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.

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-coated — beads; Batch #X0869OPP024C001; purity, 99.8%.
Formulation/vehicle: MK-0869 colloidal dispersions (200 mg/ml) were prepared by suspending the drug-coated — beads 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: Crl:CD [SD]IGS BR 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 — 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 ± 11 g and 229 ± 10 g, respectively. There were no treatment-related changes in the body weights of animals receiving MK-0869, as comapred 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 necroscopic 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 496-729-0
SUMMARY OF LAPAROTOMY DATA

TREATMENT GROUP:	CONTROL	5 MG/KG/DAY	25 MG/KG/DAY	250 MG/KG/DAY
FEMALES				
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 BRED	0	0	0	0
CORPORA LUTEA				
CORPORA LUTEA	330	392	375	328
CORPORA LUTEA/PREGNANT FEMALE	15.7 ± 1.6	17.8 ± 3.0	16.3 ± 2.3	16.4 ± 1.9
% PRE-IMPLANTATION LOSS (LITTER MEAN)	8.3 ± 17.6	11.2 ± 13.2	7.1 ± 8.8	4.6 ± 4.3
IMPLANTS				
IMPLANTS	305	346	347	310
IMPLANTS/PREGNANT FEMALE	14.6 ± 3.4	15.7 ± 2.1	15.1 ± 2.2	15.5 ± 1.4
RESORPTIONS AND DEAD FETUSES				
RESORPTIONS	14	17	10	14
% RESORPTIONS/IMPLANTS (LITTER MEAN)	5.9 ± 6.3	5.0 ± 5.6	2.9 ± 3.9	6.3 ± 6.6
DEAD FETUSES	0	0	1	0
% DEAD FETUSES/IMPLANTS (LITTER MEAN)	0.0 ± 0.0	0.0 ± 2.0	3.0 ± 0.0	0.0 ± 0.0
% POSTIMPLANTATION LOSS (LITTER MEAN)	5.9 ± 6.3	5.0 ± 5.6	2.9 ± 3.9	6.3 ± 6.6
LIVE FETUSES				
LIVE FETUSES	293	329	337	291
UNDETERMINED SEX	293	329	337	291
LIVE FETUSES/PREGNANT FEMALE	14.0 ± 3.3	15.0 ± 2.5	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 (— 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_1 generation.

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: Crl:CD [SD]IGS 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 5th 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 premating 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 os 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.

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 897-734-0
SUMMARY OF REPRODUCTIVE PERFORMANCE OF MALES

	CONTROL	25 MG/KG/DAY	125 MG/KG/DAY	250 MG/KG/DAY
FEMALES COHABITED[a]	25 (1)	25 (1)	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 BRED	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, %[b]	100	100	96	100
FECUNDITY INDEX PREGNANT FEMALES/MATED FEMALES, %	84	92	92	92
FERTILITY INDEX PREGNANT FEMALES/FEMALES COHABITED, %[b]	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 necroscopic 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 897-734-0
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)
SPERM COUNT/CAUDA EPIDIDYMIS (x 10^7)	3.11 +/- .13	NE	NE	2.98 +/- .11
SPERM COUNT/GRAM CAUDA EPIDIDYMIS (x 10^7)	9.14 +/- .38	NE	NE	9.02 +/- .39
% SPERM MOTILITY	88.9 +/- 1.5 (16)	85.8 +/- 2.4 (16)	89.2 +/- 1.4 (16)	88.6 +/- 1.0 (16)

VALUES ARE MEANS +/- S.E.M.
NE = NOT EVALUATED
( ) = GROUP SIZE, AND APPEARS ONLY IF DIFFERENT FROM PREVIOUS N. SEE INDIVIDUAL TABLES FOR EXCLUSIONS.

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 (— particle 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.

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-coated — beads; Batch #X0869OPP024C001; purity, 99.8%.
Formulation/vehicle: MK-0869 — beads 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 —

Methods:

Species/strain: Crl:CD [SD]IGS BR 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 (— 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.
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:

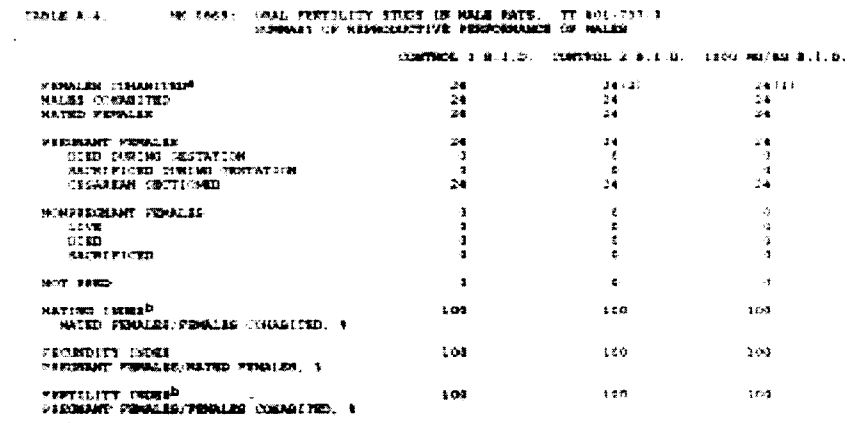
Mortality: There were no deaths of animals in any group.

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.



Terminal and necroscopic 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-6. MK-0869: ORAL FERTILITY STUDY IN MALE RATS. TT 401-737-0
SUMMARY OF CAUDA EPIDIDYMIS SPERM COUNTS AND VAS DEFERENS SPERM MOTILITY

	CONTROL 1 B.I.D.	CONTROL 2 B.I.D.	1000 MG/KG B.I.D.
SPERM COUNT/CAUDA EPIDIDYMIS (X 10^6) ± S.D.	263.8 ± 52.9(26)	269.4 ± 37.8(26)	271.3 ± 44.9(24)
SPERM COUNT/ GRAM CAUDA EPIDIDYMIS (X 10^6) ± 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. TT 401-737-0
SUMMARY OF LAPAROTOMY DATA

TREATMENT GROUP:	CONTROL 1 B.I.D.	CONTROL 2 B.I.D.	1000 MG/KG B.I.D.
FEMALES			
TOTAL FEMALES	24	24	24
PREGNANT	24	24	24
EXAMINED LIVE LITTER	24	24	24
RESORBED 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 DRED	0	0	0
CORPORA LUTEA			
CORPORA LUTEA	421	413	422
CORPORA LUTEA/PREGNANT FEMALE	18.2 ± 3.3	17.2 ± 1.9	17.6 ± 1.9
% PRE-IMPLANTATION LOSS (LITTER MEAN)	5.4 ± 9.1	4.1 ± 5.9	5.1 ± 5.8
IMPLANTS			
IMPLANTS	404	395	399
IMPLANTS/PREGNANT FEMALE	16.9 ± 2.3	16.5 ± 1.6	16.6 ± 1.9
RESORPTIONS AND DEAD FETUSES			
RESORPTIONS	16	14	15
% RESORPTIONS/IMPLANTS (LITTER MEAN)	4.9 ± 9.5	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)	4.9 ± 9.7	3.8 ± 4.7	3.7 ± 7.1
LIVE FETUSES			
LIVE FETUSES	385	380	384
UNEXAMINED SEX	385	380	384
LIVE FETUSES/PREGNANT FEMALE	16.0 ± 2.3	15.8 ± 1.9	16.0 ± 2.0

