Drug Batch No.: R-45790

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

Methods: Pregnant female rats were given MDL 73,147 orally by gavage at daily doses of 20, 60 and 100 mg/kg/day (3 ml/kg) from day 7 through 18 of gestation. Control group rats received the vehicle (water) in similar manner. The selection of the doses were based on the preliminary study in which oral doses of 0, 30, 100 and 300 mg/kg/day were used. According to sponsor significant retardation of body weight gains (20-27%) and lower fetal body weights (11.5%) were seen at 100 mg/kg/day and higher dose levels. All dams were observed for clinical signs twice daily, body weights were recorded on 2, 7, 10, 13, 19 and 21 day of gestation. Food consumptions were recorded on 7, 10, 13, 19 and 21 day of gestation. All dams were sacrificed at day 21 of gestation and was examined for number of corpora lutea, number of resorptions, number of live/dead fetuses, number of implants. The live fetuses were weighed and sexed. Approximately two-third of the fetuses eviscerated and examined for skeletal major/minor abnormalities. The remaining one-third fetuses were examined for visceral abnormalities and variations.

Results: Body weight gains were not affected by the treatment. Food consumptions in the drug treated animals were slightly higher than the control group animals (data shown graphically). The number of corpora lutea, the number of implants, numbers of live/dead fetuses and sex ratio did not show any significant difference between the treated groups and the control group. However, the fetal weights in high dose group were reduced by 6% compared to the control values. Gross examination revealed 2 fetuses had multiple malformations. One fetus in 20 mg/kg/day group had forelimb flexion deformity, aphalangia and microphthalmia; and another fetus in 60 mg/kg/day group had micrognathia, aglossia and microstomia. Upon skeletal examination, three fetuses revealed skeletal malformations (forked ribs: 1 in control group and 1 in high dose group; hemivertebra: 1 in high dose group). Additionally 8 fetuses had bent or wavy ribs (1 in control group, 2 in mid dose group and 5 in high dose group). Bent or wavy ribs are due to lack of ossification and reversible in due time. Thus, no treatment and dose related abnormalities were observed on external, skeletal, and visceral examinations in any group.

Effect of MDL 73,147 on Maternal and Fetal Parameters in Rats

Parameters Measured	Control	Low Dose	Mid Dose	High Dose
# Dam examined	20		20	
# Pregnant	19		18	
# Corpora lutea/Dam	12.63		12.78	
# Implants/Dam	10.89		11.28	
# Pre implant loss (%)	14.74		10.54	
# Post implant loss (%)	10.83		2.77	
# Resorption/dam	0.53		0.34	
# Litters examined	19		18	
# Fetuses examined	197		197	
# Live fetuses/dam	10.37		10.94	
# Dead fetuses/dam	0		0	
Fetal wt (g)	3.78	3.79	3.7	3.55
Sex Ratio (M/F)	1/0.89	1/0.88	1/0.88	1/1.03

In summary, no embryotoxic and teratogenic effects at oral dosage up to 100 mg/kg/day was observed in rats.

I.V. Segment II. Teratology Study in Rats
(Report # I-93-0008-T)

Testing Laboratories: Department of Drug safety,
Indianapolis Center.,
Marion Merrell Dow Inc.,
Kansas City, MO.

Study Started: March 20, 1992

Study Completed: January 29, 1994

GLP Requirement: A statement of compliance with GLP regulations and quality assurance unit was included.

Animals: Sprague Dawley (Crl:CD BR VAF/Plus) pregnant rats.

No. of Animals: 21-25 rats/group

Route of Administration: I.V.

Dose Levels: 0, 7, 20 and 60 mg/kg/day

Drug Batch No.: C-48616

Methods: Pregnant female rats were given MDL 73,147 intravenously at daily doses of 0 (vehicle), 7, 20 and 60 mg/kg/day (2 ml/kg) from day 6 through 17 of gestation. Because most of the rats in high dose group had lesion at the injection sites, sponsor included two additional groups, one group received vehicle (2.5 ml/kg) while the other group received 60 mg/kg/day (2.5 ml/kg) of MDL 73,147EF. It should be noted here that in the second high dose group, reduced concentration of the drug was given while total dose remained the same. The selection of the doses were based on the preliminary teratology study (report # I-93-0052-T) in which i.v. doses of 0, 5, 15, 45 and 100 mg/kg/day were used. Severe lesion at the injection sites, decreased activity in all rats and intermittent skeletal spasms were seen at 100 mg/kg/day dose level. All dams were observed for clinical signs daily, body weights and food intakes were recorded on 0, 6, 9, 12, 18 and 20 day of gestation. All dams were sacrificed at day 20 of gestation and was examined for number of corpora lutea, number of resorptions, number of live/dead fetuses, number of implants. The live fetuses were weighed and sexed. Approximately two-third of the fetuses eviscerated and examined for skeletal major/minor abnormalities. The remaining one-third fetuses were examined for visceral abnormalities and variations.

Results: Severe lesions were seen at the injection sites (tail) in most of the treated rats and severity was dose related. At high dose, 4 dams had convulsions and 1 out of 4 died. Treatment had no significant effect on body weight gains and food consumptions. The number of corpora lutea, the number of implants, numbers of live/dead fetuses, fetal weights and sex ratio did not show any significant difference between the treated groups and the control group. A total of 1027 fetuses were examined for skeletal abnormalities and 508 fetuses were examined for visceral abnormalities. No treatment and dose related abnormalities were observed on external, skeletal, and visceral examinations in any group. In summary highest i.v. dose tested (60 mg/kg/day) was maternal toxic (convulsions). However, no embryotoxic and teratogenic effects at i.v. dosage up to 60 mg/kg/day was observed in rats.

Morphologic Teratology Study of MDL 73,147EF in Rabbits (Report # C-90-0036-T)

Testing Laboratories: Merrell Dow Pharmaceuticals Inc., Cincinnati, OH.

Dates Studies Started and Completed: November 21, 1989 and July 11, 1990

Test Species: New Zealand White rabbits (4.09 ± 0.11 kg).

No. of Animals: 26 rabbits/group

Route of Administration: Oral (gavage)

Dose Levels: 0, 20, 60 and 100 mg/kg/day

Drug Batch No.: R-45790

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

Methods: Pregnant rabbits were given MDL 73,147 orally by gavage at doses of 20, 60 and 100 mg/kg/day (3 ml/kg) from day 7 through 19 of gestation. Control group rabbits received the vehicle (water) in similar manner. The selection of the doses were based on the preliminary study in which oral doses of 0, 50, 100 and 150 mg/kg/day were used. According to sponsor significant retardation of body weight gains and reduction in food consumptions were seen at 100 mg/kg/day and higher dose levels. Pregnant rabbits were observed twice daily during pregnancy, food consumptions were recorded daily and their weights were recorded on 1, 7, 10, 13, 20 25 and 29 day of gestation. All surviving dams were sacrificed at day 29 of gestation, and were examined for number of corpora lutea, number of resorptions, number of live/dead fetuses, number of implants. The live fetuses were weighed and sexed. All fetuses were eviscerated and examined for skeletal/visceral anomalies.

Results: At the end of 29 day of gestation the body weight gains were reduced by 3.3%, 2.8% and 5.6% at low, mid and high dose respectively. Food consumptions in the drug treated animals were reduced by during treatment period, and during day 20-28 of gestation the food consumptions were reduced by 22-34% in drug treated animals. There were 2 deaths (1 in control group and 1 in high dose group), and these were not treatment related. Several animals had abortion on days 25-29 (0 in control group, 3 in low dose group, 2 in mid dose group and 0 in high dose group). There was a significant dose related increase in resorptions and postimplantation losses in treated animals. The fetal body weights were 8 and 13% less at mid and high dose respectively, when compared to the control values. Seven malformed fetuses were found out of 334 fetuses (113 control and 221 treated). In control group one fetus had diaphragmatic hernia and another fetus had fused ribs, in low dose group one fetus had holoprosencephaly and hydrocephalus and another fetus had forked rib, in mid dose group, one fetus had persistent truncus arteriosus, and in high dose group, one fetus had cleft palate and its littermate had both hydrocephalus and cleft palate. Since these malformations are not dose related, therefore were not considered to be drug induced. Delayed/incomplete

ossification, extra presacral vertebrae, and rudimentary and extra 13th ribs were seen in this study and their distribution were comparable in all groups. Thus no treatment and dose related abnormalities were observed on external, skeletal, and visceral examinations in any group.

