the CDER Executive CAC Committee did not concur with the dose selection for the rat carcinogenicity study. However, in a subsequent 5-week oral toxicokinetic study, it was demonstrated that saturation of absorption was achieved at the 125 mg/kg b.i.d. dose. In this 106-week oral carcinogenicity study, treatment with aprepitant produced thyroid follicular cell adenoma (Control 1 and Control 2, 0/50 [0%]; 10 mg/kg/day, 1/50 [2%]; 50 mg/kg/day, 1/50 [2%]; 250 mg/kg/day, 3/50 [6%]; P=0.014, Trend test) and carcinoma (Control 1 and Control 2, 0/50 [0%]; 10 mg/kg/day, 1/50 [2%]; 50 mg/kg/day, 1/50 [2%]; 250 mg/kg/day, 2/50 [4%]; P=0.036, Trend test) in male rats. The incidences of these tumors at the high dose in male rats were higher than the historical control incidences from the conducting laboratory (follicular cell adenoma, 0% to 4%; follicular cell carcinoma, 0% to 2%). Treatment group females had higher incidences of hepatocellular adenoma (Control 1, 1/50 [2%]; control 2, 1/50 [2%]; 10 mg/kg/day, 1/50 [2%]; 50 mg/kg/day, 4/50 [8%]; 250 mg/kg/day, 6/50 [12%]; P=0.003, Trend test) and thyroid follicular cell adenoma (Control 1, 3/50 [6%]; control 2, 1/50 [2%]; 10 mg/kg/day, 1/50 [2%]; 50 mg/kg/day, 4/50 [8%]; 250 mg/kg/day, 6/50 [12%]; P=0.018, Trend test).

In the third 2-year carcinogenicity study with MK-0869 in Sprague-Dawley rats, the drug was administered at doses of 10, 250, 1000 and 2000 mg/kg/day. The sponsor stated that the high dose was the maximum feasible dose. Male rats treated with MK-0869 had significantly increased trends for thyroid parafollicular cell carcinoma [control 1, 1/50 (2%); control 2, 0/50 (0%); 10 mg/kg, 1/50 (2%); 250 mg/kg, 1/50 (2%); 1000 mg/kg, 2/50 (4%); 2000 mg/kg, 5/50 (10%); p=0.005, sponsor; p=0.0027, CDER statistician; trend test] and thyroid follicular cell adenoma [control 1, 1/50 (2%); control 2, 1/50 (2%); 10 mg/kg, 2/50 (4%); 250 mg/kg, 6/50 (12%); 1000 mg/kg, 2/50 (4%); 2000 mg/kg, 8/50 (16%); p=0.002, sponsor; p=0.0025, CDER statistician; trend test] and carcinoma [control 1, 0/50 (0%); control 2, 0/50 (0%); 10 mg/kg, 0/50 (0%); 250 mg/kg,

0/50 (0%); 1000 mg/kg, 2/50 (4%); 2000 mg/kg, 1/50 (2%);  $p=0.042$ , trend test]. The combined incidences of thyroid follicular cell adenoma plus carcinoma in male rats was also significant ( $p=0.0004$ , trend test). The incidence of thyroid parafollicular cell carcinoma in male rats at the high dose (10%) was higher than the historical control incidences from the conducting laboratory (1%-6%; mean, 1.7%). The incidences of thyroid follicular cell adenoma at 250 and 2000 mg/kg/day (12% at 250 mg/kg, and 16% at 2000 mg/kg) and carcinoma (4% at 1000 mg/kg, and 2% at 2000 mg/kg) in the male rats were higher than historical control incidences (adenoma, 0%-6%, mean 1%; carcinoma, 0%-2%; mean 0.3%) for this tumors in this strain of rats from the conducting laboratory.

Female rats had significantly increased trends for thyroid follicular cell adenoma [control 1, 0/50 (0%); control 2, 0/50 (0%); 10 mg/kg, 0/50 (0%); 250 mg/kg, 4/50 (8%); 1000 mg/kg, 9/50 (18%); 2000 mg/kg, 3/50 (6%);  $p < 0.001$ , sponsor;  $p=0.0018$ , CDER statistician; trend test], and hepatocellular adenoma [control 1, 0/50 (0%); control 2, 0/50 (0%); 10 mg/kg, 4/50 (8%); 250 mg/kg, 5/50 (10%); 1000 mg/kg, 4/50 (8%); 2000 mg/kg, 5/50 (10%);  $p=0.004$ , sponsor;  $p=0.0231$ , CDER statistician; trend test] and carcinoma [control 1, 0/50 (0%); control 2, 0/50 (0%); 10 mg/kg, 0/50 (0%); 250 mg/kg, 3/50 (6%); 1000 mg/kg, 5/50 (10%); 2000 mg/kg, 3/50 (6%);  $p=0.001$ , sponsor;  $p=0.0048$ , CDER statistician; trend test]. The combined incidences of hepatocellular adenoma plus carcinoma in female rats was also significant ( $p=0.0005$ , trend test). The incidence of thyroid follicular cell adenoma in female rats (8%, 18% and 6% at 250, 1000 and 2000 mg/kg, respectively) was higher than the historical control incidences for this tumor from the conducting laboratory (0%-6%; mean, 0.6%). The incidences of hepatocellular adenoma (8%, 10%, 8% and 10% at 10, 250, 1000 and 2000 mg/kg doses, respectively) and carcinoma (6%, 10% and 6% at 250, 1000 and 2000 mg/kg, respectively) in female rats were also higher than the historical control incidences in this strain of rat from the conducting laboratory (adenoma, 0%-8%, mean 2.2%; carcinoma, 0%-8%, mean 0.8%).

### Mouse Carcinogenicity Studies:

In the first 105-week oral carcinogenicity study in mice, MK-0869 doses of 2.5, 25, 125 and 500 mg/kg/day were used. The sponsor's dose selection for the mouse carcinogenicity study was based on saturation of absorption of MK-0869. As the exposure levels of only the parent compound was taken into account in determining the exposure levels, the CDER Executive CAC Committee did not concur with the doses. However in a subsequent 5-week oral toxicokinetic study, it was demonstrated that saturation was achieved at the high dose used in the carcinogenicity study. MK-0869 was found to be carcinogenic in male and female mice in the 105-week oral carcinogenicity study. There were increased incidences of fibrosarcoma (Control 1 and 2, 0/50 [0%]; 2.5 mg/kg, 0/50 [0%]; 25 mg/kg, 0/50 [0%]; 125 mg/kg, 1/50 [2%]; 500 mg/kg, 2/50 [4%];  $P=0.018$ , Trend test) of the skin in male mice. The incidences of fibrosarcoma at the high dose (4%) was higher than the historical control incidences from the sponsor's laboratory (0% to 1%; mean, 0.09%) in this strain of mice and the spontaneous incidences reported by \_\_\_\_\_ (1.54% to 2.00%; mean, 1.77%). In female mice, there were increased incidences of hepatocellular adenoma (Control 1, 1/49 [2%]; Control 2, 0/50 [0%]; 2.5 mg/kg, 2/50 [4%]; 25 mg/kg, 0/50 [0%]; 125 mg/kg, 4/50 [8%]; 500 mg/kg, 4/50 [8%];  $P=0.014$ ; Trend test) and Harderian gland adenoma (Control 1, 2/49 [4%]; Control 2, 1/50 [2%]; 2.5 mg/kg, 1/50 [2%]; 25 mg/kg, 2/50 [4%]; 125 mg/kg, 2/50 [4%]; 500 mg/kg, 4/50 [8%];  $P=0.014$ ; Trend test). The

b(4)

incidences of hepatocellular adenoma at the two higher doses (8%) were higher than the historical control incidences from the sponsor's laboratory (1% to 4%; mean, 2%). The incidence of Harderian gland adenoma at the high dose (8%) is similar to the historical control incidences from the sponsor's laboratory (4% to 6%; mean, 5.27%) and the spontaneous incidences reported (1.35% to 8.33%, ~~in the strain of mice~~) in the strain of mice. However, according to the CDER's statistical standard, the incidences of these tumors were not significant. b(4)

In the second 2-year oral (gavage) carcinogenicity study with MK-0869 in CD-1 mice, the drug was administered at dose levels of 500, 1000 and 2000 mg/kg/day. The sponsor stated that the high dose was the maximum feasible dose. Male mice treated with MK-0869 had a significantly increased trend for hepatocellular carcinoma [control 1, 10/50 (20%); control 2, 6/50 (12%); 500 mg/kg, 5/50 (10%); 1000 mg/kg, 13/50 (26%); 2000 mg/kg, 16/50 (32%);  $p=0.002$ , sponsor;  $p=0.0013$ , CDER statistician; trend test]. The combined incidences of hepatocellular adenoma and carcinoma in male mice were also significant ( $p=0.0036$ , trend test). The incidences of hepatocellular carcinoma in male mice at 1000 (26%) and 2000 (32%) mg/kg/day doses were higher than historical control incidences for this tumor in this strain of mice from the conducting laboratory (2%-22%; mean, 9%).

Female mice receiving MK-0869 also had a significantly increased trend for hepatocellular carcinoma [control 1, 2/50 (4%); control 2, 0/50 (0%); 500 mg/kg, 5/50 (10%); 1000 mg/kg, 8/50 (16%); 2000 mg/kg, 4/50 (8%);  $p=0.019$ , sponsor; trend test]. However, according to the CDER statistical standard, the incidence of hepatocellular carcinoma in female mice was not significant ( $p>0.005$ ). The incidences of hepatocellular carcinoma in treatment group female mice were higher than historical control incidences for this tumor from the conducting laboratory (0%-6.1%; mean, 0.5%).

#### Summary:

The sponsor conducted three 2-year oral carcinogenicity studies in Sprague-Dawley rats and two 2-year oral carcinogenicity studies in CD-1 mice with aprepitant (MK-0869). In the rat carcinogenicity studies, animals were treated with oral doses ranging from 0.05 to 1000 mg/kg twice daily. The highest dose produced systemic exposure to aprepitant (plasma  $AUC_{0-24 \text{ hr}}$ ) of 0.7 to 1.6 times the human exposure ( $AUC_{0-24 \text{ hr}} = 19.6 \text{ mcg.hr/ml}$ ) at the recommended dose of 125 mg/day. Treatment with aprepitant at a dose of 1000 mg/kg twice daily caused an increase in thyroid parafollicular cell carcinoma, and at doses of 5 to 1000 mg/kg twice daily it caused an increase in the incidences of thyroid follicular cell adenomas and carcinomas in male rats. In female rats, it produced hepatocellular adenomas at 5 to 1000 mg twice daily and hepatocellular carcinomas and thyroid follicular adenomas at 125 to 1000 mg/kg twice daily. In the mouse carcinogenicity studies, the animals were treated with oral doses ranging from 2.5 to 2000 mg/kg/day. The highest dose produced a systemic exposure of about 2.8 to 3.6 times the human exposure at the recommended dose. Treatment with aprepitant caused an increase in the incidences of hepatocellular adenomas and/or carcinomas at 1000 and 2000 mg/kg/day, and it produced skin fibrosarcomas at 125 and 500 mg/kg/day doses in male mice.

2.6.6.6 Reproductive and developmental toxicology

1. Segment I. Fertility and Reproductive Performance Study of I.V. L-758,298 in Female Rats (TT #96-714-0).

Testing Laboratory: Merck Research Laboratories  
West Point, PA 19486

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

Study Started: March 8, 1996

Study Completed: October 3, 1996

Animals: Female Sprague-Dawley rats (203 to 275 g; approximately 9 weeks of age).

APPEARS THIS WAY  
ON ORIGINAL

**Methods:** In an exploratory intravenous toxicity study in rats (TT #95-2559), it was determined that the highest feasible concentration of L-758,298 for repeated administration was 0.4 mg/ml; higher concentrations produced vascular irritation. In a range-finding study (TT #96-703-5) of intravenously administered L-758,298 (0, 0.5, 1, 2 and 4 mg/kg/day from Gestation Day 6 through Lactation Day 21) in female rats, the 4 mg/kg/day dose produced a decrease in body weight gain (-26% of difference from control). There were no other treatment-related effects.

Thus, 4 groups of 24 female rats each were intravenously administered 0, 1, 2 and 4 mg/kg/day of L-758,298, respectively, via the tail vein for 14 days prior to cohabitation (Days 1 to 14), during cohabitation (Day 15 up to Day 34; females were mated with untreated males on a 1:1 ratio for a maximum of 20 nights), and through Gestation Day 7 (the day of finding a vaginal plug and/or sperm in the vagina was defined as Gestation Day 0). Vehicle was 0.9% saline solution; dosing volume was 10 ml/kg; injection rate was 2 ml/min.

Dams were observed daily for clinical signs of toxicity and mortality from initiation of treatment through Gestation Day 8, 12 or 15; dams were observed for 1 to 5 hr post-dosing. Body weights were recorded on Pre-cohabitation Days 1, 4, 8, 11 and 14; during cohabitation on Days 22 and 29; if not mated, on Days 36, 43 and 49; and on Gestation Days 0, 2, 4, 6, 8, 12 and 15. Food consumption was recorded on Pre-cohabitation Days 1 to 5 and 8 to 12 and on Gestation Days 1 to 5 and 8 to 12.

Dams were euthanized on Gestation Day 15, 16 or 17 by CO<sub>2</sub> asphyxiation. Numbers of corpora lutea, implantations, pre- and post-implantation loss, resorptions, and live and dead fetuses were determined. All dams were subjected to a thoracic and visceral examination. Fetuses were euthanized by rapid induction of hypothermia.

Data were statistically analyzed by trend tests and analyses of variance or covariance.

#### **Results:**

##### **Dams**

1. **Observed Effects:** There were no treatment-related clinical signs of toxicity.
2. **Mortality:** There were no deaths.

3. Body Weight: Mean body weights of control females were 236 and 245 g on Days 1 and 14 of the pre-cohabitation period, respectively. Mean body weights of control pregnant females were 256 and 346 g on Gestation Days 0 and 15, respectively. There were no treatment-related effects on body weight.

4. Food Consumption: Mean food consumption of control females was 19 and 18 g/day on Days 5 and 12 of the pre-cohabitation period, respectively. Mean food consumption of control pregnant females was 25 and 27 g on Gestation Days 5 and 12, respectively. There were no treatment-related effects on food consumption.

5. Fertility and Reproductive Performance: As shown in the following table, there were no treatment-related effects on fertility and reproductive performance in female rats.

Summary of Fertility and Reproductive Performance of Female Rats in a Segment I. Reproductive Toxicity Study.