% PRE-IMPLANTATION LOSS = (1 NO. CORPORA LUTEA - NO. IMPLANTS) / NO. CORPORA LUTEA) X 100
% POSTIMPLANTATION LOSS = (1 NO. RESORPTIONS + NO. DEAD FETUSES) / NO. IMPLANTS) X 100

In summary, in the oral fertility study in male Sprague-Dawley rats, MK-0869 (—— particle 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.

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 Tween-sodium citrate diluent (TSCD; pH 7.5 ± 0.2). Dilutions for mid and low doses were made in sterile saline.

Methods:

Species/strain: Crl:CD [SD]IGS 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 5th 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 premating 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:

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. TT 498-704-4
SUMMARY OF REPRODUCTIVE PERFORMANCE OF MALES

	CONTROL 1	CONTROL 2	2 MG/KG/DAY	5 MG/KG/DAY	10 MG/KG/DAY
FEMALES COHABITED[a]	25 (2)	25 (3)	25 (2)	25 (3)	25 (2)
MALES COHABITED	25	25	25	25	25
MATED 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
CESAREAN SECTIONED	24	23	25	24	23
NONPREGNANT FEMALES	1	1	0	1	1
LIVE	1	1	0	1	1
DIED	0	0	0	0	0
SACRIFICED	0	0	0	0	0
NOT BRED	1	1	0	1	1
LIVE	0	1	0	1	1
MATING INDEX MATED FEMALES/FEMALES COHABITED, %[b]	100	96	100	100	96
FECUNDITY INDEX PREGNANT FEMALES/MATED FEMALES, %	96	96	100	96	96
FERTILITY INDEX PREGNANT FEMALES/FEMALES COHABITED, %[b]	96	92	100	96	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 - 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.

TABLE A-7. L-758,298: INTRAVENOUS FERTILITY STUDY IN MALE RATS. TT #98-714-0
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 (25)	0.36 ± 0.01 (25)	0.37 ± 0.01 (25)	0.36 ± 0.01 (25)	0.36 ± 0.01 (25)
SPERM COUNT/CAUDA					
EPIDIDYMIS (X 10 ⁷)	2.59 ± 0.07	2.58 ± 0.08	NE	NE	2.80 ± 0.09
SPERM COUNT/GRAM CAUDA					
EPIDIDYMIS (X 10 ⁷)	6.89 ± 0.21	7.32 ± 0.18	NE	NE	7.78 ± 0.24
% SPERM MOTILITY	87.2 ± 1.1 (16)	85.8 ± 1.3 (15)	86.3 ± 1.9 (16)	87.7 ± 1.0 (16)	85.2 ± 1.4 (16)

VALUES ARE MEANS ± S.E.M.
NE = NOT EVALUATED
( ) = SAMPLE 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 comapred 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_2 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 wee 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
Dams				
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 Implantations/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/implantation	3.5%	4.4%	6.9%	4.0%
Fetuses				
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 0	L-758,298 1	2	4
No. fetuses/litters examined	382/24	365/24	334/24	374/24
External variations				
None				
External anomalies				
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
Visceral variations				
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
Visceral anomalies				
Hydroureter	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 0	L-758,298 1	2	4
No. fetuses/litters examined	382/24	365/24	334/24	374/25
Skeletal variations				
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
Skeletal anomalies				
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.

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 though 20. Vehicle was 0.9% saline solution; dosing volume was 0.1, 0.2 and 0.4 mg/ml for the 1, 2 and 4 mg/kg/day doses, respectively. There were no treatment-related effects.

Thus, 4 groups of 18 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 0	L-758,298 1	L-758,298 2	L-758,298 4
Does				
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%
Fetuses				
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.)	Vehicle 0	L-758,298 1	2	4
No. fetuses/litters examined	156/17	145/17	146/17	133/18
External variations				
Local edema	2/1	0	0	0
External anomalies				
None				
Visceral variations				
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
Visceral anomalies				
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 0	L-858,298 1	2	4
No. fetuses/litters examined	156/17	145/17	146/17	133/18
Skeletal variations				
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
Skeletal anomalies				
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_0 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_0 females that delivered were euthanized by CO_2 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_1 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_1 animals (2 males and 2 females, when possible) were removed from dams on one of Postnatal Days 21 to 24. All F_1 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_1 females on Postpartum Days 28, 30, 32, 34, 36 and 38. The presence or absence of preputial separation of all F_1 males was recorded on Postpartum Days 38, 40, 42, 44, 46 and 48.

One male and one female from each F_1 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_1 animals were subjected to ophthalmologic examination once between Postnatal Days 47 to 59.

Beginning during Postnatal Week 10 or 11, 1 F_1 male and 1 F_1 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_1 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_1 males and females not used for mating were euthanized by CO_2 asphyxiation and discarded without further examination during Postnatal Weeks 14 to 15. All F_1 males used for mating were euthanized by CO_2 asphyxiation and discarded without further examination during Postnatal Weeks 14 to 15. F_1 females that delivered were euthanized by CO_2 asphyxiation within a week after delivery and the uterus of each female was examined for implantations and % post-implantation loss.

Pups of the F_2 generation were counted, weighed, sexed, and examined for external malformations and mortality on Postnatal Day 0. The F_2 pups were then euthanized by CO_2 asphyxiation and discarded without further examination on Postnatal Day 0.

Results:

F_0 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_0 females were 258 and 409 g on Gestation Days 0 and 22, respectively. Mean body weights of control F_0 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_0 females was 26 and 26 g/day on Gestation Days 5 and 20, respectively. Mean food consumption of control F_0 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_0 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_0 Female Rats in a Modified Segment II.-III. Reproductive Toxicity Study.

Treatment Dose (mg/kg/day, i.v.)	Vehicle 0	L-758,298 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_1 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_1 Pups During the Lactation Period in a Modified Segment II.-III. Reproductive Toxicity Study.

Treatment Dose (mg/kg/day, i.v.)	Vehicle 0	L-758,298 1	2	4
Mean No. live pups/litter				
Postnatal day 0	15.5	15.7	15.1	15.4
3[a]	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

[a]Litters were culled to 8 pups/litter (4 males and 4 females/ litter when possible).