Effect of MDL 73,147 on Maternal and Fetal Parameters in Rabbits

Parameters Measured	Control	Low Dose	Mid Dose	High Dose
Total Mated	26		26	
Pregnant	18		19	
% Pregnant	69.2		73.1	
# Surviving	17		19	
# Corpora lutea/Dam	12.71		12.47	
# Implants/Dam	7.53		6.76	
# Pre implant loss (%)	38.18		43.77	
# Post implant loss (%)	12.81		44.55	
# Resorption/dam	0.82		1.70	
# Live fetuses/dam	6.65		5.06	
# Dead fetuses/dam	0.06		0	
Fetal Weight (g)	42.22		38.72	
Sex Ratio (M/F)	1/0.85		1/1.20	
Morphological Findings of Fetuses				
# Litters examined	16		14	
# Fetuses Examined	113		86	
Gross Anomalies	0		0	
Skeletal Anomalies				
Hydrocephalus/ Cleft Palate	0		0	
Cleft Plate	0		0	
Forked Rib	0		0	
Fused Rib	1		0	
Visceral Anomalies				
Heart Superficial circumferential of the constriction around Ventricles	0		1	
Diaphragmatic Hernia	1		0	
Persistent Truncus Arteriosus/ Ventricular Septal Defect	0		1	
Holoprosencephaly	0		0	
Testicles-displaced dorsally	0		0	

In summary highest tested oral dose (100 mg/kg/day) was maternal and embryotoxic. However, no teratogenic effects at oral dosage up to 100 mg/kg/day was observed in rabbits.

I.V. Teratology Study in Rabbits
(Report # I-93-007-T)

Testing Laboratories: Department of Drug safety,
Indianapolis Center.,
Marion Merrell Dow Inc.,
Kansas City, MO.

Study Started: April 17, 1992

Study Completed: January 29, 1993

GLP Requirement: A statement of compliance with GLP regulations and quality assurance unit was included.

Test Species: New Zealand White rabbits (kg, months old).

No. of Animals: 20 rabbits/group

Route of Administration: I.V.

Dose Levels: 0, 2, 7 and 20 mg/kg/day

Drug Batch No.: C-48616

Methods: Pregnant rabbits were given MDL 73,147 intravenously at daily doses of 0 (vehicle), 2, 7 and 20 mg/kg/day (2 ml/kg) from day 6 through 18 of gestation. The selection of the doses were based on the preliminary teratology study (report # C-92-0281-T) in which i.v. doses of 0, 5, 15, 45 and 100 mg/kg/day were used. Convulsions and lethality were seen after administering of 45 or 100 mg/kg/day dose levels. Ptosis and rapid breathing were noted in rabbits treated with 5 or 15 mg/kg/day dose levels. Pregnant rabbits were observed daily during pregnancy, food consumptions were recorded daily and their weights were recorded on 1, 6, 9, 12, 19 and 28 day of gestation. All surviving dams were sacrificed at day 28 of gestation, and were examined for number of corpora lutea, number of resorptions, number of live/dead fetuses, number of implants. The live fetuses were weighed and sexed. All fetuses were eviscerated and examined for skeletal/ visceral anomalies.

Results: On gestation day 11 one high dose treated rabbit had tremors. During day 9-19 of gestation the food consumptions were reduced by in high dose treated animals and this effect was mainly caused by 3 rabbits (#75, #78 and #80). These rabbits consumed little or no food during day 9-19 of gestation. During day 19-28 of gestation (post dosing period), food intakes were decreased by 12%, 9% and 10% in low, mid and high dose groups,

when compared to control values. Body weight gains were not affected by the treatment. One rabbit (#39) from low dose group had abortion and 1 rabbit each from low dose (#25) and mid dose (#51) had 100% intra-uterine mortality. The number of corpora lutea, the number of implants, numbers of live/dead fetuses, and sex ratio did not show any significant difference between the treated groups and the control group. The mean fetal weights from treated groups were about lower than that seen in control group. A total of 502 fetuses were examined for external, skeletal and visceral abnormalities. No treatment and dose related abnormalities were observed on external, skeletal, and visceral examinations in any group except increase incidences of delayed/incomplete ossification of hyoid were seen in treated groups (control = 19/95, low dose 29/124, mid dose = 34/151 and high dose = 40/131).

Effect of MDL 73,147 on Maternal and Fetal Parameters in Rabbits

Parameters Measured	Control	Low Dose	Mid Dose	High Dose
Total Mated	20		20	
Pregnant	15		19	
% Pregnant	75		95	
# Surviving	15		18	
# Aborted	0		0	
100% Intra-uterine mortality	0		1	
# Corpora lutea/Dam	9.7		9.9	
# Implants/Dam	7.5		8.5	
# Early resorption/dam	0.9		0.1	
# Late resorption/dam	0.2		0.0	
# Live fetuses/dam	6.4		8.3	
# Dead fetuses/dam	0		0	
Mean Fetal Weight (g)	40.5		37.2	
Sex Ratio (M/F)	45		44	

In summary no teratogenic effects at i.v. dosage up to 20 mg/kg/day was observed in rabbits.

Oral Segment III. Perinatal and Postnatal Study in Rats
(Report # K-94-0547-T)

Testing Laboratories: Department of Drug safety, Indianapolis Center., Marion Merrell Dow Inc., Kansas City, MO.

Study Started: August 26, 1992

Study Completed: July 23, 1994

Laboratory:

The Dow Chemical Co., Freeport, Texas.

Dates of Conduct: Dates not given; report dated: May 19, 1988.

GLP Statement: In compliance with FDA's GLPs.

MATERIALS AND METHODS

Chemical: MDL 73,147EF, lot # not given, dissolved in DMSO; and S-9 rat liver homogenate.

Test System: Salmonella typhimuriam tester strains TA-1535, TA-1537, TA-1538, TA-100, and TA-98.

Concentrations: When using TA 100, the MDL concentrations were: 5, 15.8, 50, 158, 500, 1,580, 5,000 ug/plate of MDL. But due to precipitation and toxicity, MDL was tested at 5, 15.8, 50, 158.0 and 500 ug/plate for the remaining strains.

Negative Control: DMSO

Positive Control: Sodium azide, 25 ug/plate; -191, 10 ug/plate; 2-nitrofluorene, 100 ug/plate; and 2-anthramine, 5 ug/plate.

Methods: Standard Ames method (Ames, BN et al. Methods for Detecting Carcinogens and Mutagens with the Salmonella/Mammalian Microsomal Mutagenicity Test, Mutation Research 31:347-364, 1975) was used.

Results: MDL was toxic (cell death) to the TA 100 strain at a concentration of 1,580 and 5,000 ug/plate, while it was not mutagenic in any other strain with or without metabolic activation at any other concentration.

In summary, MDL was not mutagenic in the Ames test at concentrations of up to 500 ug/plate.

In Vitro Rat Lymphocyte Chromosomal Aberration Test
(Report # I-90-0020-T)

Testing Laboratories: The Dow Chemical Co.,
Freeport, TX

Dates Studies Started and Completed: August 16, 1989 and
February 20, 1990

Strain Employed: Rat lymphocytes cultured cells.

Concentration Employed: mcg/ml

GLP Requirement: A statement of compliance with GLP regulations and quality assurance unit was included.

Test Species: Pregnant Sprague Dawley rats (Crl: CD SD BR VAF/plus strain).