Treatment Dose (mg/kg/day, i.v.)	<u>Vehicle</u>			
	0	1	<u>L-758,298</u>	
			2	4
Females cohabited	24	24	24	24
Mated females	24	24	24	23
Pregnant females	23	20	21	20
Matings per 4-day periods of cohabitation:				
Days 1-4	21	23	21	19
5-8	1	1	0	1
9-12	1	0	0	1
13-16	1	0	3	2
17 or later	0	0	0	0
Time to mating (4- day periods)	1.25	1.04	1.38	1.39
*Mating index	0.92	0.98	0.91	0.85
Mated females/ females cohabited (%)	100%	100%	100%	96%
*Fecundity index (%)	96%	83%	88%	87%
*Fertility index (%)	96%	83%	88%	83%

\*Mean of  $(1/(\text{Time to mating}))$  for mated females and zero for females that did not mate.

\*Pregnant females/mated females.

\*Pregnant females/females cohabited.

6. Dam and Fetal Data: As shown in the following table, there were no treatment-related effects on number of pregnant females, corpora lutea, and implantations, and on % implantation loss and resorptions after euthanasia on Gestation Day 15, 16 or 17. There were no treatment-related effects on number of live fetuses.

Summary of Dam and Fetal Data After Euthanasia on Gestation Day 15, 16 or 17 in a Segment I. Study of Fertility and Reproductive Performance in Rats.

Treatment Dose (mg/kg/day, i.v.)	<u>Vehicle</u>	<u>L-758,298</u>		
	0	1	2	4
Total females	24	24	24	24
No. Pregnant females	23	20	21	20
No. Died	0	0	0	0
Mean Corpora lutea/dam	17.5	17.0	16.8	16.2
Mean Implan- tations/dam	16.1	15.8	15.8	15.3
% Pre-implantation loss/litter	7.5%	8.0%	7.5%	7.9%
% Post-implantation loss/litter	5.2%	4.5%	7.3%	9.1%
% Resorptions/im- plantation	5.2%	4.5%	7.3%	7.6%
Mean Dead fetuses/ dam	0	0	0	1.5
Mean Live fetuses/ dam	15.3	15.0	14.6	13.8

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

In summary, there were no treatment-related effects of i.v. L-758,298 (0, 1, 2 and 4 mg/kg/day) on fertility and reproductive performance of female rats. Furthermore, in a previous 4-week i.v. toxicity study of L-758,298 (0, 0.25, 1 and 4 mg/kg/day) in male and female rats, 4 mg/kg/day was the no effect dose. Thus, since the high dose of L-758,298 did not produce any toxicity in either of the above studies, it does not appear to be adequate. However, 4 mg/kg/day was originally selected as the high dose for the reproductive toxicity studies because it produced a decrease in body weight gain (-26%; % of difference from control) in a range-finding study where female rats received i.v. L-758,298 from Gestation Day 6 through Lactation Day 21. Furthermore, in an exploratory intravenous study using rats, it was determined that the highest feasible concentration of L-758,298 for repeated i.v. administration was 0.4 mg/ml; higher concentrations produced vascular irritation. Thus, based upon data from these preliminary studies, the selection of a high i.v. dose of 4 mg/kg/day for the reproductive toxicity study appeared to be reasonable.

**Study title: Oral Fertility Study in Female Rats**

**Key study findings:** MK-0869 ( \_\_\_\_\_ particle size), administered to female rats at an oral dose of 1000 mg/kg b.i.d. (2000 mg/kg/day), had no effect on the reproductive performance or fertility of the animals. b(4)

**Study no:** #01-735-0

**Volume #, and page #:** volume #21, page # B59

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

**Date of study initiation:** December 04, 2001

**GLP compliance:** Yes

**QA reports:** yes (X) no ( )

**Drug, lot #, radiolabel, and % purity:** L-754030 blended- \_\_\_\_\_ ; Batch #X0869OPP024C001; purity, 99.8% b(4)

**Formulation/vehicle:** MK-0869 colloidal dispersions (200 mg/ml) were prepared by suspending the \_\_\_\_\_ in deionized water containing hydroxypropylcellulose, sucrose and sodium lauryl sulfate. The average particle size of the drug in colloidal dispersions was approximately \_\_\_\_\_.

**Methods:**

**Species/strain:** \_\_\_\_\_ BR Sprague-Dawley rats.

**Doses employed:** 1000 mg/kg b.i.d. (2000 mg/kg/day) b(4)

**Route of administration:** Oral gavage

**Study design:** The effects of MK0869 ( \_\_\_\_\_ particle size) on the fertility of female Sprague-Dawley rats were evaluated following oral administration of the drug for 14 days prior to cohabitation, during cohabitation and through Gestation Day 7. Two control groups and one treatment group of animals were used in the study. Control 1 group received 0.5% methylcellulose in deionized water and Control 2 group received 4% hydroxypropylcellulose, 20% sucrose and 0.19% SLS in deionized water.

**Number/sex/group:** 24 females/group

**Parameters and endpoints evaluated:** The animals were observed daily for clinical signs during the treatment period and on Gestation Days 12 and 15. Body weights and food consumptions were recorded at regular intervals. Females with a confirmed mating were euthanized between Gestation Days 15 and 17, the uteri were examined for pregnancy and the total number of corpora lutea per animal were counted. Uterine implants were counted and classified as live fetus, dead fetus or resorption. Necropsy examinations, limited to thoracic and abdominal viscera, were performed on all females.

**Results:**

**Mortality:** There were no deaths of animals in any group.

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

**Body weight:** The mean body weights of the control 1 and control 2 females on Treatment Day 1 were  $231 \pm 11$  g and  $229 \pm 10$  g, respectively. There were no treatment-related changes in the body weights of animals receiving MK-0869, as compared with the controls.

**Food consumption:** The mean food consumption of control 1 and control 2 females on Premating Day 5 were 21±2 and 20±1 g/day, respectively. The food consumption of the treatment group females was 14.3% and 9.5% lower than control 1 on Premating Days 5 and 12, respectively.

**In-life observations:** Treatment with MK-0869 (1000 mg/kg b.i.d.) had no significant effect on the mating performance or fertility of the female rats. All 24 female animals in each group were pregnant.

**Terminal and necropsic evaluations:** Treatment with MK-0869 had no significant effect on the numbers of corpora lutea, implantations, resorptions or live fetuses. The pregnancy data are summarized in the sponsor's Table below.

TABLE 5. L-754,030: ORAL FERTILITY STUDY IN FEMALE RATS. TT 495-729-0  
SUMMARY OF LAPAROTOMY DATA

TREATMENT GROUP:	CONTROL	5 MG/KG/DAY	20 MG/KG/DAY	250 MG/KG/DAY
<b>FEMALES</b>				
TOTAL FEMALES	24	24	24	24
PREGNANT	21	22	23	20
EXAMINED LIVE LITTER	21	22	23	20
RESORBED OR DEAD LITTER	0	0	0	0
DIED	0	0	0	0
SACRIFICED	0	0	0	0
NOT PREGNANT	3	2	1	4
LIVE	3	2	1	4
DIED	0	0	0	0
SACRIFICED	0	0	0	0
NOT NEED	0	0	0	0
<b>CORPORA LUTEA</b>				
CORPORA LUTEA	330	392	378	326
CORPORA LUTEA/PREGNANT FEMALE	15.7 ± 1.6	17.8 ± 2.0	16.3 ± 2.3	16.3 ± 1.9
% PRE-IMPLANTATION LOSS (LITTER MEAN)	8.2 ± 17.6	11.2 ± 12.2	7.1 ± 8.8	4.6 ± 4.9
<b>IMPLANTS</b>				
IMPLANTS	306	348	347	310
IMPLANTS/PREGNANT FEMALE	14.6 ± 3.4	15.7 ± 2.1	15.1 ± 2.1	15.5 ± 1.4
<b>RESORPTIONS AND DEAD FETUSES</b>				
RESORPTIONS	13	17	30	18
% RESORPTIONS/IMPLANTS (LITTER MEAN)	3.9 ± 6.3	5.0 ± 5.6	2.9 ± 3.9	6.3 ± 6.8
DEAD FETUSES	0	0	0	0
% DEAD FETUSES/IMPLANTS (LITTER MEAN)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
% POSTIMPLANTATION LOSS (LITTER MEAN)	3.9 ± 6.3	5.0 ± 5.6	2.9 ± 3.9	6.3 ± 6.8
<b>LIVE FETUSES</b>				
LIVE FETUSES	293	329	317	291
UNDETERMINED SEX	293	329	317	291
LIVE FETUSES/PREGNANT FEMALE	14.0 ± 3.1	15.0 ± 2.3	14.7 ± 2.2	14.6 ± 1.9
% PRE-IMPLANTATION LOSS = (( NO. CORPORA LUTEA - NO. IMPLANTS ) / NO. CORPORA LUTEA ) X 100 % POSTIMPLANTATION LOSS = (( NO. RESORPTIONS + NO. DEAD FETUSES ) / NO. IMPLANTS ) X 100				

In summary, in the oral fertility study in female Sprague-Dawley rats, MK-0869 (  $\mu$ m particle size) was administered at a dose of 1000 mg/kg b.i.d (2000 mg/kg/day) for 14 days prior to mating, during mating period through Gestation Day 7. Treatment with MK-0869 was not associated with any changes in mating performance or fertility of the female rats. There were no treatment-related effects on embryonic/fetal survival in the F<sub>1</sub> generation.

b(4)

**Study title: Oral Fertility Study in Male Rats**

**Key study findings:** MK-0869 had no effect on the reproductive performance or fertility of male SD rats at oral doses up to 250 mg/kg/day.

**Study no:** #97-734-0

**Volume #, and page #:** volume #21, page # B104

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

**Date of study initiation:** September 08, 1997

**GLP compliance:** Yes

**QA reports:** yes (X) no ( )

**Drug, lot #, radiolabel, and % purity:** MK-0869, Lot # L-754, 030-004H021); purity, 99.3%.

**Formulation/vehicle:** MK-0869 was dispersed in deionized water containing 0.5% methylcellulose and 0.02% sodium lauryl sulfate.

**Methods:**

**Species/strain:** \_\_\_\_\_ BR Sprague-Dawley rats.

**Doses employed:** 25, 125 and 250 mg/kg/day.

**Route of administration:** Oral gavage

**Study design:** The effects of MK0869 on the fertility of male Sprague-Dawley rats were evaluated following oral administration for 51 to 53 days. Four groups of male animals received 0, 25, 125 and 250 mg/kg/day of MK-0869 for 28 days prior to cohabitation, during cohabitation and until 1 day prior to scheduled sacrifice (a total of 51-53 days). The males were housed with untreated females (1:1 ratio) following 4 weeks of treatment. Copulation was confirmed by the presence of a copulatory plug in the vagina or sperm in the vaginal lavage. Following the 5<sup>th</sup> night, any apparently not bred female was replaced with a virgin female, and the day of confirmed mating was considered as Gestation Day 0.

**Number/sex/group:** 25 males/group

**Parameters and endpoints evaluated:** The animals were observed daily for clinical signs during the study period. Body weights of the males were measured twice weekly and the body weights of the females were measured on pre mating day 1 and gestation days 0, 7 and 15. Food consumption was recorded twice weekly. Bred females were sacrificed on Gestation Days 15 to 17, the uterus was examined for pregnancy or implantation sites and the number of corpora lutea were counted. Uterine implants were counted and classified as live fetus, dead fetus or resorption. Females that failed to copulate during the first 5 days were sacrificed without further examination.

Males were sacrificed in Drug Week 8 and gross examinations of the thoracic and abdominal viscera were conducted. The left cauda epididymis and the weights of testes of all animals were recorded. The left cauda epididymides of all control and 250 mg/kg/day males were homogenized and the epididymal sperm heads were counted. Sperm motility was analyzed from 16 males from each group.

**Results:**

**Mortality:** There were no deaths of animals in any group.

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

**Body weight:** The mean body weight of the control males in Drug Week 1 was 306 ± 19 g. There were no treatment-related changes in the body weight of animals in any group.

b(4)

**Food consumption:** There were no treatment-related changes in the food consumption in any group.

**In-life observations:** Treatment with MK-0869 (1000 mg/kg b.i.d.) had no significant effect on the mating performance or fertility indices, as compared with the controls. There were 21 (of 25), 23 (of 25), 22 (of 25) and 23 (of 25) pregnant females in the control, low, mid and high dose, respectively. The effects of MK-0869 on the reproductive performance of the male rats are summarized in the sponsor's Table below.

TABLE 5. MK-0869: ORAL FERTILITY STUDY IN MALE RATS. TT #97-734-0  
SUMMARY OF REPRODUCTIVE PERFORMANCE OF MALES

	CONTROL	25 MG/KG/DAY	125 MG/KG/DAY	250 MG/KG/DAY
FEMALES COHABITED <sup>a</sup>	25 (11)	25 (11)	25 (2)	25 (3)
MALES COHABITED	25	25	25	25
MATED FEMALES	25	25	24	25
PREGNANT FEMALES	21	23	22	23
DIED DURING GESTATION	0	0	0	0
SACRIFICED DURING GESTATION	0	0	0	0
CESAREAN SECTIONED	21	23	22	23
NONPREGNANT FEMALES	4	2	2	2
LIVE	4	2	2	2
DIED	0	0	0	0
SACRIFICED	0	0	0	0
NOT BREED	0	0	1	0
LIVE	0	0	1	0
MATINGS PER 4-DAY PERIODS OF COHABITATION:				
DAYS 1 TO 4	25	23	23	24
DAYS 5 TO 8	0	2	1	1
DAYS 9 TO 12	0	0	0	0
DAYS 13 TO 16	0	0	0	0
DAYS 17 OR LATER	0	0	0	0
MATING INDEX MATED FEMALES/FEMALES COHABITED, % <sup>b</sup>	100	100	96	100
FECUNDITY INDEX PREGNANT FEMALES/MATED FEMALES, %	84	92	92	92
FERTILITY INDEX PREGNANT FEMALES/FEMALES COHABITED, % <sup>b</sup>	84	92	88	92

a - NUMBER IN PARENTHESIS INDICATES FEMALES THAT DID NOT MATE DURING THE FIRST 5 NIGHTS OF COHABITATION AND THAT WERE REMOVED AND REPLACED FOR THE LAST 5 NIGHTS.  
b - CALCULATIONS EXCLUDE FEMALES THAT DID NOT MATE DURING THE FIRST 5 NIGHTS OF COHABITATION.

**Terminal and necropsic evaluations:** Treatment with MK-0869 was not associated with any significant changes in average cauda epididymal weights, sperm counts, or sperm motility in any group. The effects of MK-0869 on epididymal weight, sperm count and sperm motility of male animals are summarized in the sponsor's Table below.