Mean body weights of F_1 males were 91 and 450 g during Postweaning Weeks 1 and 8, respectively. Mean body weights of F_1 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_1 pups on Lactation Day 0.

External Variations and Anomalies in F_1 Pups on Lactation Day 0 in a Modified Segment II.-III. Reproductive Toxicity Study in Rats.

Treatment Dose (mg/kg/day, i.v.)	Vehicle 0	L-758,298 1	2	4
No. pups/litters examined	388/25	376/24	377/25	386/25
External variations				
None		-		
External anomalies				
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_1 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_1 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_1 generation.

Reproductive Performance of F_1 Females in a Modified Segment II.-III. Reproductive Toxicity Study in Rats.

Treatment Dose (mg/kg/day, i.v.)	Vehicle 0	L-758,298 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_2 Generation

1. Body Weight: As shown in the following table, there were no treatment-related effects on mean body weights of F_2 pups on Lactation Day 0.

Mean Body Weights of F_2 Pups on Lactation Day 0 in a Modified Segment II.-III. Reproductive Toxicity Study in Rats.

Treatment Dose (mg/kg/day, i.v.)	Vehicle 0	L-758,298 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_2 pups on Lactation Day 0.

Fetal External Variations and Anomalies in F_2 Pups on Lactation Day 0 in a Modified Segment II.-III. Reproductive Toxicity Study in Rats.

Treatment Dose (mg/kg/day, i.v.)	Vehicle 0	L-758,298 1	2	4
No. pups/litters examined	284/19	297/20	333/21	285/19
External variations None				
External anomalies 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_0 females, and no treatment-related effects on peri- and postnatal development and reproductive performance of the F_1 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.

L-754, 030: Oral **Developmetal 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 Recruirements: 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 (-OOOZO10)

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 @'T #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 slighted 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 103/MM3) ; 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 329.5'6 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 iiig/kg/day were sacrificed prior to scheduled termination on days 23 and 24 o@gestation, while remaining animals were sacrificed on day 28. Bodv 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 groupswere reduced to 86.1, 86.3, 73.4, and 75.J.% of the control (459 x10 3 / MM3) . Aspartate transaminase activities for the 25, 125, and 250 mg/kg/day groups were increased to 252.6, 22!, and 478.96 of the control (19 IU/L), respectively. Alanirie 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 pliosphatase 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/],), 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.

Litter parameters for fetuses from F_0 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_0 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.

SUMMARY AND EVALUATION:

L-754,030 is a non-peptide NK_1 receptor (substance P) antagonist proposed for oral use in the prevention of chemotherapy-induced emesis. In support of the clinical development of this drug, the sponsor has submitted the following reproductive toxicology studies: an oral range-finding Segment III reproduction study in female rats; an oral Segment II/III developmental toxicity study in rats with postweaning evaluation; an oral Segment I fertility and reproductive performance study in female rats; an oral range-finding study in nonpregnant rabbits; an oral range-finding Segment II study in pregnant rabbits; and an oral Segment II developmental toxicity study in rabbits.

In a Segment I fertility and reproductive performance study, female rats received L-754,030 by the oral gavage at doses of 0, 5, 25, and 250 mg/kg/day. Female rats received L-754,030 for 14 days prior to cohabitation, during cohabitation, and through day 7 of gestation. L-754,030 had no effects on fertility or reproductive performance for female rats. There were no treatment-related effects on number of corpora lutea, implants, or live fetuses per dam.

In a Segment II/III study, pregnant F_0 female rats were treated with L-754,030 by oral gavage at doses of 0, 5, 25, or 250 mg/kg/day from day 6 of gestation to day 20 of lactation. There was no mortality or evidence of clinical signs of toxicity in pregnant females treated with L-754,030 at doses up to 250 mg/kg/day. For dams treated with L-754,030, hepatic weights were increased. On gestational day 21, one-half of the pregnant female rats were sacrificed. L-754,030 was not teratogenic. There was no evidence of external anomalies, delays of ossification, or skeletal/visceral malformations or variations. The remaining pregnant F_0 females were allowed to deliver naturally. For pups delivered naturally, there were no effects on length of gestation, number of implants/dam, pup survival from postnatal day 0 to 21, or pup weight from postnatal day 0 to 21. There was no evidence of external anomalies in F_1 pups delivered naturally from F_0 pups. There were no treatment-related developmental effects or behavioral changes for F_1 pups. For the F_1 generation, the mating index, fertility, and length of gestation were unaffected by L-754,030 treatment. For F_1 dams, there were no effects on numbers of implants/dam or live fetuses/dam. For F_2 pups, there were no effects on body weight and there was no evidence of external anomalies.

Study title: Segmment III Pre- and Post-natal developmental Toxicity Study in Rats.

Key study findings: In a modified oral Segment II/III reproductive toxicity study with MK-0869 (— particle size), pregnant females received the drug at a dose of 1000 mg/kg b.i.d (2000 mg/kg/day). Treatment of the F_0 females with MK-0869 was not associated with any external anomalies, delay of ossification, or skeletal or visceral malformations or variations of the F_1 pups. There were no treatment-related effects on peri- and post-natal development of the F_1 animals. For the F_1 generation, the mating index, fertility and length of gestation were unaffected by treatment with MK-0869. For F_2 pups, there were no effects on the body weights, and there was no evidence of external abnormalities.
Study no: #01-736-0
Volume #, and page #: volume #25, page # C-850
Conducting laboratory and location: Merck Research Laboratories, Merck & Co., Inc. West Point, PA
Date of study initiation: November 25, 2001
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: MK-0869 blended-coated — beads; Batch #X0869OPP024C001; purity, 99.8%.
Formulation/vehicle: MK-0869 — beads 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 —

Methods:

Species/strain: Crl:CD [SD]IGS BR Sprague-Dawley rats.

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

Route of administration: Oral gavage.

Study design: Three groups of female rats (two control groups and one treatment group) were used in the study. The treatment group received 1000 mg/kg b.i.d. (2000 mg/kg/day) of MK-0869, 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 (dosing volume, 5 ml/kg b.i.d). The doses were administered from Gestation Day (GD) 6 through GD 20 to 22 females/group scheduled for cesarean section, or GD 6 through Lactation Day 20 to 22 females/group scheduled for natural delivery. The animals scheduled for cesarean section were sacrificed on GD 21 and those scheduled for natural delivery were allowed to deliver the pups. On post-natal Day 3, the litters were culled to 8 pups (4 males and 4 females). On post-natal Day 21, the litters were culled to 2/sex, and 1 male and 1 female from each litter were used to examine the reproductive performance of the F_1 generation.