No. of Animals: 23 pregnant rats/group

Route of Administration: Oral

Dose Levels: 0, 20, 60 and 100 mg/kg/day

Drug Batch No.: 3564

Methods: Pregnant rats were given oral (gavage) doses of 0 (water), 20, 60 and 100 mg/kg/day (3 ml/kg) of MDL 73,147EF from day 15 of gestation to day 20 after parturition. All dams were observed for clinical signs daily, body weights were recorded on 0, 7, 15 and 20 day of gestation, and on days 0, 7, 14 and 21 of post partum. Food consumptions were recorded on gestation days 0, 7, 15 and 20. The number of live/dead pups were recorded, and the live pups were weighed and sexed. On day 4 of postpartum culling was carried out to make 8 offspring (4 male and 4 female) per dam. Pups were also weighed on days 4, 11 and 21 of post partum. The offspring were reared by the dams until day 21 of post partum. On day 21 of post partum all dams were sacrificed and necropsied, and examined externally and internally for abnormalities. During the nursing period the growth and differential of the pups were observed, and development parameters were assessed (pinnae detachment, upper incisor eruption, eye opening, testes descend, vagina opening and sensory-motor function tests).

Results: One dam (#83) of high dose group was found dead on day 18 of gestation. Autopsy finding could not establish the cause of death. Another female (#77) from high dose group failed to deliver offspring and was necropsied on day 28 of gestation. Her abdominal and thoracic viscera were normal and her "uterus stained negative for implantation sites". Throughout gestation and lactation period, no abnormalities were seen in clinical signs, body weight gains and food consumptions in F_0 dams. Length of gestation, number of live pups born and pre-natal loss were comparable in all groups. No abnormalities were observed at autopsy of F_0 dams which would be attributed to treatment. No drug related effects were seen in the F_1 pups during postnatal period. Development of F_1 pups were comparable in all groups.

Segment III. Perinatal and Postnatal Study in Rats

Parameters Measured	Control	Low Dose	Mid Dose	High Dose
# of Pregnant	23	23	23	21
Length of Gestation (days)	21.3	21.4	21.6	21.4
# Live fetuses/dam	14.8	15.0	14.6	14.9
Pre-natal loss (%)	6.5	4.7	5.2	5.3
Mean Fetal wt (g)				
Day 0	5.8	5.9	6.0	5.8
Day 4	8.9	9.1	9.4	9.2
Day 21	50.7	49.2	49.2	49.0

Thus no adverse effect were seen in rats following oral administration of up to 100 mg/kg/day of MDL 73,147EF during perinatal and postnatal period.

GENETIC TOXICOLOGY:

Ames Test
(Report # C-88-0019-T)

This report was submitted under (Initial Submission dated 11/18/88 and Amendment dated 6/14/89). Data were reviewed on 12/24/1889 and review text is being reproduced here:

Solvent Control: Water

Positive Control: Ethyl methane sulfonate (EMS: 1000 mcg/ml), cyclophosphamide (4.2 mcg/ml).

Drug Batch No.: Not given.

Source of Metabolic Activation: Rat liver homogenate (S-9 mix).

Criteria of Genotoxic Effect: Mutation frequency in the exposed culture must be significantly different than the frequency in the negative control and it should be dose related.

Results: Rat lymphocytes cultured cells were treated with MDL-73,147 in the presence and absence of metabolic activator (S-9 mix). At the end of the experiment 200 metaphases were examined per treatment group (100 metaphases in positive control group). There was no mitotic activity in cultures treated with 1000 mcg/ml MDL 73,147 in presence or absence of S-9 mix. Cultures treated with 33.3-333.3 mcg/ml of MDL-73,147 did not produce significant increases in the aberration frequency compared to the negative controls either in the presence or absence of S-9 mix. Significant increases in the mutation frequencies of the positive test control cultures were observed. Thus MDL-73,147 is not mutagenic in this test up to 333.3 mcg/ml.

CHO/HGPRT Cell-Forward Gene Mutation Assay
(Report # C-90-0038-T)

Testing Laboratories: The Dow Chemical Co.,
Freeport, TX

Dates Studies Started and Completed: June 23, 1989 and
January 19, 1990

Strain Employed: CHO-K1 Chinese hamster ovary cells.

Concentration Employed:

Solvent Control: Water

Positive Control: Ethyl methane sulfonate (EMS: 621 mcg/ml),
20-metylcholanthrene (20-MCA: 4 mcg/ml)

Drug Batch No.: Not given.

Source of Metabolic Activation: Rat liver homogenate (S-9 mix).

Criteria of Genotoxic Effect: Criteria of positivity was not clearly defined.

Results: A concentration of 400 mcg/ml was toxic to the cells (relative cell survival rate = 0.7%), and drug precipitated during the treatment period at 350 and 400 mcg/ml concentrations. Mutation frequencies observed in the cultures treated with MDL 73,147 were not significantly different from the negative control, irrespective of the presence or absence of S-9 mix. Significant increases in the mutation frequencies of the positive test control cultures were observed. Thus, MDL 73,147 had no mutagenic potential in this test up to 350 mcg/ml.

Unscheduled DNA Synthesis (UDS) Assay in Rat Hepatocyte
(Report # I-90-0019-T)

Testing Laboratories: The Dow Chemical Co.
Freeport, Texas

Dates Study Started and Completed: June 1 1989 and
February 20, 1990

Animals: Male CDF Fischer 344 rats

Concentration Employed:

Solvent Control: Water

Positive Control: 2-Acetyl-aminofluorene (2-AAF: 2.233 mcg/ml)

Drug Batch No.: R441274

Methods: Hepatocyte primary cultures from the liver of adult male F344 rats were incubated for 18-20 hr with MDL-73,147 , positive control 2-AAF (2.233 mcg/ml) or water (solvent control). The selection of concentrations of the drug is based on the preliminary toxicity study, in which a concentration of 500 mcg/ml was toxic to the cells. The test compound is considered positive when the mean net nuclear grain count at any dose level is greater than 5 per nucleus and also significantly higher than the negative control value.

Results: Two independent were performed. In both assay concentrations of 166.7 and 500 mcg/ml were toxic to the cells. No net increase in nuclear grain counts was observed in hepatocytes exposed to MDL-73,147 at concentrations ranging from . The positive control was genotoxic. Thus, the

results suggest that MDL-73-147 was not genotoxic in the rat hepatocyte primary culture/DNA repair test at concentration up to 50 mcg/ml.

Micronucleus Test in Mice
(Report # I-90-0021-T)

Testing Laboratories: The Dow Chemical Co.
Freeport, Texas

Dates Study Started and Completed: August 16, 1989 and
March 8, 1990

Test Strain: Male and female CD-1 (ICR) BR mice

No. of Animals: 5/sex/group/sacrifice time

Route of Administration: Oral (gavage 10 ml/kg)

Dose Levels: Single dose of 75,250 and 750 mg/kg

Negative Control: Water

Positive Control: Cyclophosphamide (120 mg/kg)

Drug Batch No.: Not given

Basis of Dose Selection: Dose selections are based on the preliminary toxicity study. In this study MDL-73,147 was administered once orally to 5 animals/sex/group at dose levels of 250, 500 and 1000 mg/kg. Nine out of 10 mice died within 2 day in 1000 mg/kg dose group, while no mortality was noted in the other two dose groups. Thus, a 750 mg/kg dose level was chosen as the highest dose for the main study and the other two remaining doses were 250 and 75 mg/kg.

Methods: Groups of mice (5/sex/dose) were given a single dose of the drug at 24, 48 or 72 hours prior to sacrifice and preparation of the bone marrow. Mice treated with cyclophosphamide were all sacrificed at 24 hr after dosing. On the Giemsa-stained slides, 1000 polychromatic erythrocytes per animal were examined for the presence of micronuclei. A dose related statistically significant increase in the number of micronucleated polychromatic erythrocytes in treated animals compared to negative control is considered as positive.