TABLE 7. MK-0869: ORAL FERTILITY STUDY IN MALE RATS. TT #97-734-0  
SUMMARY OF CAUDA EPIDIDYMAL WEIGHTS AND SPERM COUNTS AND VAS DEFERENS SPERM MOTILITY

	CONTROL	25 MG/KG/DAY	125 MG/KG/DAY	250 MG/KG/DAY	
CAUDA EPIDIDYMAL WEIGHT (GRAMS)	0.34 +/- 0.01 (25)	0.33 +/- 0.01 (25)	0.33 +/- 0.01 (25)	0.33 +/- 0.01 (25)	ITY
SPERM COUNT/GRAM CAUDA EPIDIDYMIS (x 10 <sup>6</sup> )	3.11 +/- .13	NE	NE	2.98 +/- .11	AY
SPERM COUNT/GRAM CAUDA EPIDIDYMIS (x 10 <sup>6</sup> )	2.14 +/- .30	NE	NE	2.02 +/- .30	01 (25)
% SPERM MOTILITY	88.9 +/- 1.5 (16)	85.8 +/- 2.4 (16)	89.2 +/- 1.4 (16)	89.6 +/- 1.0 (16)	11
VALUES ARE MEANS ± S.E.M. NE = NOT EVALUATED ( ) = GROUP SIZE, AND APPEARS ONLY IF DIFFERENT FROM PREVIOUS N. SEE INDIVIDUAL TABLES FOR EXCLUSIONS.					10
					0 (16)

NE = NOT EVALUATED  
( ) = GROUP SIZE, AND APPEARS ONLY IF DIFFERENT FROM PREVIOUS N. SEE INDIVIDUAL TABLES FOR EXCLUSIONS.

There were no treatment-related effects on embryonic/fetal survival as determined by the number of implants and live fetuses per pregnant females or peri- and post- implantation losses.

In summary, in the oral fertility study in male Sprague-Dawley rats, MK-0869 was administered to the animals at oral doses of 0, 25, 125 and 250 mg/kg/day. Treatment with MK-0869 was not associated with any changes in mating performance of the males. There were no effects on the epididymides weight, sperm counts or sperm motility. Treatment of the male rats with MK-0869 had no effect on embryonic/fetal survival. Thus, MK-0869 had no effect on the reproductive performance or fertility of male rats at oral doses up to 250 mg/kg/day.

**Study title: Oral Fertility Study in Male Rats**

**Key study findings:** MK-0869 (\_\_\_\_\_ article size), at an oral dose of 1000 mg/kg b.i.d (2000 mg/kg/day) had no effect on the reproductive performance or fertility of male rats. b(4)

**Study no:** #01-737-0

**Volume #, and page #:** volume #21, page # B167

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

**Date of study initiation:** December 03, 2001

**GLP compliance:** Yes

**QA reports:** yes (X) no ( )

**Drug, lot #, radiolabel, and % purity:** MK-0869- \_\_\_\_\_ ; Batch #X0869OPP024C001; purity, 99.8%.

**Formulation/vehicle:** MK-0869 \_\_\_\_\_ were suspended in deionized water containing hydroxypropylcellulose (4%), sucrose (20%) and SLS (0.19%). The average particle size of the drug in colloidal dispersions was approximately \_\_\_\_\_. b(4)

**Methods:**

**Species/strain** \_\_\_\_\_ 3R Sprague-Dawley rats.

**Doses employed:** 1000 mg/kg b.i.d. (2000 mg/kg/day)

**Route of administration:** Oral gavage

**Study design:** The effects of MK0869 (\_\_\_\_\_ nm particle size) on the fertility of male Sprague-Dawley rats were evaluated following oral administration of a 1000 mg/kg b.i.d. dose to the animals for 29 days prior to mating, during and after mating until the day prior to sacrifice (gestation days 15-17). Two control groups and one treatment group of animals were used in the study. Control 1 group received 0.5% methylcellulose in deionized water, and Control 2 group received 4% hydroxypropylcellulose, 20% sucrose and 0.19% SLS in deionized water. b(4)

**Number/sex/group:** 24 females/group

**Parameters and endpoints evaluated:** The animals were observed daily for clinical signs and mortality. Body weights and food consumptions were recorded twice weekly. All females were euthanized between Gestation Days 15 and 17, the uteri were examined for pregnancy and the total

number of corpora lutea per animal was counted. Uterine implants were counted and classified as live fetus, dead fetus or resorption.

Males were sacrificed in Drug Week 8 and gross examinations of the thoracic and abdominal viscera were conducted. The left cauda epididymis and the testes weights of all animals were recorded. The left cauda epididymides of all control and 250 mg/kg/day males were homogenized and the epididymal sperm heads were counted. Sperm motility was analysed from 16 males from each group.

**Results:**

**Mortality:** There were no deaths of animals in any group.

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

**Body weight:** The mean body weights of the control 1 and control 2 males on Treatment Week 1 were 293 ± 10 g and 296 ± 11 g, respectively. The treatment group males had slightly higher body weights during the treatment period, as compared with Control 1 group. The body weights of the treated males were 5.3%, 5.7%, 6.4% and 5.4% higher than control 1 in weeks 2, 4, 6 and 8, respectively. The mean body weight gain from Week 1 to Week 5 of the treated males was 125% of the control 1 group. However it was only 107% of the control 2 (vehicle) group.

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

**In-life observations:** Treatment with MK-0869 (1000 mg/kg b.i.d.) had no significant effect on sperm motility, mating index (mated females/females cohabitated, percent), fecundity index (pregnant females/mated females, percent) or fertility index (pregnant females/females cohabited, percent). The reproductive performances of the male animals of different groups are summarized in the sponsor's Table below.

TABLE A-4. MK-0869: ORAL FERTILITY STUDY IN MALE RATS. IT 901-737-3  
SUMMARY OF REPRODUCTIVE PERFORMANCE OF MALES

	CONTROL 1 B.I.D.	CONTROL 2 B.I.D.	1000 MG/KG B.I.D.
FEMALE COHABITATION <sup>a</sup>	24	24(10)	24(11)
MALES COHABITED	24	24	24
MATED FEMALES	24	24	24
PREGNANT FEMALES	24	24	24
DEAD DURING GESTATION	0	0	0
RESORPTIONS DURING GESTATION	0	0	0
CRESORPTIONS DURING GESTATION	24	24	24
NONPREGNANT FEMALES	0	0	0
LIVE	0	0	0
DEAD	0	0	0
SACRIFICED	0	0	0
NOT MATED	0	0	0
MATING INDEX <sup>b</sup>	100	100	100
MATED FEMALES/FEMALES COHABITED, %			
FECUNDITY INDEX	100	100	100
PREGNANT FEMALES/MATED FEMALES, %			
FERTILITY INDEX <sup>b</sup>	100	100	100
PREGNANT FEMALES/FEMALES COHABITED, %			

<sup>a</sup> NUMBER IN PARENTHESES INDICATES FEMALES THAT DID NOT MATE DURING THE FIRST 5 HOURS OF COHABITATION AND THAT WERE REMOVED AND REPLACED FOR THE LAST 3 HOURS.

<sup>b</sup> CALCULATION EXCLUDES FEMALES THAT DID NOT MATE DURING THE FIRST 5 HOURS OF COHABITATION.

**Terminal and necropsic evaluations:** Treatment of the male rats with MK-0869 had no significant effect on cauda epididymal weight and sperm count of the males, and peri- and post-implantation losses and the number of implants and live fetuses per pregnant female. The sperm counts and sperm motility data of the male animals of different groups are summarized in the sponsor's Table below.

TABLE A-4. MK-0869: ORAL FERTILITY STUDY IN MALE RATS. IT 401-737-0  
SUMMARY OF CAUDA EPIDIDYMIS SPERM COUNTS AND WAS DEFERENS SPERM MOTILITY

	CONTROL 1 B.I.D.	CONTROL 2 B.I.D.	1000 MG/KG B.I.D.
SPERM COUNT/CAUDA EPIDIDYMIS (X 10 <sup>6</sup> ) ± S.D.	263.0 ± 53.9(24)	269.4 ± 37.8(24)	271.3 ± 64.9(24)
SPERM COUNT/ GRAM CAUDA EPIDIDYMIS (X 10 <sup>6</sup> ) ± S.D.	767.4 ± 139.7(24)	771.4 ± 107.4(24)	772.7 ± 162.8(24)
‡ SPERM MOTILITY ± S.D.	89.0 ± 4.2(16)	89.4 ± 3.9(16)	87.4 ± 8.8(16)

VALUES ARE MEANS ± S.D.  
(N) = GROUP SIZE AND APPEARS ONLY IF DIFFERENT FROM PREVIOUS N.

The pregnancy data for the female rats are summarized in the sponsor's Table below.

TABLE A-5. MK-0869: ORAL FERTILITY STUDY IN MALE RATS. IT 401-737-0  
SUMMARY OF LABORATORY DATA

TREATMENT GROUP:	CONTROL 1 B.I.D.	CONTROL 2 B.I.D.	1000 MG/KG B.I.D.
<b>FEMALES</b>			
TOTAL FEMALES	24	24	24
PREGNANT	24	24	24
EXAMINED LIVE LITTER	24	24	24
ABORTED OR DEAD LITTER	0	0	0
DIED	0	0	0
SACRIFICED	0	0	0
NOT PREGNANT	0	0	0
LIVE	0	0	0
DIED	0	0	0
SACRIFICED	0	0	0
NOT NEEDED	0	0	0
<b>CORPORA LUTEA</b>			
CORPORA LUTEA	431	413	421
CORPORA LUTEA/PREGNANT FEMALE	18.0 ± 3.4	17.2 ± 1.8	17.5 ± 1.9
% PERI-IMPLANTATION LOSS (LITTER MEAN)	3.4 ± 0.1	4.1 ± 3.9	3.1 ± 3.9
<b>IMPLANTS</b>			
IMPLANTS	404	395	395
IMPLANTS/PREGNANT FEMALE	16.8 ± 2.3	16.5 ± 1.6	16.6 ± 1.9
<b>RESORPTIONS AND DEAD FETUSES</b>			
RESORPTIONS	18	14	15
% RESORPTIONS/IMPLANTS (LITTER MEAN)	4.5 ± 5.3	3.6 ± 4.7	3.7 ± 7.1
DEAD FETUSES	1	1	0
% DEAD FETUSES/IMPLANTS (LITTER MEAN)	0.3 ± 1.4	0.2 ± 1.1	0.0 ± 0.0
% POSTIMPLANTATION LOSS (LITTER MEAN)	4.8 ± 5.7	3.8 ± 4.7	3.7 ± 7.1
<b>LIVE FETUSES</b>			
LIVE FETUSES	385	389	384
UNDETERMINED SEX	385	389	384
LIVE FETUSES/PREGNANT FEMALE	16.0 ± 2.3	16.2 ± 1.8	16.0 ± 2.0

% PERI-IMPLANTATION LOSS = (( NO. CORPORA LUTEA - NO. IMPLANTS ) / NO. CORPORA LUTEA ) X 100  
% POSTIMPLANTATION LOSS = (( NO. RESORPTIONS + NO. DEAD FETUSES ) / NO. IMPLANTS ) X 100

In summary, in the oral fertility study in male Sprague-Dawley rats, MK-0869 (nanoparticle size) was administered to the male animals at a dose of 1000 mg/kg b.i.d (2000 mg/kg/day) for approximately 8 weeks. MK-0869, administered orally to male rats, had no treatment-related effects on the reproductive performance or fertility of the animals.

b(4)

**Study title: Intravenous Fertility Study with L-758298 in Male Rats**

**Key study findings:** In the i.v fertility study in male Sprague-Dawley rats, L-758-298 was administered at 0, 0, 2, 5 and 10 mg/kg/day doses for 28 days prior to cohabitation, during cohabitation and until 1 day prior to scheduled sacrifice (a total of 51-53 days). Treatment with L-758, 298 was not associated with any changes in reproductive performance or fertility of male rats.

**Study no:** #98-704-0

**Volume #, and page #:** volume #55, page # Q-2121

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

**Date of study initiation:** January 26, 1998

**GLP compliance:** Yes

**QA reports:** yes (X) no ( )

**Drug, lot #, radiolabel, and % purity:** L-758, 298, Lot # L-758, 298-003C013); purity, 99.7%.

**Formulation/vehicle:** L-758, 298 was dissolved (2 mg/ml) in \_\_\_\_\_  
\_\_\_\_\_ Dilutions for mid and low doses were made in sterile saline.

**Methods:**

**Species/strain:** \_\_\_\_\_ BR Sprague-Dawley rats.

**Doses employed:** 2, 5 and 10 mg/kg/day.

**Route of administration:** Intravenous.

**Study design:** The effects of L-758, 298 on the fertility of male Sprague-Dawley rats were evaluated following i.v. administration of the drug for 51 to 53 days. Five groups of male animals received 0 (saline), 0 (TSCD), 2, 5 and 10 mg/kg/day of MK-0869 for 28 days prior to cohabitation, during cohabitation and until 1 day prior to scheduled sacrifice (a total of 51-53 days). The males were housed with untreated females (1:1 ratio) following 4 weeks of treatment. Copulation was confirmed by the presence of a copulatory plug in the vagina or sperm in the vaginal lavage. Following the 5<sup>th</sup> night, any apparently not bred female was replaced with a virgin female, and the day of confirmed mating was considered as Gestation Day 0.

**Number/sex/group:** 25 males/group

**Parameters and endpoints evaluated:** The animals were observed daily for clinical signs. Body weights of the males were measured twice weekly and the body weights of the females were measured on pre mating day 1 and gestation days 0, 7 and 15. Food consumption was recorded twice weekly. Bred females were sacrificed on Gestation Days 15 to 17, the uterus was examined for pregnancy or implantation sites, and the number of corpora lutea were counted. Uterine implants were counted and classified as live fetus, dead fetus or resorption. Females that failed to copulate during the first 5 days were sacrificed without further examination.

Males were sacrificed in Drug Week 8 and the thoracic and abdominal viscera were grossly examined. The left cauda epididymides of all animals were weighed and frozen for subsequent sperm quantitation. Sperm counts were conducted of the control and high dose animals and expressed as number of sperm/cauda and number of sperm/g cauda epididymidis. Sperm motility was analyzed from 16 males from each group.

**Results:**

b(4)

**Mortality:** There were no deaths of animals in any group.

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

**Body weight:** The mean body weights of the control 1 and control 2 males in Drug Week 1 were 315 ± 14 and 316 ± 12 g, respectively. There were no treatment-related changes in the body weights or body weight gains in any group.

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

**In-life observations:** Treatment with L-758, 298 had no significant effect on the mating performance or fertility indices, as compared with the controls. There were 24 (of 25), 23 (of 25), 25 (of 25), 25 (of 25) and 23 (of 25) pregnant females in control 1, control 2, low, mid and high dose, respectively. The effects of L-758, 298 on the reproductive performance of the male rats are summarized in the sponsor's Table below.