Number/sex/group: 44 females/group

Parameters and endpoints evaluated: The females were observed daily for mortality. For physical signs, the animals were observed on GD 0 and then daily from GD 6 through sacrifice. Body weights were recorded on GD 0, 6, 8, 10, 12, 14, 16, 18, 20, 21, 22 and 24, and for females scheduled for natural delivery, on lactation Days 0, 3, 7, 10, 14, 17 and 21. Food consumptions were recorded at 2 to 4-day intervals. Twenty-two (22) females per group were euthanized on Gestation Day 21, 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. All fetuses were examined externally and sexes determined. The fetuses were euthanized by sodium pentobarbital and the viscera of approximately one half of the fetuses in each litter were examined. The head of one half of the fetuses were stained in Bouin's and examined after sectioning. The fetuses were fixed and stained for subsequent skeletal examinations. The thoracic and abdominal viscera of the F_0 females were grossly examined.

Twenty-two females in each group were allowed to deliver naturally, and the onset and completion of delivery and the signs of difficulty in parturition were recorded. The duration of gestation for each dam was also recorded. Females with live pups were sacrificed on Lactation Day 21 and the uterus of each female was examined for the number of metrial glands. Gross examinations of the thoracic and abdominal viscera were conducted. One female in the control 1 group did not deliver and euthanized on GD 24 and the uterus was examined for implantation sites. The F_1 pups were examined for external malformations and their genders recorded. The pups were observed daily for mortality and physical signs, and the body weights recorded on Post-natal Days (PND) 0, 7, 14 and 21. On PND 3, litters were culled to 8 pups (4 males and 4 females) per litter. On PND 21, litters were reduced to 2 pups/sex/litter. Culled pups were examined externally to confirm gender and discarded without further examinations. The body weights of the remaining F1 pups (2/sex/litter) were recorded once weekly. The female pups were examined for vaginal opening on PND 28-38 and the males were examined for preputial separation on PND 38-50. Ophthalmologic examinations of all animals were conducted between PND 47 and 51. One male and one female from each litter underwent the following behavioral assessments: passive avoidance (on PND 34 or 35 and PND 41 or 42), auditory startle habituation (on PND 62 or 63) and open field motor activity (on PND 69 or 70).

During post-natal Week 12, one female and one male from each litter (non-siblings within the same group) were cohabited to assess the reproductive performance of the F_1 generation. From GD 21 until completion of delivery, each pregnant female was observed 4 times a day, and the onset and completion of delivery and any signs of difficulty in parturition were recorded. F_1 females that delivered offspring were sacrificed within one week and the uterus of all females were examined to determine the pregnancy status. On PND 0, the F_2 pups were counted, weighed, sexed and examined for external abnormalities, and then sacrificed.

Results:

Mortality: There were no treatment-related deaths in any group. One female in control 2 group was sacrificed on GD 16 due to physical signs that included pallor, decreased motor activity, red vaginal discharge and blood in the cage pan.

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

Body weight: The mean body weights of the control 1 and control 2 F_0 females on GD 0 were 235 ± 12 g and 230 ± 16 g, respectively. There were no changes in the body weights of the treatment group animals during the gestation and lactation periods, as compared with the controls. The average body weights of control 1 and control 2 F_1 females in Postweaning Week 1 were 82 ± 4 g and 80 ± 7 g, respectively, and no treatment-related changes in the body weights were observed during the experimental period.

Food consumption: The mean food consumptions of control 1 and control 2 F_0 females on GD 5 were 27 ± 3 and 26 ± 2 g, respectively. There were no treatment-related changes in the food consumption in animals receiving the 1000 mg/kg b.i.d. dose of MK-0869.

In-life observations: Treatment of the F_0 females with MK-0869 (1000 mg/kg b.i.d.) had no effect on the length of gestation, embryonic/fetal survival, fetal sex ratio or fetal weights. The body

weights and body weight gains of the F_1 animals were not affected by treatment of the F_0 females with MK-0869. The reproductive performance, pregnancy and litter data of the F_0 female animals of different groups are summarized in the Table below.

Parameters	Control 1	Control 2	1000 mg/kg b.i.d.
Mated females	44	44	44
Pregnant females	43	44	44
Cesarean section	22	21	22
Females with live pups	21	22	22
Females with live pups (%)	100	100	100
Length of gestation (Days)	22.2 ± 0.3	22.2 ± 0.3	22.3 ± 0.3
Live fetuses	330	306	347
Sex ratio	0.50	0.48	0.51
Live fetuses/pregnant female	15.0 ± 2.9	14.6 ± 2.3	15.8 ± 2.3
Live fetal weights (G)			
Males-	4.82 ± 0.24	4.91 ± 0.39	4.90 ± 0.32
Females-	5.10 ± 0.31	5.09 ± 0.35	5.19 ± 0.34
Normal delivery			
Pregnant females	21	22	22
Implants per female	16.2 ± 1.9	16.1 ± 2.1	15.9 ± 2.0
Females with live pups on Day 0	21	22	22
Females with live pups on Day 21	21	22	22
Total pups delivered –			
Live pups	312 (98.9%)	324 (96.8%)	325 (97.9%)
Dead pups	4	12	7
Sex ratio	0.50	0.49	0.51
Live pups per litter			
Postnatal day 0	14.9± 2.0	14.7±2.4	14.8±2.7
Postnatal day 3	8.0± 0	8.0±0	7.9±0.4
	8.0±0	8.0±0	7.9±0.4
Postnatal day 7	8.0±0	8.0±0	7.9±0.4
Postnatal day 14	8.0±0	8.0±0	7.9±0.4
Postnatal day 21			
Pup deaths			
Postnatal day 0	2	4	2
Postnatal day 3	0	0	0
Postnatal day 7	1	0	0
Postnatal day 14	0	0	0
Postnatal day 21	1	0	0
Live female pup weight (G)			
Postnatal day 0	5.9± 0.4	5.9±0.4	6.0±0.6
Postnatal day 7	15.8±1.3	15.5±1.4	15.1±1.7
Postnatal day 14	33.8±2.5	32.9±2.7	30.9±3.5
Postnatal day 21	54.6±3.5	53.3±3.5	51.1±5.1
Live male pup weight (G) -			
Postnatal day 0	6.3± 0.3	6.3±0.5	6.3±0.5
Postnatal day 7	16.5± 1.3	16.2±1.7	15.6±1.5
Postnatal day 14	34.9±2.8	33.7±3.1	32.1±3.1
Postnatal day 21	56.6±4.2	55.4±4.9	53.1±4.3

Treatment with MK-0869 had no effect on the developmental (time of vaginal opening, preputial separation) and behavioral (passive avoidance, auditory startle habituation, and open field motor activity) parameters of the F_1 animals. The developmental and behavioral observations of the F_1 animals of different groups are summarized in the Table below.