Results: Five animals (2 male and 3 females) died before the scheduled sacrifice time at high dose. MDL-73,147 did not induce a significant increase of micronucleated polychromatic

erythrocytes (mn-PCE) in any treated groups compared to negative control values. In contrast, cyclophosphamide induced a significant increase in the frequencies of mn-PCE in the bone marrows of mice (both sexes). These findings suggest that MDL-73,147 is not mutagenic in this test system.

Dose (mg/kg)	At 24 hr (mn-PCE/1000 PCE)	
	Males	Females
0	2.0 ± 1.4	0.8 ± 1.2
75	1.4 ± 1.7	0.6 ± 0.5
250	2.4 ± 2.3	1.8 ± 1.1
750	1.4 ± 1.1	0 ± 0
positive control	47.2 ± 11.9	52.0 ± 13.5

Micronucleus Test in Mice
(Report # C-92-0366-T)

Testing Laboratories: The Dow Chemical Co.
Freeport, Texas

Dates Study Started and Completed: February 20, 1992 and June 3, 1992

Test Strain: Male and female CD-1 (ICR) BR mice

No. of Animals: 5/sex/group/sacrifice time

Route of Administration: I.V.Oral (5 ml/kg)

Dose Levels: Single dose of 37.5, 75 and 150 mg/kg

Negative Control: Water

Positive Control: Cyclophosphamide (120 mg/kg)

Drug Batch No.: C-48616

Basis of Dose Selection: Dose selections are based on the preliminary toxicity study. In this study MDL-73,147 was administered once intravenously to 5 animals/sex/group at dose levels of 75, 150 and 300 mg/kg. Eight out of 9 mice died within a day in 300 mg/kg dose group, while no mortality was noted in the other two dose groups. Thus, a 150 mg/kg dose level was chosen as the highest dose for the main study and the other two remaining doses were 75 and 37.5 mg/kg.

Methods: Groups of mice (5/sex/dose) were given a single dose of the drug at 24, 48 or 72 hours prior to sacrifice and preparation of the bone marrow. Mice treated with cyclophosphamide were all sacrificed at 24 hr after dosing. On the Giemsa-stained slides, 1000 polychromatic erythrocytes per animal were examined for the presence of micronuclei. A dose related statistically significant increase in the number of micronucleated polychromatic erythrocytes in treated animals compared to negative control is considered as positive.

Results: MDL-73,147 did not induce a significant increase of micronucleated polychromatic erythrocytes (mn-PCE) in any treated groups compared to negative control values. In contrast, cyclophosphamide induced a significant increase in the frequencies of mn-PCE in the bone marrows of mice (both sexes). These findings suggest that MDL-73,147 is not mutagenic in this test system.

SPECIAL TOXICITY STUDIES:

Local Tolerance Study in Beagle Dogs
(Report # I-93-0005-T)

Testing Laboratories: Department of Drug Safety,
Indianapolis Center,
Marion Merrell Dow Inc.,
Kansas City, MO

Study Started: April 21, 1992

Study Completed: January 19, 1993

GLP Requirements: A Statement of Compliance with GLP regulations was included.

Animals: months old Beagle dogs (males: kg and females: kg).

Drug Batch No.: 015F002

Methods: Groups of dogs (3/sex/group) were given a single i.v. (100 mg = 5 ml), i.a. (100 mg = 5 ml), paravenous (50 mg = 2.5 ml) or i.m. (50 mg, 2.5 ml) dose of MDL 73,147EF on left side of the dogs and equal volume of the vehicle was injected in similar fashion on right side of the dogs. One dog/sex were sacrificed at 48 hr, 96 hr and 14 days after drug administration and injection sites were examined microscopically.

Results: No treatment related local irritation was evident when the drug was given via i.v. or i.a. route. However, drug was more irritating (s.c. inflammation and collagen necrosis) than vehicle when given by paravenous or i.m. routes.

Proposed Text of the Labeling for Anzemet:

The label is according to 21 CFR, 201.50, Subpart B (April 1, 1995). However, the following changes should be incorporated:

1. Carcinogenesis, Mutagenesis, Impairment of Fertility:

Sponsor's Version:

Carcinogenesis, Mutagenesis, Impairment of Fertility

Dolasetron mesylate was not mutagenic in a variety of in vitro and in vivo oral and intravenous mutagenicity tests, including the mouse micronucleus assay. Dolasetron mesylate two-year oral carcinogenicity studies were conducted in mice and rats. Dolasetron mesylate was not carcinogenic in rats at Doses 53 and 105 times (based on mg/kg) the highest recommended human Dose (200 mg), in males and females, respectively. In mice, dolasetron mesylate was not carcinogenic at Doses 26 times the highest recommended human Dose. Liver tumors in males and endometrial polyps in females were found at 53 times the highest recommended human Dose. Fertility and reproductive performance were not affected by oral administration of dolasetron mesylate to male and female rats at Doses 140 and 35 times the highest recommended human Dose, respectively.

Evaluation:

In 2-year carcinogenicity study in Crl:CD-1 (ICR) mice, MDL 73,147 was given via diet at daily doses of 75, 150 and 300 mg/kg/day. The highest tested dose is the maximum tolerated dose, since it produced histopathological changes in the target organ (liver) of toxicity. Hence, dose selection was appropriate. Treatment had no significant effect on inter-current mortality rates and survival rates at the end of study period were comparable in all groups. In males increased incidences of hepatocellular adenomas and hepatocellular carcinomas were seen. Increase in the incidences of hepatocellular adenomas reached to statistical significance only in one sex (males: $p = 0.0001$; Peto trend test). Pairwise comparison (Fisher exact test) of incidences of hepatocellular adenomas between control and individual treatment groups showed

significance for mid and high dose treated males (p values: 0.0001 and 0.004 respectively). It should also be noted that incidence rate of hepatocellular adenoma in high dose treated male mice was within range of historical incidence rate (high dose [300 mg/kg/day] = 23.6%, published historical control incidence rate = 18.6% . In males, increase in the incidences of hepatocellular carcinoma was not statistically significant (males: p = 0.0512; Peto trend test). If one adds the incidence of hepatocellular adenomas to the incidence of hepatocellular carcinomas then the combined incidence of any liver lesion (i.e. adenomas and/or carcinomas) become statistically significant in males (p = 0.0000; Peto trend test). Sponsor testing laboratory does not have historical control incidence rate, since this is the first time they have conducted 2-year carcinogenicity study in CD-1 mice. Data indicated that MDL 73,147 is tumorigenic in male mouse. The drug is not genotoxic, therefore it is non-genotoxic carcinogen. Sponsor has not investigated the non-genotoxic mechanisms of the production of liver tumors in treated male mouse. Furthermore, tumor (hepatocellular adenomas) seen in male mice is due to drug induced liver toxicities (centrolobular hypertrophy, single cell degeneration/necrosis and altered eosinophilic foci). Based on mg/sqm, high dose treated mice (300 mg/kg/day = 900 mg/sqm) were exposed to about 6 time higher than the recommended human dose (200 mg/day, 4 mg/kg [50 kg body weight assumed] = 148 mg/sqm). Based on AUC values, high dose treated mice were exposed to fold higher levels of MDL 74,156 than human ($AUC_{0\text{-}24\ hr}$ = 3097 ng.hr/ml after a single oral dose of 200 mg of MDL 73,147 [report # K-94-0864-CDS]). Increase in hepatocellular adenomas in male mice were seen at ≥150 mg/kg/day, which on the basis of mg/sqm is about 3 time higher than the recommended human dose (200 mg/day, 4 mg/kg = 148 mg/sqm). Next lower dose (75 mg/kg/day) can be considered as threshold dose for Dolasetron's carcinogenic effect.