TABLE A-5. L-758,298: INTRAVENOUS FERTILITY STUDY IN MALE RATS. ST 498-904-8  
SUMMARY OF REPRODUCTIVE PERFORMANCE OF MALES

	CONTROL 1	CONTROL 2	2 MG/KG/DAY	5 MG/KG/DAY	10 MG/KG/DAY
FEMALES COHABITED <sup>a</sup>	25 (2)	25 (3)	25 (1)	25 (3)	25 (1)
MALES COHABITED	25	25	25	25	25
POSTED FEMALES	25	24	25	25	24
PREGNANT FEMALES	24	23	25	24	23
DIED DURING GESTATION	0	0	0	0	0
SACRIFICED DURING GESTATION	0	0	0	0	0
ORGANISM SECTIONED	0	0	0	0	0
NONPREGNANT FEMALES	1	2	0	1	1
LIVE	1	1	0	1	1
DIED	0	0	0	0	0
SACRIFICED	0	0	0	0	0
NOT BREED	0	1	0	0	1
LIVE	0	1	0	0	1
MATING INDEX	100	96	100	100	96
POSTED FEMALES/FEMALES COHABITED, % <sup>b</sup>					
FECUNDITY INDEX	96	96	100	96	96
PREGNANT FEMALES/POSTED FEMALES, %					
FERTILITY INDEX	96	92	100	96	92
PREGNANT FEMALES/FEMALES COHABITED, % <sup>b</sup>					

<sup>a</sup> = NUMBER IN PARENTHESES INDICATES FEMALES THAT DID NOT MATE DURING THE FIRST 5 NIGHTS OF COHABITATION AND THAT WERE REMOVED AND REPLACED FOR THE LAST 5 NIGHTS.

<sup>b</sup> = CALCULATION EXCLUDES FEMALES THAT DID NOT MATE DURING THE FIRST 5 NIGHTS OF COHABITATION.

**Terminal and necroscopic evaluations:** Treatment with L-758, 298 was not associated with any significant changes in average cauda epididymal weights, sperm counts, or sperm motility in any group. The effects of L-758, 298 on epidermal weight, sperm count and sperm motility of male animals are summarized in the sponsor's Table below.

APPEARS THIS WAY  
ON ORIGINAL

TABLE A-7. L-758,298: INTRAVENOUS FERTILITY STUDY IN MALE RATS. IT #39-714-0  
SUMMARY OF CAUDA EPIDIDYMAL WEIGHTS AND SPERM COUNTS AND VAS DEFERENS SPERM MOTILITY

	CONTROL 1	CONTROL 2	2 MG/KG/DAY	5 MG/KG/DAY	10 MG/KG/DAY
CAUDA EPIDIDYMAL WEIGHT (GRAMS)	0.38 ± 0.01 (23)	0.36 ± 0.01 (23)	0.37 ± 0.01 (23)	0.36 ± 0.01 (23)	0.36 ± 0.01 (23)
SPERM COUNT/CAUDA EPIDIDYMIS IN 10 <sup>6</sup>	2.59 ± 0.07	2.58 ± 0.08	NE	NE	2.80 ± 0.09
SPERM COUNT/CLAW CAUDA EPIDIDYMIS IN 10 <sup>6</sup>	0.83 ± 0.21	0.13 ± 0.13	NE	NE	0.78 ± 0.29
% SPERM MOTILITY	87.2 ± 1.3 (16)	85.8 ± 1.3 (15)	86.2 ± 0.8 (16)	87.7 ± 1.0 (16)	85.2 ± 1.4 (16)

VALUES ARE MEANS ± S.E.M.  
NE = NOT EVALUATED  
1 } = GROUP SIZE, AND APPEARS ONLY IF DIFFERENT FROM PREVIOUS N. SEE INDIVIDUAL TABLES FOR EXCLUSIONS.

No changes in the testicular weights were observed in the treated animals, as compared with the controls. Macroscopic and microscopic examinations did not reveal any abnormalities in the testes or epididymides. There were no treatment-related effects on the number of corpora lutea, implants, resorptions or live fetuses. The laparotomy data for the pregnant females are summarized in the Table below.

Observation	Control 1	Control 2	2 mg/kg/day	5 mg/kg/day	10 mg/kg/day
Corpora lutea –					
Corpora lutea/pregnant female	16.5 ± 2.4	16.7 ± 1.8	16.6 ± 1.8	16.5 ± 1.5	16.5 ± 2.2
% peri-implantation loss (litter mean)	5.3 ± 8.1	9.3 ± 12.2	8.0 ± 12.1	8.7 ± 9.8	5.8 ± 8.5
Implants/pregnant female	15.5 ± 1.6	15.0 ± 2.0	15.3 ± 2.5	15.0 ± 1.6	15.4 ± 1.4
Resorptions and dead fetuses –					
Resorptions/implants	4.3 ± 6.2	4.2 ± 6.5	4.9 ± 6.1	3.9 ± 5.7	2.9 ± 4.5
Dead fetuses/implants	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 1.4
Post-implantation loss	6.3 ± 6.2	4.2 ± 6.5	4.9 ± 6.1	3.9 ± 5.7	3.2 ± 5.3
Live fetuses-					
Live fetuses	349	331	364	346	343
Live fetuses/pregnant female	14.5 ± 1.9	14.4 ± 2.3	14.6 ± 2.6	14.4 ± 1.8	14.9 ± 1.6

In the i.v. fertility study in male Sprague-Dawley rats, L-758-298 was administered to the male animals at 0, 0, 2, 5 and 10 mg/kg/day doses for 28 days prior to cohabitation, during cohabitation and until 1 day prior to scheduled sacrifice (a total of 51-53 days). Treatment with L-758, 298 was not associated with any changes in mating performance or fertility of the male animals. There were no effects on the epididymides weight, sperm counts or sperm motility. Treatment of the male rats with L-758, 298 had no effect on the numbers of corpora lutea, implants or resorptions and embryonic/fetal survival. Thus, L-758, 298 had no effect on the reproductive performance or fertility of male rats at i.v. doses up to 10 mg/kg/day.

2. Segment II. Teratogenic Study of I.V. L-758,298 in Pregnant Female Rats (TT # 96-713-0).

Testing Laboratory: Merck Research Laboratories  
West Point, PA 19486

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

Study Started: March 24, 1996

Study Completed: August 28, 1996

Animals: Pregnant female Sprague-Dawley rats (215 to 300 g; approximately 10 weeks of age).

Methods: In an exploratory intravenous toxicity study in rats (TT #95-2559), it was determined that the highest feasible concentration of L-758,298 for repeated administration was 0.4 mg/ml; higher concentrations produced vascular irritation. In a range-finding study (TT #96-703-5) of intravenously administered L-758,298 (0, 0.5, 1, 2 and 4 mg/kg/day from Gestation Day 6 through Lactation Day 21) in female rats, the 4 mg/kg/day dose produced a decrease in body weight gain (-26%; % of difference from control). There were no other treatment-related effects.

Thus, 4 groups of 25 pregnant female rats each were intravenously administered 0, 1, 2 and 4 mg/kg/day of L-758,298, respectively, via the tail vein from Gestation Day 6 through 20. Vehicle was 0.9% saline solution; dosing volume was 10 ml/kg; injection rate was 2 ml/min.

Pregnant females were observed daily for clinical signs of toxicity and mortality from Gestation Day 6 through Day 21; rats were observed prior to and for 1 to 5 hrs after dosing. Body weights were recorded on Gestation Days 0, 6, 8, 10, 12, 14, 16, 18, 20 and 21. Food consumption was measured for 48 hrs beginning on Gestation Days 3, 6, 10, 14, and 18.

Pregnant females were euthanized on Gestation Day 21 by CO<sub>2</sub> asphyxiation. Numbers of corpora lutea, implantations, pre- and post-implantation loss, resorptions, and live and dead fetuses were determined. All pregnant females were subjected to a thoracic and visceral examination.

All fetuses were weighed and examined externally. Fetuses were euthanized by oral administration of sodium pentobarbital. Approximately one-half of the fetuses were subjected to visceral examination, while all fetuses were subjected to skeletal examination.

Data were statistically analyzed by trend tests and analyses of variance or covariance.

Results:

Dams

1. Observed Effects: There were no treatment-related clinical signs of toxicity.
2. Mortality: There were no deaths.
3. Body Weight: Mean body weights of dams were 257 and 431 g on Gestation Days 0 and 21, respectively. There were no treatment-related effects on body weight.
4. Food Consumption: Mean food consumption of dams was 27 and 29 g/day on Gestation Days 5 and 20, respectively. There were no treatment-related effects on food consumption.
5. Dam and Fetal Data: As shown in the following table, there were no treatment-related effects on number of pregnant females, abortions, corpora lutea, and implantations, and on % implantation loss and resorptions after euthanasia on Gestation Day 21. There were no treatment-related effects on number of live fetuses and fetal weight.

Summary of Dam and Fetal Data After Euthanasia on Gestation Day 21 in a Segment II. Teratogenic Study in Rats.

Treatment Dose (mg/kg/day, i.v.)	Vehicle 0	L-758,298		
		1	2	4
<u>Dams</u>				
Total females	25	25	25	25
No. Pregnant females	24	24	24	24
No. Died	0	0	0	0
Mean Corpora lutea/dam	17.0	17.7	15.7	17.7
Mean Implanta- tions/dam	16.5	15.9	14.6	16.2
% Pre-implantation loss/litter	3.1%	9.0%	3.9%	7.1%
% Post-implantation loss/litter	3.5%	4.4%	6.9%	4.0%
% Resorptions/im- plantation	3.5%	4.4%	6.9%	4.0%
<u>Fetuses</u>				
Mean Live fetuses/ dam	15.9	15.2	13.9	15.6
Mean fetal weight (g)				
Males	4.92	4.90	5.00	4.82
Females	5.18	5.15	5.24	5.08

6. Gross Pathology: There were no treatment-related gross pathological lesions in the dams.

Fetuses

1. External Variations and Anomalies, and Visceral Variations and Anomalies: As shown in the following table, there were no treatment-related effects on fetal external variations and anomalies, and fetal visceral variations and anomalies.

Fetal External Variations and Anomalies, and Visceral Variations and Anomalies in a Segment II. Teratogenic Study in Rats.

Treatment Dose (mg/kg/day, i.v.)	Vehicle	L-758,298		
	0	1	2	4
No. fetuses/litters examined	382/24	365/24	334/24	374/24
<u>External variations</u>				
None				
<u>External anomalies</u>				
Brachydactyly	1/1	0	0	0
Digit malformation	1/1	0	0	0
No. fetuses/litters examined	196/24	190/24	175/24	193/24
<u>Visceral variations</u>				
Azygos vein variation	0	0	1/1	0
Diffuse hemorrhagic kidney	0	0	1/1	0
Ureter variation	2/2	1/1	1/1	1/1
Focally hemorrhagic adrenal	1/1	1/1	2/1	0
Hemorrhagic focus on liver	0	0	0	1/1
<u>Visceral anomalies</u>				
Hydrourter	1/1	1/1	0	0
Hypoplastic lungs	0	1/1	0	0
Diaphragmatic hernia	0	1/1	0	0

2. Skeletal Variations and Anomalies: As shown in the following table, there were no treatment-related effects on fetal skeletal variations and anomalies.

Fetal Skeletal Variations and Anomalies in a Segment II.  
Teratogenic Study in Rats.

Treatment Dose (mg/kg/day, i.v.)	Vehicle	L-758,298		
	0	1	2	4
No. fetuses/litters examined	382/24	365/24	334/24	374/25
<u>Skeletal variations</u>				
Skull bone variation	0	0	0	2/2
Sacral vertebra variation	1/1	1/1	0	1/1
Vertebral count variation	1/1	0	0	1/1
Wavy rib	0	0	0	2/2
Cervical rib	0	2/2	6/3	3/3
Supernumerary rib	50/16	59/14	27/13	38/13
Incomplete ossification of:				
Thoracic vert.	0	4/3	0	2/2
Lumbar vert.	0	1	0	0
Skull bone	1/1	0	0	1/1
Sternebra	3/3	10/6	2/2	12/8
<u>Skeletal anomalies</u>				
Missing vertebra	2/1	1/1	0	0
Hypoplastic rib	1	2/2	0	0

In summary, there were no treatment-related teratogenic effects produced by i.v. L-758,298 (0, 1, 2 and 4 mg/kg/day) in pregnant female rats. Furthermore, in a previous 4-week i.v. toxicity study of L-758,298 (0, 0.25, 1 and 4 mg/kg/day) in male and female rats, 4 mg/kg/day was the no effect dose. Thus, since the high dose of L-758,298 did not produce any toxicity in either of the above studies, it does not appear to be adequate. However, 4 mg/kg/day was originally selected as the high dose for the reproductive toxicity studies because it produced a decrease in body weight gain (-26%; % of difference from control) in a range-finding study where female rats received i.v. L-758,298 from Gestation Day 6 through Lactation Day 21. Furthermore, in an exploratory intravenous study using rats, it was determined that the highest feasible concentration of L-758,298 for repeated i.v. administration was 0.4 mg/ml; higher concentrations produced vascular irritation. Thus, based upon data from these preliminary studies, the selection of a high i.v. dose of 4 mg/kg/day for the reproductive toxicity study appeared to be reasonable.

**L-754,030: Oral Developmental Toxicity Study in Rabbits** (TT #96-716-0; Section 6).

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

**Study Started:** July 16, 1996

**Study Completed:** February 5, 1997

**GLP Requirements:** A statement of compliance with the GLP regulations and quality assurance unit was included.

**Animals:** Pregnant, female New Zealand White rabbits were used in this study. Animals were approximately 24 weeks old and had a weight range of 2908 to 4341 grams.

**Drug Batch:** L-754,030 (-OOZO10)

**Methods:** In a Segment II teratogenicity study, pregnant female rabbits received L-754,030 by oral gavage at doses of 0, 1, 5, and 25 mg/kg/day from days 7 to 20 of gestation. The vehicle was 0.55% methylcellulose in deionized water with 0.02% sodium lauryl sulfate. Dose selection was based upon two dose range finding studies (TT #96-716-6 and TT #96-716-5). In the first dose range finding study (TT #96-716-C), nonpregnant female rabbits received L-754,030 by oral gavage at doses of 0, 5, 25, 125, or 250 mg/kg/day for 14 days. Changes of initial body weight and food consumption were slightly reduced (<10%) for the 125 and 250 mg/kg/day groups. Platelet counts for the 5, 25, 125, and 250 mg/kg/day groups were reduced to 53.4-77.9% of the control (429 X 10<sup>3</sup>/MM<sup>3</sup>); however, a dose response relationship was not evident. Triglyceride levels for the 5, 25, 125, and 250 mg/kg/day groups were increased to 122.7, 304.5, 306.8, and 329.5% of the control (44 mg/dL), respectively. Alanine aminotransferase activities for the 125 and 250 mg/kg/day groups were increased to 161 and 302.4% of the control (41 U/L), respectively. Alkaline phosphatase activities for the 125 and 250 mg/kg/day groups were increased to 133 and 147% of the control (109 U/L), respectively. In the second dose range finding study (TT #96-716-5), pregnant female rabbits received L-754,030 by the oral route of administration at doses of 0, 5, 25, 125, and 250 mg/kg/day from days 7 to 20 of gestation. Rabbits that received doses of 125 and 250 mg/kg/day were sacrificed prior to scheduled termination on days 23 and 24 of gestation, while remaining animals were sacrificed on day 28. Body weights for the 125 and 250 mg/kg/day groups on day 21 were 100.5 and 99.6% of values on day 7, respectively, as compared to 104% for the control group. Food consumption for 125 and 250 mg/kg/day groups was reduced. Platelet counts for the 5, 25, 125, and 250 mg/kg/day groups were reduced to 86.1, 86.3, 73.4, and 75.1% of the control (459 x 10<sup>3</sup> / MM<sup>3</sup>). Aspartate transaminase activities for the 25, 125, and 250 mg/kg/day groups were increased to 252.6, 221, and 478.96 of the control (19 IU/L), respectively. Alanine aminotransferase activities for the 25, 125, and 250 mg/kg/day were increased to 250, 697.2, and 750 of the control (36 U/L), respectively. Alkaline phosphatase activities for the 5, 25, 125, and 250 mg/kg/day were increased to 158, 204.6, 209.3, and 190.7% of the control (43 U/L), respectively. In contrast to the first range finding study, triglyceride levels for the 25, 125, and 250 mg/kg/day groups were decreased to 73, 70.8, and 46.7% of the control

(137 mg/dL) , respectively. Numbers of corpora lutea/dani, implants/dam, and live fetuses/dam as well as fetal weight were unchanged between the control and the 5 and 25 mg/kg/day groups. In the present study, there were 18 female pregnant rabbits/group. The dose volume was 5 t-,iL/kg. Body weights were measured on days C), 7, 9, 11, 13, 15, 17, 19, 21, 24, and 28 of gestation. Food consumption was measured in 24 hr intervals at days 3-4, 7-8, 8-9, 9-10, 11-12, 15-16, 19-20, 23-24, and 27-28. During the treat:mei-1L-period, animals were observed twice daily, once prior to dosing and 1-5 hr after dosing. Female rabbits were sacrificed on day 28 and the uterus of each animal was examined to determine pregnancy status. Corpora lutea and implants were counted. Each fetus was classified as a live fetus, dead fetus, or resorption. Plac,entas were examined for gross alterations. Fetuses were weighed, examined externally, aid then sacrificed. Heads from all live fetuses were examined following coronal sectioning. Following a visceral examination, all fetuses we-re fixed and stained wit!-, alizarin red for subsequent skeletal examination. A gross examination of the thoracic and abdominal viscera was performed for all F 0 females.

**Results:** There were no deaths during the study period. One female of the 1 mg/kg/day group aborted on day 25 of gestation and 1 female of the control group aborted on gestational day 26. There were no significant clinical signs of toxicity in any treatment group. Rody weight gain from days 7 to 21 for the 25 ing/kg/day group increased by 2.75% as compared to 4.52% for the control group. Food consumption for the 25 mg/kg/day group was decreased on days 16 and 20 to 91.2 and 81.5%. of control values (125 and 124 g/day/animal), respectively. There were no significant alterations of litter parameters (i.e., corpora lutea/dam, implants/dams, live fetuses/dam, male/female ratio, fetal body weight) between the control and treatment groups. Further, there were no significant maternnal, visceral, or skeletal malformations or variations. Skeletal ossification was unaffected between the control and treatment groups.

APPEARS THIS WAY  
ON ORIGINAL

Litter parameters for fetuses from F<sub>0</sub> female rabbits treated with L-754,030 at doses of 0, 1, 5, and 25 mg/kg/day from days 7 to 20 of gestation.

Parameter	0	1	5	25
# Females	18	18	18	18
# Pregnant	18	18	17	17
# Aborted	1	1	0	0
# Examined	17	17	17	17
Corpora lutea/dam	10.0 (170/17)	9.9 (169/17)	8.9 (152/17)	9.4 (159/17)
† Peri-implantation loss	17.7	15.8	11.7	17.3
Abnormal placentas /#evaluated	0/138	0/139	0/134	0/133
Implants/dam	8.2 (139/17)	8.2 (139/17)	7.9 (134/17)	7.8 (133/17)
Resorptions	5	1	1	6
Dead fetuses	2	0	1	2
Live fetuses/dam	7.8 (132/17)	8.1 (138/17)	7.8 (132/17)	7.4 (125/17)
Male/Female ratio	61/71	80/58	70/62	59/66
Male fetal body weight, g	36.9	36.7	37.0	35.9
Female fetal body weight, g	36.8	36.7	36.4	35.9

External examination of fetuses from F<sub>0</sub> female rabbits treated with L-754,030 at doses of 0, 1, 5, and 25 mg/kg/day from days 7 to 20 of gestation.

Parameter	0	1	5	25
Live fetuses/Litters examined	132/17	138/17	132/17	125/17
Dead fetuses/Litters examined	2/1	0	1/1	2/2
Omphalocele	0	0	1 (0.74)	0

In a Segment II teratogenicity study, pregnant female rabbits received L-754,030 by oral gavage at doses of 0, 1, 5, and 25 mg/kg/day from days 7 to 20 of gestation. L-754,030 was not teratogenic in this dose range.

3. Segment II. Teratogenic Study of I.V. L-758,298 in Pregnant Female Rabbits (TT #96-706-0).

Testing Laboratory: Merck Research Laboratories  
West Point, PA 19486

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

Study Started: April 8, 1996

Study Completed: October 14, 1996

Animals: Pregnant female New Zealand White rabbits (2.87 to 4.39 kg; approximately 26 weeks of age).

Methods: In a range-finding study (TT #96-706-6) of intravenously administered L-758,298 (0, 0.5, 1, 2, and 4 mg/kg/day for 14 days) in nonpregnant female rabbits, there were no treatment-related effects. Thus, in a range-ranging study (TT #96-706-5), 4 groups of 10 pregnant female rabbits each were intravenously administered 0, 1, 2 and 4 mg/kg/day of L-758,298, respectively, from Gestation Day 7 through 20. Vehicle was 0.9% saline solution; dosing volume was 0.1, 0.2 and 0.4 ml/kg for the 1, 2 and 4 mg/kg/day doses, respectively. There were no treatment-related effects.

Thus, 4 groups of 10 pregnant female rabbits each were intravenously administered 0, 1, 2 and 4 mg/kg/day of L-758,298, respectively, via the ear vein from Gestation Day 7 through 20. Vehicle was 0.9% saline solution; dosing volume was 10 ml/kg; injection rate was 15 ml/min.

Pregnant females were observed daily for clinical signs of toxicity and mortality from Gestation Day 1 through Day 28; rabbits were observed prior to and for 1 to 5 hrs after dosing. Body weights were recorded on Gestation Days 0, 7, 9, 11, 13, 15, 17, 19, 21, 24 and 28. Food consumption was measured for 24 hrs beginning on Gestation Days 3, 7, 8, 9, 11, 15, 19, 23 and 27.

Females were euthanized on Gestation Day 28 by i.v. injection of sodium pentobarbital in the marginal ear vein. Numbers of corpora lutea, implantations, pre- and post-implantation loss, resorptions, and live and dead fetuses were determined. All females were subjected to a thoracic and visceral examination.

All fetuses were weighed and examined externally. Fetuses were euthanized by oral administration of sodium pentobarbital. All fetuses were subjected to visceral and skeletal examinations.

**Results:****Does**

1. **Observed Effects:** There were no treatment-related clinical signs of toxicity.
2. **Mortality:** There were no deaths.
3. **Body Weight:** Mean body weights of control does were 3.62 and 3.95 kg on Gestation Days 0 and 28, respectively. There were no treatment-related effects on body weight.
4. **Food Consumption:** Mean food consumption of control does was 125 and 114 g/day on Gestation Days 4 and 28, respectively. There were no treatment-related effects on food consumption.
5. **Doe and Fetus Data:** As shown in the following table, there were no treatment-related effects on number of pregnant females, abortions, corpora lutea, and implantations, and on % implantation loss and resorptions after euthanasia on Gestation Day 28. There were no treatment-related effects on number of dead fetuses, live fetuses and fetal weight.

**Summary of Doe and Fetal Data After Euthanasia on Gestation Day 28 in a Segment II. Teratogenic Study in Rabbits.**

Treatment Dose (mg/kg/day, i.v.)	Vehicle	L-758,298		
	0	1	2	4
<b>Does</b>				
Total females	18	18	18	18
No. Pregnant females	17	17	18	18
No. Died	0	0	0	0
No. Aborted	0	0	1	0
Mean Corpora lutea/doe	9.9	9.1	9.4	9.2
Mean Implantations/doe	9.5	8.7	8.7	7.6
% Pre-implantation loss/litter	4.0%	4.5%	6.9%	18.0%
% Post-implantation loss/litter	3.2%	2.1%	1.4%	1.9%
% Resorptions/implantation	2.5%	2.1%	1.4%	1.9%
<b>Fetuses</b>				
Mean Live fetuses/doe	9.2	8.5	8.6	7.4
Mean fetal weight (g)				
Males	35.7	37.3	37.8	39.3
Females	37.4	37.8	38.7	38.9

6. Gross Pathology: There were no treatment-related gross pathological lesions in the does.

Fetuses

1. External Variations and Anomalies, and Visceral Variations and Anomalies: As shown in the following table, there were no treatment-related effects on fetal external variations and anomalies, and fetal visceral variations and anomalies.

Fetal External Variations and Anomalies, and Visceral Variations and Anomalies in a Segment II. Teratogenic Study in Rabbits.

Treatment Dose (mg/kg/day, i.v.)	<u>Vehicle</u>	<u>L-758,298</u>		
	0	1	2	4
No. fetuses/litters examined	156/17	145/17	146/17	133/18
<u>External variations</u>				
Local edema	2/1	0	0	0
<u>External anomalies</u>				
None				
<u>Visceral variations</u>				
Azygos vein variation	0	0	1/1	0
Reduced gallbladder	2/1	1/1	0	1/1
Lung lobation variation	10/5	5/2	7/6	4/3
Cyst	1/1	2/2	0	1/1
Hemorrhagic focus on liver	0	1/1	0	0
<u>Visceral anomalies</u>				
Ageneſis of kidney	1/1	0	0	0
Retrocaval ureter	3/3	0	7/4	3/3
Hydrocephalus	1/1	0	0	0

2. Skeletal Variations and Anomalies: As shown in the following table, there were no treatment-related effects on fetal skeletal variations and anomalies.

Fetal Skeletal Variations and Anomalies in a Segment II.  
Teratogenic Study in Rabbits.

Treatment Dose (mg/kg/day, i.v.)	Vehicle	L-858,298		
	0	1	2	4
No. fetuses/litters examined	156/17	145/17	146/17	133/18
<u>Skeletal variations</u>				
Cervical rib	1/1	0	0	0
Reduced 13th rib	23/14	16/11	33/15	26/13
Incomplete ossification of:				
Sternebra	15/8	12/7	10/5	8/3
Metacarpal	19/7	9/4	15/7	5/4
Metatarsal	1/1	0	0	0
Pelvic bone	6/3	1/1	1/1	0
Talus/Calcaneus	4/2	0	0	0
<u>Skeletal anomalies</u>				
Lumbar vertebra malformation	0	0	1/1	0
Branched rib	0	0	0	1/1

In summary, there were no treatment-related teratogenic effects produced by i.v. L-758,298 (0, 1, 2 and 4 mg/kg/day) in pregnant female rabbits. There were no other treatment-related effects. Furthermore, in a range-finding study of i.v. L-758,298 (0, 1, 2 and 4 mg/kg/day) in pregnant female rabbits, there were no treatment-related effects. However, since data in rats suggested that 0.4 mg/ml is a maximum feasible concentration for L-758,298; one could argue that 4 mg/kg/day is a maximum feasible dose in rabbits.

4. Modified Segment II.-III. Reproductive Toxicity Study of I.V. L-758,298 in Female Rats (TT #96-713-1).

Testing Laboratory: Merck Research Laboratories  
West Point, PA 19486

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

Study Started: April 28, 1996

Study Completed: February 13, 1997

Animals: Female Sprague-Dawley rats (216 to 319 g; approximately 11 weeks of age).

**Methods:** In an exploratory intravenous toxicity study in rats (TT #95-2559), it was determined that the highest feasible concentration of L-758,298 for repeated administration was 0.4 mg/ml; higher concentrations produced vascular irritation. In a range-finding study (TT #96-703-5) of intravenously administered L-758,298 (0, 0.5, 1.2 and 4 mg/kg/day from Gestation Day 6 through Lactation Day 21) in female rats, the 4 mg/kg/day decrease in body weight gain (-26%; % of difference from control). There were no other treatment-related effects.

Thus, 4 groups of 25 female rats each were intravenously administered 0, 1, 2 and 4 mg/kg/day, respectively, via the tail vein from Gestation Day 6 through Lactation Day 20. Vehicle was 0.9% saline solution; dosing volume was 10 ml/kg.

F<sub>0</sub> females were housed with males of the same strain on a 1:1 ratio. The day of finding copulatory plugs in the cage pan and/or in the vagina was considered to be Gestation Day 0. Pregnant females were observed for clinical signs of toxicity daily from Gestation Day 6 through the day of sacrifice; each pregnant female was observed prior to and 1 to 5 hrs after dosing. Mortality was checked daily. Body weights were recorded on Gestation Days 0, 6, 8, 10, 12, 14, 16, 18, 20, 21, 22 and 24 and on Lactation Days 0, 3, 7, 10, 14, 17 and 21. Food consumption was recorded on Gestation Days 3-5, 6-8, 10-12, 14-16, 18-20 and on Lactation Days 1-5 and 8-12.

F<sub>0</sub> females that delivered were euthanized by CO<sub>2</sub> asphyxiation on one of Postpartum Days 21 to 24. Number of implantations and % post-implantation loss were determined for each female. All females were subjected to complete gross pathological examinations.

F<sub>1</sub> pups were observed daily for clinical signs of toxicity and mortality from Postnatal Day 0 through Day 21. Body weights were recorded on Postnatal Days 0, 7, 14 and 21. All pups were examined externally for malformations on Postnatal Day 0. Litters were culled to 4 pups per sex on Postnatal Day 3 and to 2 pups per sex on Postnatal Day 21.

F<sub>1</sub> animals (2 males and 2 females, when possible) were removed from dams on one of Postnatal Days 21 to 24. All F<sub>1</sub> animals were observed twice weekly for clinical signs of toxicity and once weekly for mortality. Body weights of males were recorded once weekly from weaning until termination. Body weights of females were recorded once weekly from weaning until breeding or termination.

The presence or absence of vaginal canalization was recorded in all F<sub>1</sub> females on Postpartum Days 28, 30, 32, 34, 36 and 38. The presence or absence of preputial separation of all F<sub>1</sub> males was recorded on Postpartum Days 38, 40, 42, 44, 46 and 48.

One male and one female from each F<sub>1</sub> litter were subjected to behavioral assessment of passive avoidance (Postnatal Days 35 and 42), auditory startle habituation (Postnatal Day 63), and open field motor activity (Postnatal Day 70). All F<sub>1</sub> animals were subjected to ophthalmologic examination once between Postnatal Days 47 to 59.

Beginning during Postnatal Week 10 or 11, 1 F<sub>1</sub> male and 1 F<sub>1</sub> female per litter (non-siblings) were cohabited for a maximum of 16 days. The day on which spermatozoa were detected in vaginal lavage and/or copulatory plugs were found in the cage pan and/or in the vagina was considered to be Gestation Day 0, and the mated females were removed and individually caged.

All F<sub>1</sub> rats were examined twice weekly for clinical signs of toxicity and once weekly for mortality until sacrifice. Body weights were recorded once weekly, except during cohabitation. For females that mated, body weights were recorded on Gestation Days 0, 7, 14, 20 and 24, and on Lactation Day 0.

All F<sub>1</sub> males and females not used for mating were euthanized by CO<sub>2</sub> asphyxiation and discarded without further examination during Postnatal Weeks 14 to 15. All F<sub>1</sub> males used for mating were euthanized by CO<sub>2</sub> asphyxiation and discarded without further examination during Postnatal Weeks 14 to 15. F<sub>1</sub> females that delivered were euthanized by CO<sub>2</sub> asphyxiation within a week after delivery and the uterus of each female was examined for implantations and % post-implantation loss.

Pups of the F<sub>2</sub> generation were counted, weighed, sexed, and examined for external malformations and mortality on Postnatal Day 0. The F<sub>2</sub> pups were then euthanized by CO<sub>2</sub> asphyxiation and discarded without further examination on Postnatal Day 0.

## Results:

### F<sub>0</sub> Generation

1. Observed Effects: There were no treatment-related clinical signs of toxicity.
2. Mortality: There were no deaths.
3. Body Weight: Mean body weights of control F<sub>0</sub> females were 258 and 409 g on Gestation Days 0 and 22, respectively. Mean body weights of control F<sub>0</sub> females were 303 and 336 g on Lactation Days 0 and 21, respectively. There were no treatment-related effects on body weight.

4. Food Consumption: Mean food consumption of control F<sub>0</sub> females was 26 and 26 g/day on Gestation Days 5 and 20, respectively. Mean food consumption of control F<sub>0</sub> females was 40 and 62 g/day on Lactation Days 5 and 12, respectively. There were no treatment-related effects on food consumption.

5. Pregnancy Data: As shown in the following table, there were no treatment-related effects on number of mated or pregnant F<sub>0</sub> female rats. There were no deaths. There were no treatment-related effects on mean length of gestation, % females with live pups, mean implantations/dam and % post-implantation loss/litter.

Summary of Pregnancy Data for F<sub>0</sub> Female Rats in a Modified Segment II.-III. Reproductive Toxicity Study.

Treatment Dose (mg/kg/day, i.v.)	<u>Vehicle</u>	<u>L-758,298</u>		
	0	1	2	4
Total females	25	25	25	25
No. Mated females	25	25	25	25
No. Pregnant females	25	24	25	25
No. Died	0	0	0	0
Mean length of gestation (days)	22.3	22.3	22.3	22.3
Females with live pups/pregnant females (%)	100	100	100	100
Mean Implantations/dam	16.8	17.5	16.2	16.8
% Post-implantation loss/litter	7.3	10.4	7.1	8.2

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

F<sub>1</sub> Generation (Birth until Mating):

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

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

3. Body Weight: As shown in the following table, there were no treatment-related effects on body weight of pups during the lactation period.

Mean Body Weights of F<sub>1</sub> Pups During the Lactation Period in a  
Modified Segment II.-III. Reproductive Toxicity Study.

Treatment Dose (mg/kg/day, i.v.)	Vehicle	L-758,298		
	0	1	2	4
Mean No. live pups/litter				
Postnatal day 0	15.5	15.7	15.1	15.4
3 <sup>a</sup>	8.0	8.0	8.0	8.0
7	7.9	8.0	8.0	8.0
14	7.9	8.0	7.9	7.9
21	7.9	8.0	7.9	7.9
Mean male pup weight (g)				
Postnatal day 0	6.5	6.5	6.5	6.6
7	18.3	17.8	17.5	18.0
14	36.5	35.2	35.1	35.8
21	61.7	59.9	59.4	61.4
Mean female pup weight (g)				
Postnatal day 0	6.1	6.1	6.2	6.2
7	17.6	17.1	17.1	17.1
14	35.3	34.4	34.7	34.6
21	59.1	58.2	58.2	58.7

<sup>a</sup>Litters were culled to 8 pups/litter (4 males and 4 females/litter when possible).

Mean body weights of F<sub>1</sub> males were 91 and 450 g during Postweaning Weeks 1 and 8, respectively. Mean body weights of F<sub>1</sub> females were 85 and 291 g during Postweaning Weeks 1 and 8, respectively. There were no treatment-related effects on body weight during Postweaning Weeks 1 through 8.

4. External Variations and Anomalies: As shown in the following table, there were no treatment-related external variations and anomalies in F<sub>1</sub> pups on Lactation Day 0.

APPEARS THIS WAY  
ON ORIGINAL

External Variations and Anomalies in F<sub>1</sub> Pups on Lactation Day 0 in a Modified Segment II.-III. Reproductive Toxicity Study in Rats.

Treatment Dose (mg/kg/day, i.v.)	<u>Vehicle</u>	<u>L-758,298</u>		
	0	1	2	4
No. pups/litters examined	388/25	376/24	377/25	386/25
<u>External variations</u> None		-		
<u>External anomalies</u>				
Displaced ear	0	1/1	0	0
Agnathia	0	1/1	0	0
Microstomia	0	1/1	0	0
Craniorachischisis	0	1/1	0	0
Shortened torso	0	1/1	0	0

5. Developmental Signs: There were no treatment-related effects on sexual maturity (vaginal canalization in females; preputial separation in males).

6. Behavioral Assessment: There were no treatment-related effects on passive avoidance, auditory startle habituation and open field motor activity.

7. Ophthalmologic Examination: There were no treatment-related effects.

F<sub>1</sub> Generation (Mating Until Sacrifice)

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

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

3. Body Weight: Mean body weights of control F<sub>1</sub> pregnant females were 297, 345, 387 and 460 g on Gestation Days 0, 7, 14 and 20, respectively, and 360 g on Lactation Day 0. There were no treatment-related effects on body weight.

4. Reproductive Performance: As shown in the following table, there were no treatment-related effects on reproductive performance in the F<sub>1</sub> generation.

Reproductive Performance of F<sub>1</sub> Females in a Modified Segment II.-  
III. Reproductive Toxicity Study in Rats.

Treatment Dose (mg/kg/day, i.v.)	<u>Vehicle</u>	<u>L-758,298</u>		
	0	1	2	4
Total Cohabited females	25	25	25	25
No. Mated females	25	24	25	25
No. Pregnant females	19	20	21	19
No. Died	0	0	0	0
Mean length of gestation (days)	22.4	22.3	22.5	22.6
Females with live pups/pregnant females (%)	95	95	100	100
Pregnant females/mated females (%)	79	91	88	90
Pregnant females/females cohabited (%)	76	83	84	76
Mean Implantations/dam	16.5	15.9	7.9	9.1
% Post-implantation loss/litter	9.6	6.5	7.1	8.2

F<sub>2</sub> Generation

1. Body Weight: As shown in the following table, there were no treatment-related effects on mean body weights of F<sub>2</sub> pups on Lactation Day 0.

Mean Body Weights of F<sub>2</sub> Pups on Lactation Day 0 in a Modified Segment II.-III. Reproductive Toxicity Study in Rats.

Treatment Dose (mg/kg/day, i.v.)	<u>Vehicle</u>	<u>L-758,298</u>		
	0	1	2	4
Mean No. live pups/litter Postnatal day 0	15.8	15.6	15.9	15.0
Mean male pup weight (g) Postnatal day 0	6.7	6.7	6.7	6.7
Mean female pup weight (g) Postnatal day 0	6.4	6.4	6.4	6.3

2. External Variations and Anomalies: As shown in the following table, there were no treatment-related external variations and anomalies in F<sub>2</sub> pups on Lactation Day 0.

Fetal External Variations and Anomalies in F<sub>2</sub> Pups on Lactation Day 0 in a Modified Segment II.-III. Reproductive Toxicity Study in Rats.

Treatment Dose (mg/kg/day, i.v.)	Vehicle	L-758,298		
	0	1	2	4
No. pups/litters examined	284/19	297/20	333/21	285/19
<u>External variations</u> None				
<u>External anomalies</u> Tail malformation	0	0	0	1/1

In summary, in this modified Segment II.-III. reproductive toxicity study of L-758,298 (0, 1, 2 and 4 mg/kg/day), there were no treatment-related effects on pregnancies of F<sub>0</sub> females, and no treatment-related effects on peri- and postnatal development and reproductive performance of the F<sub>1</sub> generation. Furthermore, in a previous 4-week i.v. toxicity study of L-758,298 (0, 0.25, 1 and 4 mg/kg/day) in male and female rats, 4 mg/kg/day was the no effect dose. Thus, since the high dose of L-758,298 did not produce any toxicity in either of the above studies, it does not appear to be adequate. However, 4 mg/kg/day was originally selected as the high dose for the reproductive toxicity studies because it produced a decrease in body weight gain (-26%; % of difference from control) in a range-finding study where female rats received i.v. L-758,298 from Gestation Day 6 through Lactation Day 21. Furthermore, in an exploratory intravenous study using rats, it was determined that the highest feasible concentration of L-758,298 for repeated i.v. administration was 0.4 mg/ml; higher concentrations produced vascular irritation. Thus, based upon data from these preliminary studies, the selection of a high i.v. dose of 4 mg/kg/day for the reproductive toxicity study appeared to be reasonable.

#### Reproductive and Developmental Toxicology Summary:

Reproductive and developmental toxicology studies were conducted with both fosaprepitant (pro-drug; L-758,298) and aprepitant (active drug; L-754,030; MK-0869) by the intravenous and oral routes of administration. In the i.v. Segment I fertility and general reproductive performance study in male rats, fosaprepitant doses of 2, 5 and 10 mg/kg/day were used. It had no treatment-related effects on the fertility and general reproductive performance of male rats at i.v. doses up to 10 mg/kg/day. In the oral Segment I fertility and general reproductive performance study with MK-0869 in male rats, it had no effects on the fertility and general reproductive performance at doses up to 2000 mg/kg/day (1000 mg/kg b.i.d). In the i.v. Segment I fertility and general reproductive

performance study with L-758,298 in female rats, it had no treatment-related effects at i.v. doses up to 4 mg/kg/day. In the oral Segment I fertility and general reproductive performance study with MK-0869 in female rats, it had no treatment-related effects at doses up to 2000 mg/kg/day (1000 mg/kg, b.i.d.).

In the i.v. Segment II teratogenicity study with L-758, 298 in rats, the drug was administered at doses of 1, 2 and 4 mg/kg/day from gestation day 6 through 20. L-758, 298 had no teratogenic effects in rats at i.v. doses up to 4 mg/kg/day. In the i.v. Segment II teratogenicity study with L-758, 298 in rabbits, the drug was administered to pregnant animals at doses of 1, 2 and 4 mg/kg/day. It was not teratogenic in rabbits at i.v. doses up to 4 mg/kg/day.

In a modified i.v. Segment II/III reproductive toxicity study with L-758, 298 in female rats, the drug was administered to pregnant females at doses of 1, 2 and 4 mg/kg from gestation day 6 through lactation day 20. There were no treatment-related effects on pregnancies of F0 animals, and no effects of the peri- and post- natal development and reproductive performance of the F1 generation were observed. In an oral Segment II/III reproductive toxicity study with MK-0869 in rats, it had no effects on the peri- and post- natal development or reproductive performance of the F1 animals.

**Reproductive and Development Toxicology Conclusions:** Fosaprepitant (L-758, 298) or its active metabolite, aprepitant had no treatment-related effects on the fertility and general reproductive performance of male and female rats at i.v and oral doses up to 10 mg/kg/day and 2000 mg/kg/day, respectively. L-758, 298 was not teratogenic in rats and rabbits at i.v. doses up to 4 mg/kg/day. L-758, 298 had no effects on pre- and post- natal development in rats at i.v. doses up to 4 mg/kg/day.

**Labeling Recommendations:** None.

#### 2.6.6.7 Local tolerance

##### 3. Local I.v. Irritation by L-758,298 (Study #95-2559)

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

**Compliance with Good Laboratory Practice and Quality Assurance Requirements:** Sponsor did not provide statements of compliance.

**Date Study Started:** Not provided

**Date Study Completed:** April 26, 1995

The methods and data for a local i.v. irritation study were provided in a summary report by the sponsor; thus, certain details are lacking.

**Animals:** Female (body weight range of 237 to 393 g; 14 weeks of age) Sprague-Dawley rats.

**Methods:** Four groups of 3 rats each were intravenously administered 1, 2, 3 and 5 mg/kg/day of L-758,298 via the tail vein for 7 days. Vehicle was 0.9% saline. Injection rate was 1 ml/min and dosing volume was 2.5 ml/kg.

Clinical signs of toxicity were assessed daily, and body weights were recorded before initiation of the study and on Day 7. At the end of the study, rats were euthanized with carbon dioxide and discarded without examination.

**Results:** No deaths occurred in this study.

On Days 1 through 7, prolonged bleeding at the injection site was noted at all dose levels. Difficulty in injecting animals occurred at all dose levels on Days 2 through 7.

One rat at the 1 mg/kg/day dose had a reddish-colored tail on Days 6 and 7. Injection sites were red in rats at the 3 and 5 mg/kg/day doses on Day 2, 6 and/or 7. Tails were purple at the 2, 3 and 5 mg/kg/day doses on Days 4, 5, 6 and 7. On Day 7, white blotches were noted on the tail of 1 rat each at the 2 and 5 mg/kg/day doses.

Thus, local irritability was minimal and acceptable at the 1 mg/kg/day dose. I.v. doses of 2 mg/kg/day and greater produced more pronounced irritability that might prohibit chronic studies longer than 7 days in duration.

#### **Exploratory Vascular Irritation Study in Rats (TT #96-2697)**

**Methods:** The irritation potential of L-758,298 was determined in rats (3 animals/group) following intravenous administration of 0.5, 1, 2, 3 and 5 mg/kg/day doses for seven days (dosing volume, 2.5 ml/kg). The control group received the vehicle (0.9% sodium chloride) at a dosing volume of 2.5 ml/kg.

**Results:** There were no deaths in any group. Intravenous administration of L-758,298 to male and female rats at intravenous doses up to 5 mg/kg/day for 7 days was not associated with signs of vascular irritation at the injection sites of the animals.

#### **L-000758298: Exploratory 5-Day Intravenous Tolerability Study in Rats (Study # 03-2622)**

**Methods:** L-000758298 was administered intravenously once daily as bolus injection into the tail veins of female Sprague-Dawley rats (5 animals/group; body wt., 244-297 g) at doses of 2.5, 5, 10 or 25 mg/kg/day for 5 consecutive days to determine tolerability of the dosing formulation. Lyophilized L-000758298 was reconstituted with saline (0.9%) to concentrations of 25 mg/ml and 50 mg/ml. Five female rats in the control group were administered the vehicle at a dosing volume

of 0.5 ml/kg. The 25 mg/kg/day dose group was terminated on Day 3 due to severe irritation (tail discoloration and stiffness, first observed on Day 1) at the injection site that precluded further dose administration. On Day 3, two additional groups of 5 animals each were added to the study which received the 25 mg/kg/day dose (25 mg/ml and 50 mg/ml concentrations), with an increased post-dose vehicle flush of approximately 1.5 ml.

**Results:** Beginning Day 1, treatment-related purple tails were noted in 5 of 5 rats receiving the 25 mg/kg/day dose with a 0.2 or 1.5 ml post-dose vehicle flush. Purple tails were also observed in the 10 mg/kg/day group (3 of 5 animals), beginning Day 2 of dosing. The incidences of discolored tail in different groups are summarized in the Table below.

Concentration (mg/mL)	Incidence of Discolored Tails (#affected/#total)					
	Dose (mg/kg/day)	Day				
		1	2	3	4	5
25	2.5 <sup>b</sup>	0/5	0/5	0/5	0/5	0/5
	5 <sup>b</sup>	0/5	0/5	0/5	0/5	0/5
	10 <sup>b</sup>	3/5	4/5	4/5 [1]	4/5 [1]	4/5 [1]
	25 <sup>b,c</sup>	5/5	5/5 [3]	5/5	-	-
	25 <sup>d</sup>	5/5	5/5	5/5 [3]	N/A	N/A
50	2.5 <sup>b</sup>	0/5	0/5	0/5	0/5	0/5
	5 <sup>b</sup>	0/5	0/5	1/5	2/5	3/5
	10 <sup>b</sup>	1/5	1/5	2/5	3/5	4/5
	25 <sup>b,c</sup>	4/5	5/5 [1]	5/5	-	-
	25 <sup>d</sup>	3/5	3/5 [1]	5/5 [1]	N/A	N/A
Vehicle Control <sup>a</sup>	0	0/5	0/5	0/5	0/5	0/5

[ ] Number in brackets indicates number of animals not dosed due to condition of the tail.  
<sup>a</sup> Vehicle control was sodium chloride 9.0 mg/mL, disodium EDTA 0.76 mg/mL, 1N sodium hydroxide q.s. to pH 8.0, and water for injection q.s.  
<sup>b</sup> 0.2 mL vehicle postdose flush.  
<sup>c</sup> Dose group terminated predose on Day 3.  
<sup>d</sup> 1.5 mL vehicle postdose flush.  
N/A = Not applicable.

Vascular irritation along the tail, evidenced by discoloration, stiffness, sloughing of the skin, discharge at the injection site, and/or the inability to administer the dose, was observed in 10 mg/kg/day and 25 mg/kg/day groups receiving both 25 mg/ml and 50 mg/ml formulations, and in the 5 mg/kg/day group receiving the 50 mg/ml formulation. Signs of vascular irritation were observed beginning Day 1 of administration. No treatment related physical signs were observed in the control group or at 2.5 mg/kg/day (25 and 50 mg/ml formulations) and 5 mg/kg/day (25 mg/ml formulation) doses. The physical signs of irritation at the injection sites of the tail of different groups of rats are shown in the Table below.

Treatment-Related Antemortem Findings

	L-000758298 (mg/kg/day)										
	25 mg/mL						50 mg/mL				
	0a	2.5	5	10	25	25†	2.5	5	10	25	25‡
Mortality	-	-	-	-	‡	-	-	-	-	‡	-
Physical Signs:											
Purple tails	-	-	-	P	P	P	-	P	P	P	P
Tails black and stiff	-	-	-	P	P	P	-	-	P	P	-
Discharge at injection site	-	-	-	-	-	P	-	-	-	-	-
Sloughing of skin at injection site	-	-	-	P	-	P	-	-	P	-	P

† = 1.5 mL postdose vehicle flush.  
‡ = Dose group sacrificed on Day 3 due to physical signs associated with the injection of the test article.  
- = No treatment-related change.  
P = Present.  
a Control group was administered saline-based vehicle (L-001209189-000Y).

Study conducted by the applicant: Yes

Study in compliance with GLP: No

**L-000758298: Exploratory 5-Day Intravenous Tolerability Study in Female Rats (TT #04-0228).**

**Methods:** L-000758298 was administered intravenously once daily as a bolus injection into the tail veins female Sprague-Dawley rats (75 days old; 5 animals/group) at doses of 5, 10 or 25 mg/kg/day for up to 5 days to determine the tolerability of the dosing formulation. Lyophilized L-000758298 was reconstituted with Liposyn II 20% at concentrations of 25 mg/ml and 50 mg/ml. The dosing volumes were 0.2, 0.4 and 1 ml (25 mg/ml), and 0.1, 0.2 and 0.5 ml/kg, for 5, 10 and 25 mg/kg doses, respectively. The control group was administered the vehicle at a dosing volume of 1 ml/kg. The animals were observed twice daily for clinical signs and mortality. Following the 4-hour post-dose observation on Day 5, the animals were euthanized and discarded without further examination.

**Results:** There were no mortalities in any group. Vascular irritation at the injection sites (tail) was observed in all drug-treated groups, and included discoloration, scabbing, swelling, discharge at the injection site, missing tip of tail, and/or inability to administer the dose. In general, the incidence and severity of the physical signs were dose-dependent, but not dependent on the concentration of the formulation. No physical signs were observed in the vehicle control group. The incidences of discolored tails in animals from different groups receiving the 25 mg/ml and 50 mg/ml formulations are shown in the Table below.

**Incidence of Discolored Tails**  
(Number of Affected Rats/Total Number of Rats)  
L-000758298 – 25 mg/mL

mg/kg/day	Drug Day				
	1	2	3	4	5
5	0/5	0/5	0/5	0/5	1/5
10	1/5	0/5	2/5	3/5 <sup>a</sup>	5/5 <sup>a</sup>
25	5/5	5/5 <sup>a</sup>	5/5 <sup>a</sup>	5/5 <sup>a</sup>	5/5 <sup>a</sup> [4]

<sup>a</sup> In addition, scabbing, discharge, swollen tail, and/or missing tip of tail.  
[ ] Number in brackets indicates number of animals not dosed due to condition of tail.

**Incidence of Discolored Tails**  
(Number of Affected Rats/Total Number of Rats)  
L-000758298 – 50 mg/mL

mg/kg/day	Drug Day				
	1	2	3	4	5
5	0/5	0/5	1/5	1/5	1/5
10	1/5	2/5	3/5	4/5	4/5
25	5/5	5/5 <sup>a</sup>	5/5 <sup>a</sup>	5/5 <sup>a</sup>	5/5 <sup>a</sup>

<sup>a</sup> In addition, scabbing, discharge, swollen tail, and/or missing tip of tail.

### **Single-Dose Intravenous Vascular Tolerability and Toxicokinetic Study in Male Rats (TT #04-0138).**

**Methods:** L-000758298 was administered intravenously to male Sprague-Dawley rats as a single dose to determine its vascular tolerability. The toxicokinetic profiles of L-000758298 (pro-drug) and L-000754030 (active compound) were also determined. The rats were randomized into 4 groups and each group was administered the vehicle (normal saline) or 2.5, 5.0 or 7.5 mg/kg of L-000758298. Lyophilized samples of L-000758298 were reconstituted with sterile sodium chloride (0.9%) for injection to a concentration of 25 mg/ml. L-000758298 was administered into a tail vein at dosing volumes of 0.1, 0.2 and 0.3 ml/kg at doses of 2.5, 5.0 and 7.5 mg/kg, respectively. The animals were observed daily for clinical signs and mortality.

For plasma drug concentration analyses, blood samples were collected (from 3-4 animals at each time point) at approximately 2, 5, 15 and 30 minutes and 1, 4, 8 and 24 hours post-dose on Day 1. The plasma concentrations of L-000758298 and L-000754030 were determined by liquid chromatography-tandem mass spectrometry.

At study termination (Day 2), the animals were sacrificed and necropsies performed on all rats, limited to the examination and collection of tail (injection sites). Sections of injection sites were fixed and stained with hematoxylin and eosin for microscopic examinations.

**Results:** There were no mortalities in any group, and no treatment-related physical signs or gross changes were observed at necropsy. Increased incidences of treatment-related edema, hemorrhage and/or cellular infiltration in the subcutis at the injection sites were observed in animals administered the 5.0 and 7.5 mg/kg doses. In addition, one rat each in the mid and high dose

groups had necrosis of a medium sized vessel. The overall damage scores for the injection sites of the mid and high dose groups were higher than the control or the low dose group. The histomorphologic changes at the injection sites of the vehicle control and 2.5 mg/kg groups were less severe, and had lower incidences, consistent with the route of administration (venipuncture). Histomorphologic changes at the injection sites of different groups are summarized in the Table below.

Treatment-Related Postmortem Findings

Histomorphologic Changes:	L-000758298 (mg/kg)		
	2.5	5	7.5
Injection sites			
Vein, Necrosis	-	I	I
Subcutis, cellular infiltration	-	I	I
Subcutis, edema	-	I	I
Subcutis, hemorrhage	-	I	I
- = No treatment-related change. I = Increased based on incidence and/or severity.			

The incidences of histomorphologic changes for different groups of rat are shown in the Table below.

TABLE B-1. L-000758298: Single-Dose Intravenous Vascular Tolerability and Toxicokinetic Study in Male Rats. TT #04-0138  
Summary of Histomorphology

	GROUP 1	GROUP 2	GROUP 3	GROUP 4
NUMBER NECROPSIED	15	15	15	15
Injection Site NO. EXAMINED MICROSCOPICALLY	15	15	15	15
Number Not Remarkable	3	2	1	-
Vein, Necrosis	-	-	1	1
Subcutis, Cellular infiltration	10	8	13	10
Subcutis, Edema	9	11	12	15
Subcutis, Hemorrhage	8	6	9	13

KEY: GROUP 1 = Control  
GROUP 2 = 2.5 mg/kg  
GROUP 3 = 5 mg/kg  
GROUP 4 = 7.5 mg/kg

The maximum plasma concentrations ( $C_{max}$ ) and plasma exposure levels (AUC) of L-000758298 and its active metabolite, L-000754030 increased with increasing dose of L-758298 in male rats. The  $C_{max}$  values for both L-000758298 and L-000754030 were reached at the first sampling time (2.0 min). Overall, the mean  $AUC_{0-24hr}$  values for L-000754030 were substantially (75 to 228-fold) higher than the mean  $AUC_{0-24hr}$  values for L-000758298. Plasma elimination of L-000758298 was very rapid for all groups. No measurable plasma concentration of L-000758298 was observed following the 5 min time point at 2.5 mg/kg, following 15 minutes time point at 5 mg/kg, and following 1 hr time point at 7.5 mg/kg. The toxicokinetic parameters of male rats receiving 2.5, 5.0 and 7.5 mg/kg doses are shown in the Table below.

## Mean Plasma L-000758298 Toxicokinetic Parameters – Drug Day 1

	L-000758298 (mg/kg)		
	Males		
	2.5	5	7.5
AUC <sub>0-24 hr</sub> (µg/mL•hr) <sup>a</sup>	0.0104 ± 0.00286	0.0250 ± 0.00634	0.0835 ± 0.0125
C <sub>max</sub> (µg/mL) <sup>a</sup>	0.222 ± 0.0678	0.284 ± 0.0894	1.58 ± 0.189
T <sub>max</sub> (hr)	0.0333	0.0333	0.0333
<sup>a</sup> Mean ± SEM.			

## Mean Plasma L-000754030 Toxicokinetic Parameters – Drug Day 1

	L-000758298 (mg/kg)		
	Males		
	2.5	5	7.5
AUC <sub>0-24 hr</sub> (µg/mL•hr) <sup>a</sup>	2.37 ± 0.271	3.84 ± 0.583	6.26 ± 0.851
C <sub>max</sub> (µg/mL) <sup>a</sup>	0.848 ± 0.0693	1.26 ± 0.370	3.64 ± 0.196
T <sub>max</sub> (hr)	0.0333	0.0333	0.0333
<sup>a</sup> Mean ± SEM.			

Thus, single i.v. injection of L-000758298 to rats at doses of 5.0 and 7.5 mg/kg, was associated with increased incidences and severity of edema, hemorrhage and/or cellular infiltration at the injections sites. The 2.5 mg/kg day dose was the NOAEL, as there were no treatment-related histomorphologic changes at this dose.

**Study title: Exploratory Acute Dermal Irritation Study in Rabbits.**

**Key study findings:** Dermal application of L-754, 030 (500 mg/5 cm<sup>2</sup>) to rabbits for 24 hours was not irritating to the skin.

**Study no:** #96-2584

**Volume #, and page #,** Volume #48, page #H-21

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

**Date of study initiation:** April 09, 1996.

**GLP compliance:** Yes

**QA reports:** yes (X) no ( )

**Drug, lot #, radiolabel, and % purity:** L-754,030 (L-754,030-004H), Lot #1, purity 99.7%.

**Formulation/vehicle:** N/A

**Methods:** L-754, 030 was applied to the back of the rabbit in an approximately 5 cm<sup>2</sup> site and moistened with 0.5 ml of saline. The hair was removed from the application site before application of the drug. The area was covered with a gauze pad and then wrapped with an occlusive dressing. The dressings were removed after 24 hours and the residual drug was removed with tap water. The treatment sites were examined daily for 8 days, after which the animals were euthanized and discarded without any necropsy.

**Results:** There were no treatment-related clinical signs observed in any group. Topical application of the drug for 24 hours did not produce any dermal changes during the 8-day observation period.

**Summary:** Dermal application of L-754, 030 (500 mg/5 cm<sup>2</sup>) to rabbits for 24 hours was not irritating to the skin.

APPEARS THIS WAY  
ON ORIGINAL

2.6.6.8 Special toxicology studies

**Study title:** Effect of L-754, 030 on the Bovine Corneal Opacity and Permeability.

**Key study findings:** In the *in vitro* corneal irritancy assay, L-754, 030 was a severe irritant to bovine corneas, as determined by an arbitrary scoring method.

**Study no:** #96-4272

**Volume #, and page #,** Volume #48, page #H-32

**Conducting laboratory and location:** Laboratoires Merck Sharp & Dohme-Chibret, Centre de Recherche, Riom, France.

**Methods:** Ocular irritation potential of L-754, 030 was examined using an *in vitro* assay of ocular irritancy, the bovine corneal opacity and permeability (BCOP) assay. Corneas from fresh bovine eyes, collected from local abattoir, were used in the study. The corneas were incubated in Minimal Essential Medium (MEM) containing 1% fetal bovine serum, with or without the drug, for 4 hr at 32°C. L-754, 030 was suspended in the medium at a concentration of 20% (w/v). At the end of the incubation period, changes in corneal transparency were determined. To determine the corneal permeability, a solution of fluorescein dye was applied to the same corneas for 1.5 hr at 32°C. The amount of dye that passed through the corneas was measured spectrophotometrically. An *in vitro* score of irritancy, combining values for both opacity and permeability, was calculated:  
 Irritancy score = opacity + O.D. (optical density).

Ocular irritancy was then classified into one of the three categories: scores ≤ 25 = mild, from 25.1 to 55 = moderate, ≥ 55.1 = severe.

**Results:** Incubation of the bovine corneas with L-754, 030 (20% suspension) produced severe effects on the opacity and permeability, as determined by the ocular irritancy scores. The effects of L-754, 030 on corneal opacity, permeability and irritancy score are summarized in the sponsor's Table below.

Effect of L-755446-002G005 in the BCOP Assay

COMPOUND	CONC.	N	OPACITY (a)	PERMEABILITY (b)	SCORE	IRRITANT
L-755446-002G005 pH: 5.16	20%	4	102.00 ± 3.74	4.483 ± 0.116	169.2	SEVERE
MEM (CONTROLS)	100%	4	3.67 ± 1.53	0.021 ± 0.004	4.0	

(a) Arbitrary score  
 (b) O.D. score

**Summary:** The effects of L-754, 030 on bovine corneal opacity and permeability were examined by incubating the cornea with the drug for 4 hours. L-754, 030 was a severe irritant to bovine cornea in this *in vitro* assay, as determined by an arbitrary scoring method.

**Study title: Five-Week Oral Thyroxine Clearance Study in Rats.**

**Key study findings:** In a 5-week oral thyroxine clearance study with MK-0869 in rats, three groups of animals received 0 (vehicle), 0.25 mg/kg b.i.d (0.50 mg/kg/day) and 125 mg/kg b.i.d. (250 mg/kg/day) of the drug. The TSH levels of the high dose animals were higher than that of controls and the thyroxine clearance of the high dose animals was also higher.

APPEARS THIS WAY  
ON ORIGINAL

**Study no:** #98-153-0

**Volume #, and page #,** Volume #50, page #Q-188.

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

**Date of study initiation:** October 26, 1998.

**GLP compliance:** yes

**QA report:** yes (X) no ( )

**Drug, lot #, radiolabel, and % purity:** MK-0869 (L-754, 030), lot #L-754, 030-004H026.

**Formulation/vehicle:** MK-0869 was suspended in deionized water containing 0.5% methylcellulose and 0.02% sodium lauryl sulfate (SLS).

**Methods:** The study was conducted to evaluate the effects of MK-0869 on thyroxine clearance and serum thyroid hormone concentrations in Sprague-Dawley rats after oral b.i.d. administration of the drug for 5 weeks.

**Dosing:** Three groups of animals (25/sex/group) received the vehicle (deionized water containing 0.5% methylcellulose and 0.02% SLS) or 0.25 mg/kg and 125 mg/kg b.i.d. doses of MK-0869 for 5 weeks.

**Observations and times:** The animals were observed daily for mortality and clinical signs and the body weights were measured once to twice weekly. Blood was collected from 20 non-fasted rats/sex/group during the pretest period and approximately 4 hours after the first daily dose in Weeks 2 and 4 for determinations of serum triiodothyronine (T3), thyroxine (T4) and thyroid stimulating hormone (TSH) levels. Five rats/sex/group received an i.v. injection of  $^{125}\text{I}$ -thyroxine (160  $\mu\text{Ci}/\text{kg}$ ) on Drug Day 24 after the first daily dose of the drug or the vehicle. Blood samples were collected approximately 8, 22, 34, 48 and 72 hours after administration of radiolabeled thyroxine. Plasma  $^{125}\text{I}$ -thyroxine concentrations were determined by HPLC analysis. The animals continued to receive the drug and were sacrificed on Day 27. Gross pathological examination was limited to the liver and the thyroid gland. The weights of the liver and the thyroid gland were also recorded. The following toxicokinetic parameters for plasma thyroxine levels were also determined: elimination rate ( $K_{el}$ ), half life ( $t_{1/2}$ ), volume of distribution ( $V_d$ ) and plasma clearance ( $Cl_s$ ).

**Results:** The liver weights of the high dose males and females were higher than that of controls (males: absolute, 40.5%; relative, 37.3%; females: absolute, 57%; relative, 49%). Thyroid weights of the treatment group animals were higher than that of controls (males: 8.2% and 34.8% absolute and 11.4% and 34.1% relative, at low and high dose, respectively; females: 19.5% and 42.5% absolute and 13.6% and 35.6% relative, at low and high dose, respectively). There were increases in the TSH levels in Weeks 2 and 4 in males and females receiving the 125 mg/kg b.i.d. dose of MK-0869 (in week 2, 112% and 58%, and week 4, 92% and 73%, in males and females, respectively). Males had higher increases in the TSH levels as compared with females. There were no changes in the T3 and T4 levels in males and females at any time of the treatment. The rate of plasma  $^{125}\text{I}$ -thyroxine clearance was higher (about 2-fold) in animals receiving the 125 mg/kg b.i.d. dose of MK-0869 for 4 weeks, as compared with the controls. The increased thyroxine clearance in the high dose rats was associated with an increase in the volume of distribution. The mean thyroxine clearance and volume of distribution in female and male rats are summarized in the sponsor's Table below.

Mean Thyroxine Clearance and Volume of Distribution in Female and Male Rats

	Clearance (mL/hr) <sup>1</sup>		Volume of Distribution (mL) <sup>1</sup>	
	Females	Males	Females	Males
Vehicle Control	1.58±0.15	2.03±0.07	32.2±2.5	48.1±1.3
MK-0869				
0.25 mg/kg b.i.d.	1.81±0.25	2.06±0.10	43.9±7.4	46.1±3.8
125 mg/kg b.i.d.	2.84±0.53	3.88±0.15	58.4±13.5	72.7±4.8

Values are the Mean ± Standard Error of the Mean.  
<sup>1</sup> = Parameter normalized for 100 g body weight.

**Summary:** In a 5-week oral thyroxine clearance study with MK-0869 in rats, three groups of animals received 0 (vehicle), 0.25 mg/kg b.i.d (0.50 mg/kg/day) and 125 mg/kg b.i.d. (250 mg/kg/day) of the drug. Animals receiving the high dose had higher thyroid and liver weights. The TSH levels of the high dose animals were higher than that of controls and the thyroxine clearance of the high dose animals was also higher. However, there were no changes in the T3 and T4 levels in animals receiving MK-0869. This may be due to a compensatory effect of increased TSH levels.

1. In vitro Hemolysis Assay of L-758,298 in Washed Red Blood Cells and Whole Blood (Study TT #94-4905)

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

Compliance with Good Laboratory Practice and Quality Assurance Requirements: Sponsor did not provide statements of compliance.

Date Study Started: Not provided

Date Study Completed: June 30, 1994

The methods and data for an in vitro hemolysis study were provided in a summary report by the sponsor; thus, certain details are lacking.

APPEARS THIS WAY  
ON ORIGINAL

**Methods:** Whole blood samples were obtained from rats, dogs and humans; washed red blood cells were prepared from portions of the whole blood samples.

In the washed red blood cell hemolytic assay, a 3.0% red blood cell suspension (amount was not specified) was added to serially diluted concentrations (0.009, 0.018, 0.036, 0.072, 0.144, 0.288, 0.575, 1.15, 2.3, 4.6 and 9.1 mg/ml) of L-758,298 (amount was not specified) and the saline vehicle. Precipitation of red cell stroma was assessed immediately after mixing. Hemolysis was assessed (details were not provided) immediately after mixing and after 15 min of incubation at room temperature. After 30 min of incubation, test samples were centrifuged at 2000 rpm for 5 min, and hemolysis was assessed.

In the whole blood hemolytic assay, 0.9 ml of whole blood was added to 0.1 ml of serially diluted concentrations (0.001, 0.002, 0.004, 0.008, 0.016, 0.031, 0.062, 0.125, 0.25, 0.5 and 1.0 mg/ml) of L-758,298 and the saline vehicle. Test samples were incubated for 15 min at room temperature. Test samples were then centrifuged at 2000 rpm for 5 min, and hemolysis was assessed (details were not provided).

**Results:** In washed red blood cells from the rat and dog after 30 min of incubation and subsequent centrifugation, hemolysis was observed at L-758,298 concentrations of 1.15 to 9.1 mg/ml; in humans at L-758,298 concentrations of 2.3 to 9.1 mg/ml.

In whole blood from the rat, hemolysis was observed at L-758,298 concentrations of 0.5 to 1.0 mg/ml; in the dog, at the L-758,298 concentration of 1.0 mg/ml; in the human whole blood, no hemolysis was observed.

#### **L-758,298: Exploratory Enzyme Induction Studies in Mice (TT #94-261-0, -1)**

**Methods:** Liver microsomes prepared from CD-1 mice (4 male and 4 female) treated with L-758,298 for 4 days were assayed for Cytochrome P450-mediated 7-ethoxy-4-trifluormethylcoumarin O-deethylase (EFCOD) and peroxisomal fatty-acyl-CoA-oxidase (FACO) activities. Phenobarbital and Bezafibrate, each at 75 mg/kg/day, were used as a combined positive control for both EFCOD and FACO activities. Control animals received the vehicle. L-758,298, and the positive and negative controls were administered by oral gavage once a day, for 4 days before preparing the liver microsomes.

**Results:** The positive control compounds caused significant increases in EFCOD and FACO activities in the liver microsomes from male and female mice. With positive controls, there were 300% and 329% increases in EFCOD activities and 210% and 182% increases in FACO activities in male and female mice, respectively. L-758,298 caused a slight increase in the liver weights (about 12%) in both male and female mice. EFCOD activity was increased by 62% in females, but not in males. FACO activity was not affected in male or female mice. The EFCOD activities in male and female mice are shown in the Table below.

**L-758,298: EXPLORATORY ENZYME INDUCTION STUDY IN MICE. TR094-261-0,-1**

**TABLE 1 INDUCTION OF EFC-O-DENITRILATION**

MALES	NEGATIVE FB/BS		L-758,298	
	CONTROL	75/75 MPK/DAY	100 MPK/DAY	
Rates	1102.93	4183.76	1910.65	
	1026.09	4324.52	1039.82	
	1125.08	4587.51	1313.60	
	1373.17	5433.34	1768.05	
Mean	1156.82	4632.28	1508.03	
StDev	150.34	559.64	402.80	
T Value*		11.69	2.04	
Sig Level*		<0.01	0.21	
t Change		300.43	30.36	
<b>FEMALES</b>				
Rates	1369.54	6333.78	3033.03	
	1601.69	6910.21	2449.71	
	1321.14	6282.42	2321.21	
	1867.02	6877.49	2157.28	
Mean	1539.85	6600.98	2490.31	
StDev	250.14	339.09	301.09	
T Value*		12.49	4.11	
Sig Level*		<0.01	<0.01	
t Change		320.68	61.72	

Rates are in  $\mu\text{moles}/\text{min}/\text{mg}$  microsomal protein.

\*Comparison of treatment groups use one-way analysis of variance on log-transformed data. P-values are based on Dunnett's one-sided procedure for control and 6 treatment groups. Not all treatment groups appear above. Levels of 5% or below are considered statistically significant ( $p \leq 0.05$ ).

Thus, L-758,298 caused a weak induction of EFCOD activities in mice administered an oral dose of 100 mg/kg/day for 4 days.

**2. Enzyme Induction by L-758,298 in Mice (Study TT #94-258-0,-4)**

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

Compliance with Good Laboratory Practice and Quality Assurance Requirements: Sponsor did not provide statements of compliance.

Date Study Started: Not provided

Date Study Completed: August 15, 1995 (Revision of reports dated April 7, 1994 and July 6, 1995)

The methods and data for an enzyme induction study were provided in a summary report by the sponsor; thus, certain details are lacking.

Animals: Male and female (body weight range of 20 to 30 g; ages and species were not provided by the sponsor) mice.

Methods: Three groups of 8 mice each (4 males and 4 females) were orally administered L-758,298 (350 mg/kg/day), phenobarbital/benzafibrate (PB/BZ, 75/75 mg/kg/day) and 0.5% methylcellulose (10 ml/kg), respectively, for 4 days. On Day 5 (24 h after last dosing), animals were sacrificed, and livers were removed and weighed. Microsomal pellets were prepared from liver.

$P_{450}$ -mediated 7-ethoxy-4-trifluoromethylcoumarin O-deethylase (EFCOD) activity and fatty acyl-CoA oxidase (FACO) activity in microsomal pellet aliquots were assessed spectrophotometrically.

Results: L-758,298 had no significant effect on liver weight in males or females. PB/BZ increased liver weight by 43% (% of difference from control) and 22% in males and females, respectively.

L-758,298 increased EFCOD activity by 30% and 62% in males and females, respectively. PB/BZ increased EFCOD activity by 387% (% of difference from control) and 329% in males and females, respectively.

L-758,298 had no significant effect FACO activity in males or females. PB/BZ increased FACO activity by 210% and 182% in males and females, respectively.

Thus, L-758,298 moderately induced  $P_{450}$  enzyme activity, but had no effect on peroxisomal proliferation. The positive control pentobarbital induced  $P_{450}$  enzyme activity and the positive control benzafibrate induced FACO activity; FACO is a biochemical marker for peroxisomal proliferation.