Parameters	Control 1	Control 2	1000 mg/kg b.i.d.

Developmental Observations			
Vaginal Canalization – Mean day of occurrence	31.5 ± 2.0	32.4 ± 1.8	32.7 ± 1.9
Preputial Separation – Mean day of occurrence	43.2 ± 2.6	44.4 ± 2.4	44.0 ± 2.1
Behavioral Observations			
Passive Avoidance – Females			
Mean trials to criterion (Days 34-35 postnatal)	7.8 ± 3.0	6.9 ± 2.9	6.8 ± 2.4
No. not achieving criterion (Days 34-35 postnatal)	1	2	2
Passive Avoidance – Males			
Mean trials to criterion (Days 34-35 postnatal)	8.0 ± 2.9	7.6 ± 3.1	7.3 ± 2.4
No. not achieving criterion (Days 34-35 postnatal)	0	2	1
Auditory Startle Habituation – Females			
Mean V max (Trials 1-50)	341 ± 180	342 ± 132	347 ± 192
Auditory Startle Habituation – Males			
Mean V max (Trials 1-50)	378 ± 225	370 ± 169	439 ± 306
Open Field Testing – Females			
Mean horizontal activity (0-60 minutes)	2732 ±858	2888 ± 646	2548 ± 538
Open Field Testing – Males			
Mean horizontal activity (0-60 minutes)	2064 ± 541	1919 ± 455	2033 ± 444

There were no changes in the mean body weights of the treatment group F_1 females during gestation and lactation periods, as compared with the control groups. Treatment of the F_0 females with MK-0869 had no significant effect on the reproductive performance, fertility and pregnancy parameters of the F_1 animals. The reproductive performance of the F_1 females of different groups is summarized in the sponsor's Table below.

TABLE 22. MK-0869: ORAL DEVELOPMENTAL TOXICITY STUDY IN RATS WITH POSTWEANING EVALUATION. TT 401-736-0
SUMMARY OF REPRODUCTIVE PERFORMANCE OF F1 FEMALES

	CONTROL 1 B.I.D.	CONTROL 2 B.I.D.	1000 MG/KG B.I.D.
FEMALES COHABITED	21	22	22
MALES COHABITED	21	22	22
MATED FEMALES	20	22	21
PREGNANT FEMALES	20	22	21
DIED DURING GESTATION	0	0	0
SACRIFICED DURING GESTATION	0	0	0
DIED OR SACRIFICED DURING PARTURITION	0	0	0
DIED OR SACRIFICED POSTPARTUM	0	0	0
FEMALES WITH LIVE PUPS PND 0 (A)	20	22	21
FEMALES WITH NO LIVE PUPS PND 0 (A)	0	0	0
NONPREGNANT FEMALES	0	0	0
LIVE	0	0	0
DIED	0	0	0
SACRIFICED	0	0	0
NOT MATED	1	0	1
LIVE	1	0	1
MATINGS PER 4-DAY PERIODS OF COHABITATION:			
DAYS 1 TO 4	17	19	21
DAYS 5 TO 8	3	0	0
DAYS 9 TO 12	0	0	0
DAYS 13 TO 16	0	3	0
TIME TO MATING (4-DAY PERIODS) ± S.D.	1.15± 0.37	1.41± 1.05	1.06± 0.00
MATING INDEX MATED FEMALES/FEMALES COHABITED, %	95	100	95
FECUNDITY INDEX PREGNANT FEMALES/MATED FEMALES, %	100	100	100
FERTILITY INDEX PREGNANT FEMALES/FEMALES COHABITED, %	95	100	95
FEMALES WITH LIVE PUPS/PREGNANT FEMALES, % (A)	100	100	100
LENGTH OF GESTATION (DAYS) (A)	22.2 ± 0.3	22.4 ± 0.6	22.3 ± 0.6

A. INCLUDES ONLY FEMALES COMPLETING DELIVERY

BEST POSSIBLE COPY

The pregnancy data for F_1 females and the status of F_2 generation at parturition are summarized in the sponsor's Table below.

TABLE 23. MK-0869: ORAL DEVELOPMENTAL TOXICITY STUDY IN RATS WITH POSTWEANING EVALUATION. TT #21-736-0
SUMMARY OF STATUS OF F2 GENERATION AT PARTURITION

	CONTROL 1 B.I.D.	CONTROL 2 B.I.D.	1000 MG/KG B.I.D.
PARENTAL FEMALES	20	22	21
IMPLANTS PER FEMALE ±S.D.	16.4± 2.6	18.3± 3.0	16.9± 1.5
% POSTIMPLANTATION SURVIVAL TO DAY 0 (L.M. ±S.D.)	89.4± 7.9	90.0± 5.3	91.1±10.5
FEMALES WITH LIVE PUPS DAY 0 POSTPARTUM	20	22	21
TOTAL PUPS DELIVERED			
LIVE PUPS (SEX RATIO, L.M.)	297 (1.47)	314 (0.43)	325 (0.44)
DEAD PUPS (%, L.M.±S.D.)	27 (3.57± 1.76)	31 (0.77± 2.00)	4 (1.15± 1.15)
% LIVE PUPS DELIVERED (L.M.) ±S.D.	99.4± 1.9	99.2± 2.0	99.4± 1.1
LIVE PUPS PER LITTER ±S.D. POSTNATAL DAY 0	14.9± 3.6	15.2± 3.1	15.2± 2.9
LIVE FEMALE PUP WEIGHT (GM) (L.M.) ±S.D. POSTNATAL DAY 0	6.4 ± 0.6	6.1 ± 0.4	6.0 ± 0.5
LIVE MALE PUP WEIGHT (GM) (L.M.) ±S.D. POSTNATAL DAY 0	6.3 ± 0.5	6.5 ± 0.4	6.3 ± 0.5

%POSTIMPLANTATION SURVIVAL TO DAY 0 = (NO. LIVE PUPS DELIVERED/TOTAL NO. MATERIAL GLANDS) X 100
L.M. = LITTER MEAN
SEX RATIO = TOTAL NO. LIVE FEMALE PUPS / TOTAL NO. LIVE PUPS
TOTAL INCLUDES PUPS FOR WHICH SEX COULD NOT BE DETERMINED

Terminal and necroscopic evaluations:

Dams: Treatment of the F_0 females with a 1000 mg/kg b.i.d. dose of MK-0869 had no effect on the number of resorptions, number of corpora lutea and peri- and post- implantation losses. The reproductive parameters for pregnant F0 female rats sacrificed on Gestation Day 21 by cesarean section are summarized in the Table below.