In 2-year carcinogenicity study in Crl:CD(SD)BR rats, MDL 73,147 was given via diet at daily doses of 75, 150 and 300 mg/kg/day in males and 150, 300 and 600 mg/kg/day in females (it should be noted that MTD in 3-month dose ranging study was close to 250 mg/kg/day). More than 85% of high dose (300 mg/kg/day in males and 600 mg/kg/day in females) treated rats had hematuria, therefore, all rats in high dose group were killed and discarded on day 228/229 of the study. On day 176 of the study, sponsor added 4 additional groups (male control group, female control group, male treated with 25 mg/kg/day and female treated with 50 mg/kg/day) and dosed for 2-years (day 176 was designated as study day 1 for these groups). Hence, the selection of top dose in the initial experiment exceeded MTD. The new top doses (i.e. 150 mg/kg/day in males and 300 mg/kg/day in females) are close to MTD. Hence,

dose selection was appropriate. Even though experiment was conducted in two "time period" i.e. one of the control group and low dose group were started on day 176 of the study and continued for full 2-years, overall conduct of the study is acceptable. In all the analysis, initial top doses (i.e. 300 mg/kg/day in males and 600 mg/kg/day in females) were excluded. For analysis purposes only doses 25, 75 and 150 mg/kg/day in males and 50, 150 and 300 mg/kg/day in females were used. The treatment had no significant effect on intercurrent mortality rates and survival rates at the end of treatment period were comparable in all groups. At the end of treatment period, final body weights in males were 14%, 13% and 19% lower than control final body weight at low, mid and high dose respectively and the corresponding values in females were 15%, 15% and 27% respectively. Based on mg/sqm, highest tested dose in males (150 mg/kg/day) and females (300 mg/kg/day) were 5.98 and 11.96 fold higher than the recommended daily dose in human (200 mg/day = 148 mg/sq.m.; 50 kg body wt. assumed) respectively. Based on AUC values, high dose treated male and female rats were exposed to 3.9 and 7.7 fold higher levels of MDL 74,156 respectively than human ($AUC_{0\text{-}24\ hr}$ = 3097 ng.hr/ml after a single oral dose of 200 mg of MDL 73,147 [report # K-94-0864-CDS]). With respect to non-neoplastic findings, increased incidences of thymus involution and cystic glandular hyperplasia in the mammary gland were seen in high dose treated females. No treatment related neoplastic findings were evident in this study. Thus, MDL 73,147 did not show carcinogenic effect in 2-year carcinogenicity study in rats.

Anzemet was not mutagenic in Ames test, rat lymphocyte chromosomal aberration test, Chinese hamster ovary cell/HGPRT forward gene mutation assay, mouse bone marrow micronucleus test (oral and i.v.) and in vitro rat hepatocyte

<u>Proposed Version</u>:

In 104-week dietary carcinogenicity study, mice (Crl:CD-1 [ICR]) were treated orally with Anzemet 75, 150 and 300 mg/kg/day (225, 450 or 900 mg/sq. m./day). For a 50 kg person of average height (1.46 sq.m. body surface area), these doses represent 1.52, 3.04 and 6.08 times the recommended clinical dose (148 mg/sq. m., p.o.) on a body surface area basis. Based on AUC values, high dose treated mice were exposed to fold higher levels of MDL 74,156 than human ($AUC_{0\text{-}24\ hr}$ = 3097 ng.hr/ml after a single oral dose of 200 mg of MDL 73,147). Increase in hepatocellular adenomas only in male mice were seen at ≥150 mg/kg/day, which on the basis of mg/sqm is about 3 time higher than the recommended

human dose (200 mg/day, 4 mg/kg = 148 mg/sqm). The drug is not genotoxic, therefore it is non-genotoxic carcinogen. Next lower dose (75 mg/kg/day) can be considered as threshold dose for Dolasetron's carcinogenic effect.

In 104-week dietary carcinogenicity study, male rats (SD) were treated orally with Anzemet 25, 75, 150 mg/kg/day while females were treated with 50, 150 and 300 mg/kg/day (147.5, 442.5 and 885 mg/sq. m./day in males and 295, 885 and 1770 mg/sq.m./day). For a 50 kg person of average height (1.46 sq.m. body surface area), these doses represent 0.99, 2.99, 5.98 times the recommended clinical dose (148 mg/sq. m., p.o.) on a body surface area basis in males and the corresponding ratio in females were 1.99, 5.99 and 11.96 respectively. Based on AUC values, high dose treated male and female rats were exposed to 3.9 and 7.7 fold higher levels of MDL 74,156 respectively than human ($AUC_{0\text{-}24\ hr}$ = 3097 ng.hr/ml after a single oral dose of 200 mg of MDL 73,147). Anzemet did not show carcinogenic effect in 104-week carcinogenicity study in rats.

Anzemet was not mutagenic in Ames test, rat lymphocyte chromosomal aberration test, Chinese hamster ovary cell/HGPRT forward gene mutation assay, mouse bone marrow micronucleus test (oral and i.v.) and in vitro rat hepatocyte unscheduled DNA synthesis (UDS) assay.

Anzemet at oral doses up to 400 mg/kg/day (2360 mg/sq. m./day, 15.9 times the recommended human dose based on body surface area) was found to have no effect on the fertility and reproductive performance of male rats. Anzemet at oral doses up to 100 mg/kg/day (590 mg/sq. m./day, 3.9 times the recommended human dose based on body surface area) was found to have no effect on the fertility and reproductive performance of female rats.

2. Pregnancy:

Sponsor's Version:

Pregnancy

Teratogenic Effects. Pregnancy Category B: Reproduction studies have been performed in rats and rabbits at oral Doses up to 35 times the 200 mg human Dose and have revealed no evidence of impaired fertility or harm to the fetus due to dolasetron mesylate. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Nonteratogenic Effects. Slight (6% and 13%) reductions in fetal weights at oral dolasetron Doses of 100 mg/kg were observed in rats and rabbits, respectively. In rabbits, oral Doses ≥ 20 mg/kg/day resulted in early resorptions and postimplantation losses. These changes were not observed at comparable Doses administered intravenously

Evaluation:

The text is not according to 21 CFR, 201.50, Subpart B (April 1, 1995).

Proposed Version:

Pregnancy: Teratogenic effects. Pregnancy category B.

Reproduction studies have been performed in rats (up to 100 mg/kg/day) and rabbits (up to 100 mg/kg/day) at oral doses up to 4 times the human dose (200 mg = 4 mg/kg/day = 148 mg/sq. m.; 50 kg body weight assumed) on the basis of mg/kg/day and up to 3.99 and 5.8 times the human dose on the basis of mg/sq. m. respectively which revealed no evidence of impaired fertility or harm to the fetus due to Anzemet. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

3. Overdosage:

Sponsor's Version:

OVERDOSAGE

A 7-year-old male received 6 mg/kg orally before surgery. No symptoms occurred and no treatment was required.

It is not known if dolasetron is removed by hemodialysis or peritoneal dialysis.

Following a suspected overDose of ANZEMET, a patient found to have 2-degree or higher AV conduction block should undergo Cardiac telemetry monitoring.

There is no known specific antidote for dolasetron mesylate, and patients with suspected overDose should be managed with supportive therapy. Individual Doses as large as 5 mg/kg intravenously or 400 mg orally have been safely given to healthy volunteers or cancer patients.

APPEARS THIS WAY
ON ORIGINAL

Evaluation:

Sponsor did not provide any clinical or preclinical overdose data. Acute oral toxicity of Dolasetron mesylate was studied in mice and rats. The minimum oral lethal doses were 525 and 400 mg/kg for mice and rats respectively. Clinical signs in both species were tremors, depression and convulsions.

Proposed Version:

The following sentences should be added to the sponsor's version:

The minimum oral lethal doses were 525 and 400 mg/kg for mice and rats respectively. Clinical signs in both species were tremors, depression and convulsions.