Parameters	Control 1	Control 2	1000 mg/kg b.i.d.
Pregnant females	22	22	22
Resorbed or dead litter	0	0	0
Corpora lutea/dam	17.3 ± 2.3	16.0 ± 1.9	17.4 ± 1.7
Peri-implantation loss/litter	7.5 ± 9.2	5.3 ± 8.0	4.8 ± 5.8
Implants/pregnant female	15.9 ± 1.9	15.1 ± 2.1	16.5 ± 2.0
Resorptions/dam	6.6 ± 11.7	3.7 ± 5.2	4.8 ± 7.4
Post-implantation loss/litter	6.6 ± 11.7	4.0 ± 5.6	4.8 ± 7.4
Dead fetuses/implant	0	0.3 ± 1.5	0
Live fetuses/pregnant female	15.0 ± 2.9	14.6 ± 2.3	15.8 ± 2.3

Offspring: There were no treatment-related external malformations of the fetuses derived from F_0 females receiving the 1000 mg/kg b.i.d. dose of MK-0869. Results of the external examinations of the fetuses from F0 females derived by cesarean section on Day 21 of gestation are summarized in the Table below.

Parameters	Control 1	Control 2	1000 mg/kg b.i.d.
Live fetuses/litters examined	330/22	306/21	347/22
Dead fetuses/litters examined	0	1/1	0
Fetuses with malformations (% litter mean)	1 (0.28)	0 (0.0)	2 (0.52)
Fetuses with variations	0	0	0
No. of abnormal placentas	2/330	2/306	0/347
Type and number of fetal alterations (% litter mean)			
Microphthalmia	0	0	1 (0.25)
Micrognathia	1 (0.28)	0	0
Polydactyly	0	0	1 (0.27)

Visceral examinations of fetuses derived by cesarean section from the drug-treated F_0 females on Gestation Day 21 did not show any abnormalities, as compared with the control groups. Results of visceral examination of fetuses are summarized in the Table below.

Parameters	Control 1	Control 2	1000 mg/kg b.i.d.
Thoracic and abdominal examinations			
Live fetuses/litters examined	171/22	158/21	179/22
Dead fetuses/litters examined	0	1/1	0
Fetuses with malformations (%)	0	0	0
Litters with malformations (%)	0	0	0
Fetuses with variations (%)	0	0	1 (0.65%)
Litters with variations (%)	0	0	1 (4.5%)
Coronal examination			
Live fetuses/litters examined	170/22	158/21	179/22
Fetuses with malformations (%)	0	0	0
Litters with malformations (%)	0	0	0
Fetuses with variations (%)	0	0	0
Litters with variations (%)	0	0	0

Type and Number of fetal alterations Focally hemorrhagic adrenal (%)	0	0	1 (0.65%)

Skeletal examination of fetuses derived by cesarean section from the treatment-group F_0 females on Day 21 of gestation did not show any abnormalities. The data is summarized in the Table below.

Parameters	Control 1	Control 2	1000 mg/kg b.i.d.
Live fetuses/litters examined	330/22	306/21	247/22
Dead fetuses/litters examined	0	1/1	0
Cervical vertebra malformation	1 (0.28%)	1 (0.37%)	0
Absent rib	1 (0.38%	1 (0.34%)	0
Hypoplastic rib	0	1 (0.34%)	1 (0.35%)
Vertebral count variation	1 (0.27%)	0	0
Cervical rib	12 (3.8%)	3 (1.1%)	7 (2.1%)
Supernumerary rib	22 (6.6%)	44 (15%)	20 (5.7%)
Sternebral variation	0	1 (0.32%)	0

The number of fetuses with incomplete ossification was slightly higher in the treatment group as compared with the controls (control 1, 13/330; control 2, 15/306; 1000 mg/kg b.i.d, 20/347). However, this was not significant as compared with the controls. Fetal ossification data for fetuses derived by cesarean section from F_0 females are summarized in the sponsor's Table below.

TABLE 9. MK-0869: ORAL DEVELOPMENTAL TOXICITY STUDY IN RATS WITH POSTNATAL EVALUATION. TT 401-718-8
SUMMARY OF FETAL OSSIFICATION DATA

TREATMENT GROUP:	CONTROL 1 B.I.D.	CONTROL 2 B.I.D.	1000 MG/KG B.I.D.
TORSO AND LIMB EXAMINATION			
LIVE FETUSES/LITTERS EXAMINED	330/22	306/21	247/22
FETUSES WITH INCOMPLETE OSSIFICATION	13	15	20
% (LM ±S.D.)	4.4 ± 5.4	4.9 ± 9.2	8.9 ± 7.5
LITTERS WITH INCOMPLETE OSSIFICATION (%)	11 (50)	7 (35)	11 (50)
NUMBER IDENTIFIED SACRAL/CAUDAL VERTEBRAE			
LITTER MEAN ±S.D.	10.4 ± 0.8	10.4 ± 0.9	10.4 ± 0.8
HEAD EXAMINATION			
LIVE FETUSES/LITTERS EXAMINED	140/22	149/21	158/22
FETUSES WITH INCOMPLETE OSSIFICATION	0	0	0
% (LM ±S.D.)	0.00± 0.00	0.00± 0.00	0.00± 0.00
LITTERS WITH INCOMPLETE OSSIFICATION (%)	0	0	0
SITE AND NUMBER OF FETUSES WITH INCOMPLETE OSSIFICATION % LM			
INCOMP OSS. CERVICAL VERTEBRA	1 (0.30)	0	0
INCOMP OSS. THORACIC VERTEBRA	3 (0.84)	2 (0.62)	3 (1.2)
INCOMP OSS. LUMBAR VERTEBRA	2 (0.55)	0	0
INCOMP OSS. STERNEBRA	8 (2.3)	14 (4.7)	17 (6.8)

(LM) = LITTER MEAN

In a modified Segment II/III reproductive toxicity study in rats, three groups of pregnant F_0 females received 0 (0.5% methylcellulose in deionized water), 0 (4% hydroxypropylcellulose, 20% sucrose and 0.19% SLS in deionized water) and 1000 mg/kg b.i.d (2000 mg/kg/day) MK-0869 (— — particle size). The doses were administered by oral gavage from gestation Day 6 through gestation Day 20-22 (22 female/group), or though lactation Day 20-22 (22 females/group). MK-0869, at an oral dose of 1000 mg/kg b.i.d., was not teratogenic. There was no evidence of external anomalies, delay of ossification, or skeletal or visceral malformations or variations of the F_1 pups. Treatment of the F_0 females with MK-0869 had no effect on peri- and post-natal development of the F_1 animals. For the F_1 generation, the mating index, fertility and length of gestation were unaffected by treatment with MK-

0869. For F_2 pups, there were no effects on the body weights, and there was no evidence of external abnormalities.

Reproductive and developmental toxicology summary:

Reproductive and developmental toxicology studies were conducted with both the pro-drug, L-758, 298 and the active drug, MK-0869 (L-754, 030) by the i.v. or oral routes. In the i.v. Segment I fertility and reproductive performance study with L-758, 298 (prodrug of MK-0869) in male rats, 2, 5 and 10 mg/kg/day doses were used. It had no treatment-related effects on the fertility and reproductive performance of the male animals at i.v. doses up to 10 mg/kg/day. In the oral Segment I fertility and reproductive performance study with MK-0869 in male rats, 25, 125 and 250 mg/kg/day doses were used. MK-0869 had no effects on the fertility and reproductive performance of the male rats at oral doses up to 250 mg/kg/day. In a third Segment I fertility and reproductive performance study in male rats, MK-0869 (——— particle size formulation) at an oral dose of 1000 mg/kg b.i.d (2000 mg/kg/day), had no effects on the fertility and reproductive performance of the animals.

In the i.v. Segment I fertility and reproductive performance study with L-758, 298 (pro-drug of MK-0869) in female rats, 0, 1, 2 and 4 mg/kg/day doses were used. L-758, 298 had no treatment-related effects on the fertility and reproductive performance of the female rats at i.v. doses up to 4 mg/kg/day. In a second oral Segment I fertility and reproductive performance study with MK-0869 (——— particlle size) in female rats, a 1000 mg/kg b.i.d (2000 mg/kg/day) dose was used. No treatment-related effects on the fertility and reproductive performance of the female rats were observed.

In the i.v. Segment II teratogenicity study with L-758, 298 in rats, pregnant animals received 0, 1, 2 and 4 mg/kg/day doses of the drug from Gestation Day 6 through 20. Intravenous L-758, 298 had no teratogenic effects in rats at doses up to 4 mg/kg/day.

In the i.v. Segment II teratogenicity study with L-758, 298 in rabbits, pregnant animals received 0, 1, 2 and 4 mg/kg/day doses. There were no treatment-related teratogenic effects produced by i.v. L-758, 298 at doses up to 4 mg/kg/day. In an oral Segment II study in rabbits, it was not teratogenic.

In a modified i.v. Segment II/III reproductive toxicity study with L-758, 298 in female rats, doses of 0, 1, 2 and 4 mg/kg/day were administered to pregnant animals from Gestation Day 6 through Lactation Day 20. There were no treatment-related effects on pregnancies of F_0 females, and no effects on the peri- and post- natal development and reproductive performance of the F_1 generation were observed. In an oral Segment II/III reproductive toxicity study with MK-0869 (——— particle size) in rats, pregnant females received 0 and 1000 mg/kg b.i.d. (2000 mg/kg/day) dose of the drug. MK-0869 at an oral dose of 2000 mg/kg/day, had no teratogenic effects. It had no effects on the peri- and post-natal development or the reproductive performance of the F_1 animals. Treatment of the F_0 females with MK-0869 had no effects on body weights or external appearance of the F_2 animals.

Reproductive and developmental toxicology conclusions: MK-0869 or its pro-drug, L-758, 298 had no treatment-related effects on the fertility and reproductive performance of male and female rats. L-758, 298, at i.v. doses up to 4 mg/kg/day, had no teratogenic effects in rats or rabbits. MK-0869, at the 1000 mg/kg b.i.d (2000 mg/kg/day) dose, had no teratogenic effects, and had no effects on the peri- and post-natal development of the F_1 animals.

Labeling recommendations: None.

VIII. SPECIAL TOXICOLOGY STUDIES:

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.

Study title: Exploratory Acute Dermal Irritation Study in Rabbits.

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

Study title: Exploratory Acute Dermal Irritation Study with L-755,446 in Rabbits.

Key study findings: Dermal irritation potential of L-755, 446, a metabolite of MK-0869, was assessed after dermal application to rabbits for 24 hours. L-755, 446 was mildly irritating to the skin of rabbits.

Study no: #00-2547 and #99-2503
Volume #, and page #, Volume #48, page #H-136 and page #H-218.
Conducting laboratory and location: Merck Research Laboratoires, Merck & Co., Inc., West Point, PA 19486.

Methods: L-755446, a major metabolite of MK-0869 (and a chemical process intermediate), was examined for its dermal irritation potential, when applied to the skin of rabbits for 24 hours, in two separate studies. Three New Zealand White rabbits (2 males, 1 female) were used in each study. The drug (500 mg) was applied on an approximately 5 cm^2 shaved area at the back of each rabbit, moistened with saline and then wrapped with an occlusive dressing. After 24 hours, the dressing was removed, the area was washed with tap water and blotted dry. The animals were observed daily for clinical signs, and the treatment sites were examined for dermal changes approximately 24 hours after treatment and daily thereafter. On Day 8, all animals were euthanized and discarded without necropsy.

Results: No treatment-related clinical signs were observed in any animal. Slight erythema was observed in 1 (of 3) animal at 24 hours. At 48 hours, all animals appeared normal and no changes were observed during the 8-day observation period. In the second study, 2 (of 3) rabbits had slight erythema at 24 and 48 hours, and no changes were observed on study days 4 through 8.

Summary: Dermal irritation potential of L-755, 446 was examined after application to the skin of rabbits for 24 hours in two separate studies. In one study, slight erythema was observed in 1 (of 3) animals at 24 hours after application. In the second study, slight erythema was observed in 2 (of 3) animals at 24 and 48 hours. Thus, the MK-0869 metabolite, L-755, 446 was a mild irritant to the skin of rabbits.

Study title: Exploratory Primary Ocular Irritation Study in Rabbits.

Key study findings: Application of L754, 030 into the conjunctival sacs of rabbits eye caused very slight to slight conjunctival redness in all eyes that lasted for up to 24 hours. L-754, 030 was practically non-irritating to the eyes of rabbits.
Study no: #96-4275
Volume #, and page #, Volume #48, page #H-61
Conducting laboratory and location: Laboratoires Merck Sharp & Dohme-Chibret, Centre de Recherche, Riom, France.
Date of study initiation: May 28, 1996.
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: L-754,030 (L-754,030-004H), Lot #001, purity 99.7%.
Formulation/vehicle: N/A
Methods: Ocular irritation potential of L-754, 030 was examined in rabbits (2 females, 1 male) after application of a single dose of the drug into the conjunctival sac. One eye was used for the drug and the other eye was used as a control (untreated). After application of L-754, 030 (100 mg/rabbit), the eyelids were held together for 20 seconds, so that the test material remained in contact with the conjunctiva and cornea. The animals were observed daily for clinical signs. General examinations of both eyes and scoring for ocular reactions of the drug-treated eye were conducted before initiation of treatment, 15 min, 120 min, 24 hr, 48 hr, 72 hr, 4 days and 7 days after administration of the drug. Direct reaction of the pupil to light and anesthesia of the cornea were also checked at the same time intervals. On study Day 7, all animals were euthanized and discarded without any necropsy.