SUMMARY AND EVALUATIONS:

The mechanism of action of different stimuli on emetic center is not well understood to date. Recent studies have suggested that chemotherapeutic agents or radiation has a cytotoxic action on enterochromaffin cells in the upper G.I. tract, which results in a large release of 5-HT. This release of 5-HT stimulates 5-HT_3 receptors on vagal afferent fibers close to enterochromaffin cells, and 5-HT then acts centrally as a vegal neurotransmitter or co-transmitter to initiate vomiting reflex. Any agent which block 5-HT_3 receptors either at vagal or area postrema should be able to prevent emesis. It has been shown that 5-HT_3 antagonists are very effective in treating chemotherapy induced nausea and vomiting. MDL 73,147EF is a selective 5HT_3 antagonist without any significant dopamine antagonist activity, thus void of any significant extrapyramidal side effects, and it inhibits cisplatin-induced nausea and vomiting.

In anesthetized dogs, intravenous dose of 1 mg/kg of MDL 73,147E' had no effect on heart rate, blood pressure or ECG. However, 2 4 mg/kg (i.v.) dose produced transient (<90 sec) reduction in blood pressure and a cumulative dose of 6 mg/kg and higher dose levels start affecting cardiac hemodynamic (decrea left ventricular BP, dP/dt max, systemic BP and heart rate) ar cardiac conduction (significantly increased PQ and RR interva

In conscious dogs, a cumulative i.v. dose of 1 mg/kg of MDL 73,147EF had no effect on heart rate, blood pressure or However, significant increased PR intervals were seen at i. cumulative doses of of MDL 73,147EF (increas interval: 9 m sec at 3 mg/kg, 17 m sec at 10 mg/kg and 47

at 30 mg/kg [cumulative dose of 15 mg/kg ondansetron significantly decreased PR interval by 10 m sec]). It is clear that at 30 mg/kg of MDL 73,147EF value of PR interval is approaching first degree heart block. MDL 73,147EF (cumulative dose: 30 mg/kg) and ondansetron (cumulative dose: 15 mg/kg) both significantly also increased QTc interval (MDL: 47 m sec, ondansetron: 35 m sec) compared to baseline.

Thus, in dogs (anesthetized or conscious), 2 mg/kg (i.v.) of MDL 73,147EF was no effect dose for cardiac toxicities.

Oral administration of MDL 73,147EF (10 mg/kg/day for 4 days) in conscious dogs, did not significantly affect arterial blood pressure but heart rate was slightly increased (about 16 bpm) at 3 and 4 hours after drug administration and ECG recording was normal. Thus in conscious dogs, 10 mg/kg (oral) of MDL 73,147EF was considered to be the no effect dose for cardiac toxicities.

MDL 74,156 (the main metabolite of MDL 73,147) is also pharmacologically active (anti-emetic in ferrets, inhibition of Bezold-Jarisch Reflex in anesthetized rats, in vitro binds to 5-HT_3 receptors). Sponsor did not report ED50 values for the drug or its main metabolite (MDL 74,156).

In support of the new drug application for dolasetron, sponsor has submitted preclinical data from the pharmacology studies; absorption, distribution, metabolism and excretion (ADME) studies in rats, rabbits, dogs and monkeys; acute toxicity studies in mice, rats, dogs and monkeys; 1-month (oral and i.v.), 3-month oral and 1-year oral toxicity studies in rats; 1-month (i.v.) and 1-year oral toxicity studies in dogs; 1-month (i.v.) and 3-month oral toxicity studies in monkeys; 3-month oral (diet) dose-ranging study in mice and rats; 2-year oral dietary carcinogenicity studies in mouse and rat; Segment I. fertility and general reproductive performance study in rat, oral and i.v. Segment II. teratology studies in rats and rabbits and Segment III. oral prenatal and postnatal studies in rats; genotoxicity studies: Ames test, micronucleus test in mice (oral and i.v.), chromosomal aberration test in rat lymphocytes, in vitro UDS assay in rat hepatocytes and CHO/HGPRT forward mutation assay and special toxicity study (local tolerance study in dogs).

ADME studies have been conducted in rats, dogs, rabbits and monkeys. In all 4 species drug was absorbed rapidly (T_{max} ≤2 hr) and completely. Based on urinary excretion data after oral and i.v. dose, absorption ranged from (human: 90%). However, the absolute bioavailability was low due to extensive first pass effect (rat: 3.5%, dog: 13.2%, monkey: 9% and human:

not determined). Irrespective of species and route of administration the plasma $t_{½}$ of MDL 73,147 was close to 0.5 hr (human: not determined) and plasma $t_{½}$ of MDL 74,156 (major metabolite) was about 5 hr (human: 7-9 hr).

In rats, administered radioactivity was distributed throughout the body. Radioactivity levels in liver, kidney, stomach and small intestine were higher than that seen in plasma. In Long-Evans rats levels of radioactivity in eyes were about 4-fold higher than that seen in SD rat's eyes, which suggests ^{14}C-labeled drug binds to uveal tract melanin ($t_{½}$ = 130 hrs). Based on Vd, it is evident that the parent drug is not widely distributed (Vd = 1.1 L/kg) while MDL 74,156 (the main metabolite) was widely distributed (Vd = 27.0 L/kg).

Irrespective of species and route of administration, drug is metabolized rapidly. No parent drug was seen in urine or feces. MDL 74,156 is the main metabolite (it is also pharmacologically active) and the other metabolites were N-oxide, 5'-OH, 6'-OH and 7'-OH derivatives of MDL 74,156 and their corresponding conjugates.

Irrespective of species and route of administration about -- and of radioactivity were excreted in urine and feces (mainly biliary), and most of the excretion occurred during the first 48 hr period.

In vitro about of the drug (MDL 73,147) was bound to rat, dog, monkey and human plasma, while binding of MDL 74,156 ranged in all 4 species. In rat, MDL 73,147 is not a hepatic enzyme inducer.

In acute toxicity study, the minimum oral lethal doses were 525 and 400 mg/kg for mice and rats respectively, and the corresponding i.v. doses were 160 mg/kg for male mice, 140 mg/kg for female mice and rats of both sexes. The highest nonlethal oral doses were 400 mg/kg and 300 mg/kg in mice and rats respectively while 126 mg/kg was the highest nonlethal i.v. dose in female mice and rats of both sexes. The highest nonlethal i.v. dose in male mice was 140 mg/kg. Clinical signs in both species were tremors, depression and convulsions at high doses. In dogs and monkeys oral/i.v. minimum lethal doses could not be determined since no animal died during the study period. However in dogs, higher doses produced emesis and salivation lacrimation, in addition the i.v. doses also produced tremors, chewing movements and panting. These clinical signs regress within 3.5 hours after dosing. In contrast no clinical signs were evident in monkeys at 200 mg/kg oral dose.

In 1-month i.v. toxicity study in rats, doses of 15, 30, 60 and 120 mg/kg/day were used. In this study, no effect dose was not identified. In this study, no effect dose was not identified. The lowest tested dose produced reduction in body weight gains in males (3.8%) and ulceration/necrosis in 1 out of 10 females at the injection sites. This dose level can be considered as well tolerated dose. Higher dose levels produced decreased activity, convulsions and lesions (inflammation, hemorrhage, thrombosis and necrosis) at the injection site. CNS is the target organ of toxicities.

In 1-month oral toxicity study in rats, doses of 200, 300, 400 and 500 mg/kg/day were used. Lethality was seen at 300 mg/kg in females and at 400 mg/kg in males and cause of death was not established. A 200 mg/kg/day and higher dose level produced clinical signs (salivation, depression, ataxia and tremors) in rats of both sexes. Limited histopathological examinations indicated that kidney could be the target organ of toxicity. In this study, a no effect dose was not established, CNS and kidneys are the target organs of toxicities.

In three months oral toxicity study in rats, doses of 10, 30, 100 mg/kg/day were used. In female rats, spleen, liver, brain, heart and kidney weights were increased by 8.6%, 28.8%, 4.5%, 14.9%, and 14.8% respectively at 100 mg/kg without any histopathological changes. The "no effect dose" was 30 mg/kg/ day, and no target organ(s) of toxicity was identified in this study.

In 1-year oral toxicity study in rats, doses of 30, 75 and 400 mg/kg/day were used. In this study CNS (convulsions, depression and tremors), kidney (proximal convoluted tubule degeneration) and urinary tract epithelium irritation (inflammation and/or reactive hyperplasia of the renal pelvic epithelium) are the target organ of toxicities. Lethality was evident at ≥75 mg/kg/ day. Lowest tested dose (30 mg/kg/day) did not produce any toxicity except irritation in lower urinary tract epithelium in males (control = 3/40 and low dose = 5/43) and sporadic convulsions in males (control = 0/68 and low dose = 3/68).

In 1-month i.v. toxicity study in dogs, doses of 1, 3 and 6 mg/kg/day were used. The highest tested dose only produced clinical signs (salivation, vomiting, tremors and ataxia). In this study, CNS is the target organ of toxicities and mid dose (3 mg/kg/day) was the no effect dose.

In 1-year oral toxicity study in dogs, doses of 3 mg/kg/day (1 mg/kg t.i.d.), 10 mg/kg/day (5 mg/kg b.i.d.), 15 mg/kg/day (7.5 mg/kg b.i.d.) or 20 mg/kg/day (10 mg/kg b.i.d.) were used. In this study no target organ of toxicity was identified. Highest tested dose produced only emesis and salivation.

In 1-month i.v. toxicity in monkeys, doses of 2, 5 and 10 mg/kg/day were used. Two out of 3 female monkeys from high dose group had "small focal lesions of chronic interstitial myocarditis". No such lesions were found in the control, low or mid dose group monkeys. Sponsor also indicated that this kind of lesion is often found in control monkeys. Hence, sponsor considered this finding to be not treatment related. Because of cardiac lesions seen at high dose, mid dose should be considered as the no effect dose.

In three months oral toxicity in monkeys, doses of 5, 15 and 50 mg/kg/day were used. No clinical signs were noted except all treated animals on 1 or 2 occasions vomited just before or during dosing. The "no effect dose" was 50 mg/kg/day and no target organ(s) of toxicity was identified in this study.

In 3-month dietary range finding study in mice doses of 100, 250, 500, 1000 and 2000 mg/kg/day were used. In this study, liver is the target organ of toxicity. Based on hepatocellular hypertrophy at ≥100 mg/kg/day, significantly increased liver weights at 250 mg/kg/day in males (28%) and increased serum alanine aminotransferase and serum aspartate aminotransferase activities in treated mice (both sexes), sponsor selected 75, 100 and 300 mg/kg/day for the main carcinogenicity study in mice.

In 2-year carcinogenicity study in Crl:CD-1 (ICR) mice, MDL 73,147 was given via diet at daily doses of 75, 150 and 300 mg/kg/day. The highest tested dose is the maximum tolerated dose, since it produced histopathological changes in the target organ (liver) of toxicity. Hence, dose selection was appropriate. Treatment had no significant effect on inter-current mortality rates and survival rates at the end of study period were comparable in all groups. In males increased incidences of hepatocellular adenomas and hepatocellular carcinomas were seen. Increase in the incidences of hepatocellular adenomas reached to statistical significance only in one sex (males: $p = 0.0001$; Peto trend test). Pairwise comparison (Fisher exact test) of incidences of hepatocellular adenomas between control and individual treatment groups showed significance for mid and high dose treated males (p values: 0.0001 and 0. respectively). It should also be noted that incidence rate of hepatocellular adenoma in high dose treated male mice was within range of historical incidence rate (high dose [300 mg/kg/day] = 23.6%, published historical control

incidence rate = 18.6%, range In males, increase in the incidences of hepatocellular carcinoma was not statistically significant (males: p = 0.0512; Peto trend test). If one adds the incidence of hepatocellular adenomas to the incidence of hepatocellular carcinomas then the combined incidence of any liver lesion (i.e. adenomas and/or carcinomas) become statistically significant in males (p = 0.0000; Peto trend test). Sponsor testing laboratory does not have historical control incidence rate, since this is the first time they have conducted 2-year carcinogenicity study in CD-1 mice. Data indicated that MDL 73,147 is tumorigenic in male mouse. The drug is not genotoxic, therefore it is non-genotoxic carcinogen. Sponsor has not investigated the non-genotoxic mechanisms of the production of liver tumors in treated male mouse. Furthermore, tumor (hepatocellular adenomas) seen in male mice is due to drug induced liver toxicities (centrolobular hypertrophy, single cell degeneration/necrosis and altered eosinophilic foci). Based on mg/sqm, high dose treated mice (300 mg/kg/day = 900 mg/sqm) were exposed to about 6 time higher than the recommended human dose (200 mg/day, 4 mg/kg [50 kg body weight assumed] = 148 mg/sqm). Based on AUC values, high dose treated mice were exposed to fold higher levels of MDL 74,156 than human ($AUC_{0\text{-}24\ hr}$ = 3097 ng.hr/ml after a single oral dose of 200 mg of MDL 73,147 [report # K-94-0864-CDS]). Increase in hepatocellular adenomas in male mice were seen at ≥150 mg/kg/day, which on the basis of mg/sqm is about 3 time higher than the recommended human dose (200 mg/day, 4 mg/kg = 148 mg/sqm). Next lower dose (75 mg/kg/day) can be considered as threshold dose for Dolasetron's carcinogenic effect.

In 3-month dietary range finding study in rats doses of 100, 250, 500, 1000 and 2000 mg/kg/day were used. In this study, lethality was evident at ≥1000 mg/kg/day. A dose of 250 mg/kg/day could be considered as well tolerated dose since it produced only reduction in body weight gains (males: 8.5% and females: 7.6%) and slight hematuria in 1 out of 10 males. The dose level (500 mg/kg/day) was more toxic to rats of both sexes reduction in body weight gains, increased incidence of hematuria and 1 out of 10 males ureter was inflamed). Based on these findings it appears that maximum tolerated dose (MTD) will be close to 250 mg/kg/day.

In 2-year carcinogenicity study in Crl:CD(SD)BR rats, MDL 73,147 was given via diet at daily doses of 75, 150 and 300 mg/kg/day in males and 150, 300 and 600 mg/kg/day in females (it should be noted that MTD in 3-month dose ranging study was close to 250 mg/kg/day). More than 85% of high dose (300 mg/kg/day in males and 600 mg/kg/day in females) treated rats had hematuria, therefore,

all rats in high dose group were killed and discarded on day 228/229 of the study. On day 176 of the study, sponsor added 4 additional groups (male control group, female control group, male treated with 25 mg/kg/day and female treated with 50 mg/kg/day) and dosed for 2-years (day 176 was designated as study day 1 for these groups). Hence, the selection of top dose in the initial experiment exceeded MTD. The new top doses (i.e. 150 mg/kg/day in males and 300 mg/kg/day in females) are close to MTD. Hence, dose selection was appropriate. Even though experiment was conducted in two "time period" i.e. one of the control group and low dose group were started on day 176 of the study and continued for full 2-years, overall conduct of the study is acceptable. In all the analysis, initial top doses (i.e. 300 mg/kg/day in males and 600 mg/kg/day in females) were excluded. For analysis purposes only doses 25, 75 and 150 mg/kg/day in males and 50, 150 and 300 mg/kg/day in females were used. The treatment had no significant effect on intercurrent mortality rates and survival rates at the end of treatment period were comparable in all groups. At the end of treatment period, final body weights in males were 14%, 13% and 19% lower than control final body weight at low, mid and high dose respectively and the corresponding values in females were 15%, 15% and 27% respectively. Based on mg/sqm, highest tested dose in males (150 mg/kg/day) and females (300 mg/kg/day) were 5.98 and 11.96 fold higher than the recommended daily dose in human (200 mg/day = 148 mg/sq.m.; 50 kg body wt. assumed) respectively. Based on AUC values, high dose treated male and female rats were exposed to 3.9 and 7.7 fold higher levels of MDL 74,156 respectively than human ($AUC_{0-24\ hr}$ = 3097 ng.hr/ml after a single oral dose of 200 mg of MDL 73,147 [report # K-94-0864-CDS]). With respect to non-neoplastic findings, increased incidences of thymus involution and cystic glandular hyperplasia in the mammary gland were seen in high dose treated females. No treatment related neoplastic findings were evident in this study. Thus, MDL 73,147 did not show carcinogenic effect in 2-year carcinogenicity study in rats.

In the oral Segment I. fertility and general reproductive performance study in male rats doses of 50, 200 and 400 mg/kg/day were used. There were no abnormal effects on the fertility and mating performance of the treated male rats at oral doses up to and including 400 mg/kg/day of MDL 73,147EF. However, this dose level was toxic to males (decreased body wt. gain [16.4%] and about 28% mortality rate).

In the oral Segment I. fertility and general reproductive performance study in female rats doses of 20, 60 and 100 mg/kg/day were used. There were no abnormal effects on the fertility and mating performance of the treated female rats at oral doses up to and including 100 mg/kg/day of MDL 73,147EF.

In oral Segment II. teratology study in rats, doses of 20, 60 and 100 mg/kg/day were used. No teratogenic effects at doses up to 100 mg/kg/day was observed.

In i.v. Segment II. teratology study in rats, doses of 7, 20 and 60 mg/kg/day were used. No teratogenic effects at doses up to 60 mg/kg/day was observed. However, the highest i.v. dose tested (60 mg/kg/day) was maternal toxic (convulsions and death).

In oral Segment II. teratology study in rabbits, doses of 20, 60 and 100 mg/kg/day were used. The highest dose tested (100 mg/kg/day) was maternal (reductions in body weight gains and food consumptions) and embryotoxic (dose related increase in resorptions, post implantation losses and lower fetal body weights). However, no teratogenic effects at dosage up to 100 mg/kg/day was observed.

In i.v. Segment II. teratology study in rabbits, doses of 2, 7 and 20 mg/kg/day were used. No teratogenic effects at doses up to 20 mg/kg/day was observed.

In oral Segment III. perinatal and postnatal study in rats, doses of 20, 60 and 100 mg/kg/day were used. No adverse effect were seen in rats following oral administration of up to 100 mg/kg/day of MDL 73,147EF during perinatal and postnatal period.

No mutagenic potential was demonstrated when MDL 73,147 was tested in five different tests: Ames test, rat lymphocyte chromosomal aberration test, Chinese hamster ovary cell/HGPRT forward gene mutation assay, mouse bone marrow micronucleus test (oral and i.v.) and in vitro rat hepatocyte unscheduled DNA synthesis (UDS) assay.

In dogs, no treatment related local irritation was evident when the drug was given via i.v. or i.a. route. However, drug was more irritating (s.c. inflammation and collagen necrosis) than vehicle when given by paravenous or i.m. routes.

In humans the proposed route of administration is oral. Sponsor has adequately characterized Anzemet and conducted sufficient preclinical toxicity studies in different species. In rats, CNS (convulsions, depression and tremors), kidney (proximal convoluted tubule degeneration) and urinary tract epithelium irritation (inflammation and/or reactive hyperplasia of the renal pelvic epithelium) are the target organ of toxicities. In dogs, CNS (salivation, vomiting, tremors and ataxia) is the target organ of toxicity.

MDL 73,147 did not show carcinogenic effect in 2-year carcinogenicity study in rats. In 2-year carcinogenicity study in Crl:CD-1 (ICR) mice, significant increase incidence of hepatocellular adenomas were seen in male mice at ≥150 mg/kg/day. Tumor (hepatocellular adenomas) seen in male mice is due to drug induced liver toxicities (centrolobular hypertrophy, single cell degeneration/necrosis and altered eosinophilic foci). Furthermore, the incidence rate of hepatocellular adenoma in high dose treated male mice was within range of historical incidence rate (see above). The drug is non-genotoxic carcinogen and no significant increase in liver tumor was seen at low dose (75 mg/kg/day = 225 mg/sq.m.) which on the basis of mg/sq.m. is about 1.5 time higher than the recommended human dose (200 mg/day, 4 mg/kg [50 kg body weight assumed] = 148 mg/sq.m.). A dose of 75 mg/kg/day can be considered as threshold dose for Dolasetron's carcinogenic effect.

MDL 73,147 and its main metabolite (MDL 74,156) binds selectively to 5-HT_3 receptors (Ki=0.8-20 nM). MDL 73,147 and MDL 74,156 blocked rat brain 5-HT_4 receptors with Ki values of 330 nM and 67 nM respectively. However, in isolated guinea pig ascending distal colon preparation (a in vitro system in which actions of drug(s) at 5-HT_4 receptors can be assessed), MDL 73,147 and MDL 74,156 had no significant agonist activity. Both compounds (MDL 73,147 and MDL 74,156) at a concentration of 10 mcM had no significant affinity for dopamine D_2-receptors and dopamine D_3-receptors in conventional radioligand binding assays. Chronic treatment with MDL 73,147 or ondansetron down regulated the activity of specific dopaminergic systems in the rat brain. In cloned alpha-subunit of the human cardiac muscle sodium channel (expressed in Xenopus Oocytes), significant sodium channel blocking activity was only observed at concentration >10 μM of MDL 73,147EF or its metabolites. Sponsor suggested that conduction defect seen in dogs (see above) may be related to the effect of drug (&/or its main metabolite: MDL 74,156) on cardiac muscle voltage-dependent sodium channels.

This application is for the prevention of cancer chemotherapy-induced nausea and vomiting, and for the prevention of postoperative nausea and vomiting. The proposed duration of treatment is of short-term (i.e. single dose).

From a preclinical standpoint the application is approvable.

The label is according to 21 CFR, 201.50 Subpart B (April 1, 1991), however, it needs minor changes in the text as outlined in the review portion.

RECOMMENDATION:

From a preclinical standpoint the application is approvable. Sponsor should be asked to change the labeling as outlined in the review portion.

/S/ 5/24/96

Tanveer Ahmad, Ph.D.
Pharmacologist, HFD-180

cc:
Orig. NDA
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HFD-006/CAC/Ms. Olmstead
HFD-150/CAC/Dr. DeGeorge

1. Concur
2. See the attached addendum (Page 109a).

- /S/ 6/28/96

TA/hw/5/24/96
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ATTACHMENTS:

APPENDIX I. **Non-Neoplastic Findings in Mice**

Unscheduled Deaths - Males
Unscheduled Deaths - Females
Two-Year Necropsy - Males
Two-Year Necropsy - Females

Neoplastic Findings in Mice

Unscheduled Deaths - Males
Unscheduled Deaths - Females
Two-Year Necropsy - Males
Two-Year Necropsy - Females

APPENDIX II. **Non-Neoplastic Findings in Rats**

Unscheduled Deaths - Males & Females
Two-Year Necropsy - Males & Females

Neoplastic Findings in Rats

Unscheduled Deaths - Males & Females
Two-Year Necropsy - Males & Females

NDA 20,623 ANZEMET Tablets

Pharmacology Team Leader's Addendum to Pharmacology Review of Dr. T. Ahmad

1. Concur.

2. The proposed indications for ANZEMET tablets are (a) the prevention of nausea and vomiting associated with emetogenic cancer therapy and (b) the prevention of postoperative nausea and vomiting. These indications require only single dose treatments for which carcinogenicity studies are usually not required.

3. The following corrections should be noted. They are indicated by reviewer's page numbers.

a. Page 13 "f. Effect on ---------Fuinea Pig Trachea" The tissue used in this in vitro study was guinea pig tracheal ring segments, but not "guinea pig atria" (Volume 1.26, Page 1, Reference 53).

b. Pages 29, 41, 45 and 88 Reviewer incorporated the previous IND 32,386 Pharmacology review of toxicology studies. The correct date of the Pharmacology review is December 24, 1989, but not "12/24/1889".

/S/

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