Results: Fifteen (15) minutes after application of the drug, very slight conjunctival redness was observed in all animals, and slight discharge was observed in 2 (of 3) animals. At 120 minutes post-dosing, very slight to slight conjunctival redness was observed in all animals and after 24 hours, slight redness was still present in 2 animals. By 48 hours, the eyes of all animals were normal. The individual total Draize scores were low, and never exceeded 4 (the maximum achievable score being 110). There was a normal response of the pupil to light beam and a positive blink response in all animals during the entire experimental period.

Summary: Ocular irritation potential of L-754, 030 was examined in rabbits after application of a single dose (100 mg) into the conjunctival sac. Very slight to slight conjunctival redness was observed in all eyes that lasted for up to 24 hours. L-754, 030 was practically non-irritating to the eyes of rabbits, as assessed by the methods of Kay and Calandra (Journal of the Society of Cosmetic Chemists 13: 281-289, 1962).

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 32^0C. 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 fluorescin dye was applied to the same corneas for 1.5 hr at 32^0C. The amount of dye that passed through the corneas was measured 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: Exploratory Bovine Corneal Opacity and Permeability (BCOP) Assay with L-755,446.

Key study findings: The irritation potential of L-755, 446, a metabolite of MK-0869, was assessed using bovine corneal opacity and permeability (BOCP) assay. L-755, 446 was a severe irritant to bovine cornea in this in *vitro assay*, in all three experiments.

Study no: #00-4267, #99-4251, #96-4258
Volume #, and page #, Volume #48, page #H-100, H-188, H-257
Conducting laboratory and location: Laboratoires Merck Sharp & Dohme-Chibret, Centre de Recherche, Riom, France.

Methods: L-755446, a major metabolite of MK-0869, was examined for its ocular irritation potential using an *in vitro* test of ocular irritancy, the bovine corneal opacity and permeability (BCOP) assay. Three assays were conducted with three different batches of the metabolite (L-755, 446-001E001, L-755, 446-004L001 and L-755, 446-002G005). 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^{0}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 fluorescin dye was applied to the same corneas for 1.5 hr at 32^{0}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: *In vitro* incubation of bovine corneas with L-755, 446 (20% suspension), a metabolite of MK-0869 produced severe irritant effects. as assessed by corneal opacity and permeability scores, in all three experiments. The mean values for opacity, permeability and irritancy scores for the test agent and the vehicle in three different experiments are summarized in the Table below.

Treatment Group	Opacity	Permeability	Irritancy Score
Study #96-4258			
L-755,446-001E001 (20% w/v)	160 ± 8.68	2.044 ± 0.321	190.7 (severe)
Vehicle (control)	0.67 ± 0.58	0.014 ± 0.001	0.9
Study #99-4251			
L-755, 446-004L001 (20% w/v)	85.5 ± 9.47	3.429 ± 0.384	136.9 (severe)
Vehicle (control)	0.67 ± 1.15	0.013 ± 0.002	0.9
Study #00-4267			
L-755, 446-002G005 (20% w/v)	102.00 ± 3.74	4.483 ± 0.116	169.2 (severe)
Vehicle (control)	3.67 ± 1.53	0.021 ± 0.004	4.0

Summary: The effects of L-755, 446 on bovine corneal opacity and permeability were examined in three different experiments after incubating the cornea with the drug for 4 hours. L-755, 446 was found to be a severe irritant to bovine cornea in this in *vitro assay*, in all three experiments.

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.

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}I-thyroxine (160 µCi/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}I-thyroxine concentrations were determined by ⟵ 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 ($t_{½}$), volume of distribution (Vd) and plasma clearance (Cls).

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}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)[1]		Volume of Distribution (mL)[1]	
	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.
1 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.

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.

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-trifluromethylcoumarin O-deethylase (EFCOD) activity and fatty acyl-CoA oxidase (FACO) activity in microsomal pellet aliquots were assessed

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.

Conclusions:

L-758, 289 (pro-drug of MK-0869) caused minimal local irritation in rats when administered at i.v. doses up to 5 mg/kg/day for 7 days. Topical application of L-754, 030 was not irritating to the skin of rabbits, and it was mildly irritating to the eyes of rabbits. In an *in vitro* bovine corneal opacity and permeability assay, L-745, 030 was a severe irritant to the cornea. The nonpolar netabolite, L-755, 446 was also a severe corneal irritant in this assay. However, the significance of this finding is not clear, as in the toxicology studies with L-754, 030, it had no effects on corneal opacity, and in the eye irritation study, it was a mild irritant to the rabbits eye. L-758, 298 caused hemolysis of washed washed red blood cells from rats, dogs and humans. However, it did not cause any hemolysis in human whole blood. The effects of oral MK-0869 on the plasma T_3, T_4 and TSH levels, and thyroxine clearance were examined in rats. It caused an increase in the TSH levels in both males and females; thyroxine clearance was also increased in animals receiving MK-0869. The increases in the TSH levels may be due to increased liver enzyme activity that causes an increased metabolism of thyroid hormones. The pro-drug, L-758, 298 caused moderate induction P_{450} enzyme activity (7-ethoxy-4-

trifluromethylcoumarin-O-deethylase and fatty acyl-CoA oxidase activity) in the mouse liver, when administered orally. The liver enzyme inducing capability of the drug may also be responsible for the hepatocellular hypertrophy and thyroid follicular cell hyperplasia, observed in rats in subchronic and chronic toxicity studies.

Labeling:
Carcinogenesis, Mutagenesis, Impairment of Fertility

Sponsor's version:

DRAFT

Evaluation: In the 106-week oral carcinogenicity study in rats, treatment with aprepitant produced thyroid follicular cell adenoma and carcinoma in the male rats, and there were increased incidences of hepatocellular adenoma, thyroid follicular cell adenoma and adenocarcinoma of the uterus in the female rats. In the 105-week oral carcinogenicity study in mice, aprepitant treatment produced skin fibrosarcoma in the male mice, and there were increased incidences of hepatocellular adenoma and Harderian gland adenoma in the female mice. These findings should be included in the labeling. Plasma exposure levels in rodents should be expressed in comparison to that in humans at the recommended clinical dose.

Proposed version:

DRAFT

Draft

Pregnancy
Sponsor's version:

Teratogenic Effects: Pregnancy Category B.

Draft

Evaluation: The doses used in the teratology studies in rats and rabbits should be expressed in comparison with the recommended human dose on a body surface area basis.

Proposed version:

Draft

Nursing mothers

Draft

OVERDOSAGE:
Sponsor's version: