Dog:

1-Month I.V. Toxicity Study in Dogs

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:

Laboratory: Merrell Dow's Cincinnati Labs.

Dates of Conduct: Initiated 9/24/87, completed 10/28/87.

GLP Statement: In compliance with FDA's GLPs.

MATERIALS AND METHODS

Chemical: MDL 73,147EF, sample # 09; analyzed for purity and stability periodically (102.5% pure); dissolved in buffered saline.

Animals: Beagle dogs, kg; 3/sex/dose.

Doses: 0 (control saline), 1, 3, and 6 mg/kg/d, iv once daily for 30 days. The doses were selected from the single dose iv dose-range finding tolerance study (T-88-01), where single doses of MDL were tested in 1 dog/sex/dose at 3, 4.5, 6, 20, and 30 mg/kg, iv. Salivation, emesis, chewing movements, tremors, and panting were noted at 6, 20, and 30 mg/kg but not at lower doses. There were no deaths. The concentration of the drug solution was 10 mg/ml and was injected at a rate of 20 ml/min.

Methods: The control vehicle or the test agent was injected as mentioned above and the animals were observed for morbidity and mortality. Various parameters were recorded that are mentioned in the respective sections below. Plasma samples were collected at 2 and 24 hr post-dosing on days 1 and 26 for pharmacokinetics (see above under ADME section). At termination all the dogs were necropsied followed by complete histopathology of control and high dose (6 mg/kg/d) dogs.

Observed Effects: Frequent emesis, salivation, loose stools, hyper-excitability and infrequently, ataxia were seen in all the 6 mg/kg/d treated dogs. No such effects were seen with lower doses.

Mortality: None.

Body weight gain and food consumption were unremarkable.

EKGs taken at 2 hr post-dosing on days 5, and 2 and 24 hr post-dosing on day 27 did not reveal any abnormal findings.

Similarly, ophthalmic examinations on pre-test and terminal days were normal.

Hematology, clinical chemistries, and urinalyses on pre-test and terminal days were all within normal limits.

Plasma levels: No unchanged parent drug could be detected in the plasma at 2 and 24 hr after dosing on both day 1 and 26 of the study due the extensive metabolism of the drug. It was also not detected in the plasma at 2 hr on days 1 to 26. However, its metabolite, MDL 28,577, was detected in the plasma for up to 18 hr. The 2-hr plasma levels of the metabolite after receiving 26 doses of MDL at doses of 1, 3, and 6 mg/kg/d were 137, 402, and 568 ng/ml, respectively. Further, small amounts (38 ng/ml) of MDL 28,577 were also present at 24 hr after receiving the 26th iv dose of 6 mg/kg/d. It is thus possible to suggest that the frequent development of emesis, and infrequent salivation, loose stools, hyperexcitability, and ataxia seen in the high dose dogs within 30-33 min of dosing was due to the metabolite and not the parent drug.

Necropsy and Organ weights did not reveal any abnormal trend and were all within the normal limits. No drug-related effects were seen at any dose.

Histopathology of selected tissues/organs also did not reveal any significant drug-related findings.

Thus, iv administration of MDL in the dogs at doses of 1, 3, and 6 mg/kg/d once daily for one month resulted in salivation, vomiting, tremors, ataxia with 6 mg/kg/d but not at lower doses. There was no significant histopathological finding at any dose that could be attributed to the drug treatment, since no parent drug was detected in the plasma at 2 and 24 hr after drug administration. However, the clinical symptoms could be attributed to the metabolite that was detected in the plasma for up to 24 hr after 26th dose of 6 mg/kg/d. The no-toxic dose and the maximum tolerated dose (MTD) was 3 mg/kg/d.

1-year Oral Toxicity Study in Dogs
(Report # I-92-0168-T)

Testing Laboratories: Drug Safety,
Cincinnati Center
Marion Merrell Dow Inc.,
Kansas City, MO

Study Started: August 13, 1990

Study Completed: November 27, 1992

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

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

Drug Batch No.: C-46711, C-47320, C-47341, C-47342, C-47344, C-48351, C-48616 and R-48613.

Methods: Groups of 10 male and 10 female beagle dogs were given orally (capsules) MDL 73,147EF at daily doses of 0 (empty gelatin capsules), 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.) for 1 year. Four dogs/sex/group were sacrificed at the end of 6-month and 1-year of treatment. Additionally, 1 dog/sex/group was used for 1-month recovery study after 6-month and 1-year treatment period. In this study dose selection was based on 1-week (5, 1.5, 10.5, 25.0, 50.0 or 100.0 mg/kg/day via capsule or 10.0, 25.0 or 50 mg/kg/day via gavage) and 2-week (10.0, 15.0 or 20.0 mg/kg/day given in divided doses via capsule) range finding study (report # C-90-0037-T) in dogs, in which 100.0 mg/kg produced convulsions and death, ≥50.0 mg/kg/day produced depression, ≥20 mg/kg/day produced tremors and ≥10 mg/kg/day produced increased incidence of emesis. No effect was seen at 7.5 mg/kg/day given once or twice daily. All animals were observed for clinical signs twice daily, body weights were recorded weekly and food consumptions were evaluated subjectively. Ophthalmoscopic examinations were performed on all dogs once pre-test, at 6, 12 and 13 (recovery dogs) months of the study. ECG recordings were taken at pre-test, at 6 months (2, 15 or 18 hr post dose) at 12 months (2, 15 or 18 hr post dose) and 13-month of the study. Blood samples were collected from jugular vein at pre-test, at 1, 3, 6, 9 and 12 months (approximately 18 hr post dose) and from 1/sex/group at the end of the recovery period for hematological and serum chemistry tests. Additionally, blood samples were collected from 4 dogs/sex/group on days 1, 30 and 366 at 2 and 6 hours after the first daily dose

for measuring MDL 73,147EF (drug) and its metabolite (MDL 74,156) levels in plasma. At the end of study/recovery period all dogs were sacrificed and subjected to complete necropsy and histopathological examinations.

Results:

1. Observed Effects: Increased incidence of emesis was seen at ≥10 mg/kg/day (5 mg/kg b.i.d.) and excessive salivation occurred sporadically at ≥15 mg/kg/day (7.5 mg/kg b.i.d.).

2. Mortality: None

3. Body Weight/Food Consumption/Water Consumption: No treatment related effects were seen.

4. Hematology/Coagulation/Bone Marrow: No treatment related effects were seen.

5. Blood Chemistry/Urinalysis: No treatment related effects were seen.

6. Vital Signs/Physical Examination/Ophthalmic Examination/ECG: No treatment related effects were seen.

7. Organ Weights: No treatment related effects were seen.

8. Gross Pathology: No treatment related effects were seen.

9. Histopathology: No treatment related effects were seen.

10. Plasma Levels of Drug (MDL 73,147) and its Metabolite (MDL 74,156): MDL 73,147 (drug) levels in plasma at 2 hr after drug administration were generally below detection limit (10 ng/ml). However, on day 366, 2 hr after drug administration, plasma levels of MDL 73,147 ranged between 10-45 ng/ml.

MDL 74,156 (main metabolite) levels were comparable in both sexes and increased with increasing dosages. In addition, MDL 74,156 levels were higher on day 366 than on day 1 or 30 suggesting significant decrease in MDL 74,156 clearance over 1-year period.

Mean MDL 74,156 Plasma Concentrations of Males and Females Combined

Dose (mg/kg/d)		Concentration (ng/ml) Day 1 2 hr	Day 1 6 hr	Day 30 2 hr	Day 30 6 hr	Day 366 2 hr	Day 366 6 hr
3 (1 t.i.d.)	Mean	< 17.8	*	29.6	<11.6	65.0	< 32.0
	S.D.	> 4.7	-	4.8	> 1.8	17.2	> 13.8
10 (5 b.i.d.)	Mean	< 105.6	< 43.6	183.3	84.8	368.0	162.0
	S.D	> 55.4	> 26.7	46.2	29.3	158.0	60.1
15 (7.5 b.i.d.)	Mean	245.1	113.9	300.6	156.4	653.0	284.5
	S.D.	44.4	29.5	120.0	64.3	88.2	66.4
20 (10 b.i.d.)	Mean	300.0	171.4	472.1	276.0	779.0	475.6
	S.D	143.8	93.8	182.0	135.6	191.0	157.0

Mean values were calculated with LLQ set equal to 10 ng/ml
* Five of the eight values were < LLQ (10 ng/ml)

Sponsor's Table 17, Vol. 1.51, Page 95

In this study no target organ of toxicity was identified. Highest tested dose produced only emesis and salivation.

Monkey:

1-Month I.V. Toxicity Study in Monkeys
(Report # C-88-0015-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:

Laboratory: Merrell Dow's Cincinnati Labs.

Dates of Conduct: Initiated 9/25/87, completed 10/30/87.

GLP Statement: In compliance with FDA's GLPs.

MATERIALS AND METHODS

Chemical: MDL, sample # 9; dissolved in normal saline, 10 mg/ml.

Animals: Cynomolgus monkeys; ; 3/sex/dose.

Doses: 0 (control), 2, 5, and 10 mg/kg/d, given by iv injection at a rate of 20 ml/min. The doses were selected from the dose-range finding tolerance study (T-88-01) with one monkey/sex/dose given MDL in single iv doses of 3, 6, 10, and 20 mg/kg for one day. There was no significant toxicity in any animal at any dose.

Methods: The control vehicle or MDL was given as indicated once daily for 30 days and the monkeys were observed daily for general appearance, morbidity and mortality. Various other standard parameters were recorded that are discussed in individual sections below. In addition, plasma samples were collected for pharmacokinetics at 2 and 24 hr post-dosing on days 1 and 26 of the study. At termination, all animals were necropsied but tissues/organs of only control and high dose (10 mg/kg/d) monkeys were examined for histopathological changes. No bone marrow smears were prepared.

Observed Effects: General appearance and behavior of all monkeys was normal.

Mortality: None.

Body weight gains and food consumption were not affected by drug treatment.

Clinical chemistries, hematology and urinalyses were all unremarkable.

Plasma levels: No parent drug was detected in the plasma after 1 or 26 doses possibly due the extensive metabolism of the drug . But the plasma levels of its metabolite, MDL 28,577 at 2 hr after the 26th dose of 2, 5, or 10 mg/kg/d were 69, 136, and 530 ng/ml, respectively. At 24 hr after the 26th dose of 10 mg/kg/d, the metabolite was barely detectable (10 ng/ml). There was no evidence of accumulation of the drug after multiple dosing that could explain the absence of any significant systemic toxicity in the monkeys except interstitial myocarditis (see below).

Organ weights were unremarkable.

Histopathology: An unusual but significant finding was the presence of mineralized foci (stained positive for iron, phosphate and/or carbonate) in the basal nuclei (cerebrum) of 2 of 3 control male monkeys and 4 of 6 high dose monkeys (2M,2F). In addition, 2 of 3 high dose female monkeys had small focal lesions of chronic interstitial myocarditis. Neither control females nor any male monkey developed such lesions.

In summary, MDL injected at doses of 2, 5, and 10 mg/kg/d iv into monkeys for 30 days did not produce any systemic toxicity at any dose, possibly due to extensive metabolism of the drug since no drug could be found in the plasma at 2 or 24 hr post-dosing on day 1 of the study. A number of the mineralized foci were seen in the brains of 4 of 6 treated and 2 of 6 control monkeys. In addition, 2 of 3 high dose female monkeys had small focal lesions of chronic interstitial myocarditis. Neither control females nor any male monkey developed such lesions. Whether this could be attributed to its metabolite, remains unclear at this time. Thus, 10 mg/kg/d could be considered as the no-toxic dose for the monkey. The maximum tolerated dose could not be determined in the absence of clear dose-related toxicity in the monkey.

3-Month Oral Toxicity Study in Monkeys
(Report # C-89-0020-T)

This report was submitted as Amendment (dated 2/15/90) to . Data were reviewed on 7/9/90 and review text is being reproduced here:

Date of the Study: December 28, 1988 to July 26, 1989.

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

Animals: Cynomolgus monkeys weighing were used.

Methods: Four groups of animals each consisting of 4 males and 4 females were given MDL73147EF in water by gavage at dose levels of 0, 5, 15 and 50 mg/kg/day for 3 months. The monkeys were bled pretest, 2 and 24 hrs after dosing on days 1 and 79 for pharmacokinetic study. The individual body weight, hematology, blood chemistry and urinalysis were presented. No statistical analysis was performed.

Results:

Clinical signs (daily): Emesis was observed in all treatment groups but it only occurred on 1-2 occasions except one 15 mg/kg male vomited on 39 occasions.

Mortality: None.

Body weight (weekly): Normal.

Hematology (weeks 0 and 1, months 1 and 3): According to sponsor, decrease in white blood cells and neutrophil were observed in the 50 mg/kg/day group.

Blood chemistry and urinalysis (weeks 0 and 1, months 1 and 3): Normal.

Organ weights and pathology: Normal.

Pharmacokinetics: No accumulation of parent drug or metabolites was seen after 79 single daily doses and there were no sex difference in plasma concentration.

In conclusion, MDL 73147EF given by gavage at doses up 50 mg/kg/day for three months did not produce any toxicity in monkeys except occasional emesis. Thus, no effect dose was 50 mg/kg/day. However, complete toxicity profile of MDL 73147EF was not revealed in the monkey. Data were not analyzed statistically.

3-Month Dietary Toxicity Study in Mice
(Report # I-93-0022-T)

Testing Laboratories: Department of Toxicology
Cincinnati Center
Marion Merrell Dow Inc.
Kansas City, MO

Study Started: December 3, 1991

Study Completed: April 2, 1993

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

Animals: Male and female Crl:CD-1 (ICR) mice (g, weeks old).

Drug Batch No.: C-49319

Methods: Groups of mice (22/sex/group) were given MDL 73,147EF via diet at daily doses of 0, 100, 250, 500, 1000 and 2000 mg/kg/day for 3 months. All animals were observed twice daily for clinical signs and mortality. Body weights and food intakes were recorded weekly. Just before sacrifice, blood samples were collected by cardiac puncture for hematological and serum chemistry tests. Twelve mice/sex/group were used for toxicokinetic studies. Blood samples were collected at 5 hr before the end of light cycle (3:00 p.m.) and at 1 hr after the beginning of dark cycle (9:00 p.m.) on days 31 and 91 of the study for monitoring drug levels in plasma (3 mice/sex/group/time point). At the end of study period all surviving mice were sacrificed and subjected to complete necropsy and histopathological examinations were limited to control and high dose groups. Liver was examined microscopically from remaining dose groups.

Results:

1. Observed Effects: No treatment related effects were seen.

2. Mortality: None

3. Body Weight/Food Consumption/Water Consumption: Body weight gains were reduced by 6.0% and 14.9% in 1000 and 2000 mg/kg/day treated males respectively, when compared to control values. In females body weight gains were reduced by 2.5% in 2000 mg/kg/day treated group, when compared to control values. Treatment had no adverse effect on food intakes.

4. Achieved Doses: Based on food consumption data, achieved doses were 96, 242, 491,, 975 and 1933 mg/kg/day in males and 97, 248, 511, 978 and 2018 mg/kg/day in females at 100, 250, 500, 1000 and 2000 mg/kg/day theoretical dose levels respectively.

5. Hematology/Coagulation/Bone Marrow: No treatment related effects were seen.

6. Blood Chemistry/Urinalysis: Alanine aminotransferase activities were increased dose dependently in treated mice (both sexes). Additionally, serum aspartate aminotransferase and phosphorus levels were also increased in ≥1000 mg/kg/day treated mice (both sexes).

Parameters	Sex (M/F)	Percent Increase Over Control Values				
		100 mg/kg/day	250 mg/kg/day	500 mg/kg/day	1000 mg/kg/day	2000 mg/kg/day
Alanine Aminotransferase	M	11	55	175	149	492
	F	36	55	80	192	189
Aspartate Aminotransferase	M	5	-9	51	28	79
	F	18	25	31	91	62
Phosphorus	M	15	5	16	26	23
	F	-3	10	9	27	43

Urinalysis were normal.

7. Organ Weights: In males, liver weights were increased by (relative wt: in ≥250 mg/kg/day treated mice, when compared to control values. In females, liver weights were increased by (relative wt:) in ≥1000 mg/kg/day treated mice, when compared to their control values.

8. Gross Pathology: No treatment related effects were seen.

9. Histopathology: Centrolobular hepatocellular hypertrophy were seen in treated mice (both sexes).

			Dose (mg/kg/day)				
Liver	Sex (M/F)	Control	100	250	500	1000	2000
Hepatocellular Hypertrophy (slight-moderate)	M	0/10	7/10	9/10	9/10	10/10	10/10
	F	0/10	3/10	3/10	4/10	10/10	10/10

10. Plasma Levels of the Drug (MDL 73,147) and its Metabolite (MDL 74,156) (report # K-95-0432-D): Levels of MDL 73,147 and MDL 74,156 were determined Levels of MDL 73,147 (drug) in mice plasma were below detection limit (50 ng/ml). Levels of MDL 74,156 (the main metabolite) increased with increasing dosages. There were no sex differences and levels on day 31 and day 91 were comparable.

	Mean Levels of MDL 74,156 (ng/ml) in Mice			
	3:00 p.m.		9:00 p.m.	
Dose (mg/kg/day)	Day 31	Day 91	Day 31	Day 91
100	158±111	93±45	165±84	162±55
250	373±199	254±189	529±297	677±314
500	738±184	639±793	930±282	941±356
1000	1022±459	503±178	1079±396	1073±351
2000	1112±660	1635±553	1370±690	2039±387

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.

FDA CDER CARCINOGENICITY ASSESSMENT COMMITTEE (CAC/CAC-EC) RODENT CARCINOGENICITY FACTSHEET

NDA: 20-623 DRUG CODE #:
CAS #: DATE:
DIVISION(s): HFD-180
DRUG NAME(s): Anzemet (Dolasetron)
SPONSOR: Hoechst Marion Roussel, Inc.
LABORATORY: Health and Environmental Sciences
The Dow Chemical Co.
Indianapolis, IN 46268-0470

P/T REVIEWER(s): Tanveer Ahmad, Ph.D.
P/T REVIEW DATE: May 24, 1996
CARCINOGENICITY STUDY REPORT DATE: 8-1-95

THERAPEUTIC CATEGORY: Prevention of cancer chemotherapy-induced nausea and vomiting and prevention of post-operative nausea and vomiting.

PHARMACOLOGICAL/CHEMICAL CLASSIFICATION: 5-HT_3 receptor antagonist.

PRIOR FDA DOSE CONCURRENCE (Div./CAC)? (Y/N; Date): No

MUTAGENIC/GENOTOXIC (Y/N/equivocal/na; assay): Negative 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.

MOUSE CARCINOGENICITY STUDY (multiple studies? Std1; Std2 etc):

MOUSE STUDY DURATION (weeks): 104
STUDY STARTING DATE: September 1, 1992
STUDY ENDING DATE: August 1, 1995
MOUSE STRAIN: Crl:CD-1 (ICR)
ROUTE: Diet
DOSING COMMENTS:

No. Mice in Control1 (C1): 55 Control2 (C2):
Low Dose (LD): 55 Middle Dose (MD): 55
High Dose (HD): 55 High Dose2 HD2):

MOUSE DOSE LEVELS (mg/kg/day)

Mouse Low Dose: 75 Mouse Middle Dose: 150
Mouse High Dose: 300 Mouse High Dose2:
*Dose adjusted during study.

Basis for Doses Selected (MTD; AUC ratio; saturation; maximum feasible): MTD

MOUSE CARCINOGENICITY (negative; positive; MF; M; F): Positive (M)

MOUSE TUMOR FINDINGS: Hepatocellular adenomas

MOUSE STUDY COMMENTS: In 2-year carcinogenicity study in Crl:CD-1) 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 hepato-cellular 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%, 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 2.3-3.8 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.

COVERSHEET FOR CARCINOGENICITY STUDY IN MICE

1. No. of Studies: K-95-0571-T

2. Name of Laboratory: Health and Environmental Sciences, The Dow Chemical Co.

3. Strain: Crl:CD-1 (ICR) Mice

4. No./sex/group: 60 (5 mice/sex were sacrificed on day 90 of the study).

5. Doses (O, L, M, H): 0, 75, 150 and 300 mg/kg/day

6. Basis for dose selection stated: Yes

7. Interim sacrifice: Yes

8. Total duration (weeks): 104

9. Week/site for first tumor:

	Male	Female
0	52/Malignant Lymphosarcoma (marrow)	54/Malignant Broncho-alveolar carcinoma (lung)
L	36/Malignant Lymphosarcoma (thymus)	20/Malignant Lymphosarcoma (thymus)
M	51/Malignant Myeloid Cell Sarcoma (marrow)	35/Malignant Lymphosarcoma (thymus)
H	41/Malignant Myeloid Cell Sarcoma (marrow)	51/Malignant Myeloid Cell Sarcoma (marrow)

10. No. alive at termination:

	Male	% Survival	Female	% Survival
O	31/55	56.4	20/55	36.4
L	25/55	45.4	27/55	49.1
M	35/55	63.6	22/55	40.0
H	31/55	56.4	34/55	61.8

11. Statistical Methods Used: Tumor data were analyzed according to Peto etal (IARC Supplement 2, 1980).

12. Attach tumor and non-tumor data for each tissue (i.e., benign; malignant; hyperplastic): See Appendix I.

Two-Year Dietary Carcinogenicity Study in CD-1 Mice
(Report # K-95-0571-T)

Testing Laboratories: Health and Environmental Sciences
The Dow Chemical Co.,
Indianapolis, IN 46268-0470

Study Started: September 01, 1992

Study Completed: August 01, 1995

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

Test Species: Crl:CD-1 (ICR) mice (_. g; age not given).

Route of Administration: Via diet.

Dose Levels: 0, 75, 150 and 300 mg/kg/day.

Drug Batch No.: .3601, .3602, 69550 and 69551.

Methods: In this study dose selection was based on 3-month oral (dietary) toxicity study (report # I-93-0022-T) in mice in which a dose of 250 mg/kg/day produced increased serum alanine amino-transferase and serum aspartate aminotransferase activities (both sexes), increased liver weights (only in males) and hepato-cellular hypertrophy (both sexes). This dose level is considered to be maximum tolerated dose (MTD). Based on these findings sponsor selected 0, 75, 100 and 300 mg/kg/day dose (via diet) levels for the main carcinogenicity study in mice. The duration of treatment was 2 years and control group mice were given unmedicated diet. There were 60 mice/sex/group and 5 mice/sex/group were necropsied after 3-month of treatment for assessing adequacy of doses based on clinical chemistry and liver changes. Additionally, twelve mice/sex in control group and 30 mice/sex in treatment groups were included in the study and used exclusively for toxicokinetic studies. Blood samples from these mice were collected at the end of 3-month and 12-month of treatment and time of collections were 8:00 p.m., 2:00 a.m., 8:00 a.m. and 2:00 p.m. (3 mice/sex/group/time point were used). All animals (except mice assigned for toxicokinetic studies) were observed daily for clinical signs and mortality. Body weights and food consumptions were recorded weekly during the first 3 months, bi-weekly during the second 3 months and every 4 weeks thereafter. Just before sacrifice blood samples were collected by cardiac puncture for hematological tests. All surviving mice were sacrificed at the end of treatment period and subjected to complete necropsy and histopathological examinations. Tumor data were analyzed according to Peto etal (Supplement 2, 1980).

Results:

1. Achieved Doses: The mean intakes of MDL 73,147 were 70.0 (range), 140.5 ( ) and 291.2 (range) mg/kg/day for males and 70.6 (range), 139.5 (and 290.5 for females at low, mid and high dose respectively.

2. Observed Effects: No treatment related effects were seen.

3. Mortality: Treatment had no significant effect on intercurrent mortality rates. Survival rates at the end of treatment period were comparable in all groups.

INTERCURRENT MORTALITY RATES

Male Mice								
Weeks	Control	%	Low Dose	%	Mid Dose	%	High Dose	%
0 - 52	2/55	3.6	5/55	9.1	3/55	5.4	3/55	5.4
53 - 78	5/53	9.4	9/50	18.0	6/52	11.5	8/52	15.4
79 - 104	17/48	35.4	16/41	39.0	11/46	23.9	13/44	29.5
Terminal	31	--	125	--	135	--	131	--
Survival rate	--	56.4	--	45.4	--	63.6	--	56.4
Female Mice								
0 - 51	3/55	5.4	4/55	7.3	6/55	10.9	2/55	3.6
52 - 78	9/52	17.3	5/51	9.8	8/49	16.3	4/53	7.5
79 - 104	23/43	5.3	19/46	41.3	19/41	46.3	15/49	30.6
Terminal	20	--	27	--	22	--	34	--
Survival rate	--	36.4	--	49.1	--	40.0	--	61.8

Note: There were 60 mice/sex/group, however, 5 mice/group were sacrificed on test day 90 for the 3-month necropsy.

4. 3-Month Interim Sacrifice:

a. Serum Chemistry: Treatment had no effect on serum alanine aminotransferase and aspartate aminotransferase activities.

b. Organ Weights: Only in males, liver weights were increased by 15% (relative wt.: 19%) and 24% (relative wt.: 24%) at mid and high dose respectively, when compared to control values.

c. Histopathology: Only in males, hepatocellular hypertrophy (control = 0/5, low dose = 2/5, mid dose = 2/5 and high dose = 5/5) and hepatocellular degeneration/ necrosis (control = 0/5, low dose = 2/5, mid dose = 1/5 and high dose = 1/5) were seen.

5. Body Weight/Food Consumptions/Water Consumptions: Treatment had no significant effect on body weight gains and food intakes.

Body Weight (g) of Male Mice				
Days	Control	Low Dose	Mid Dose	High Dose
-1	28.6 ± 1.5	28.6 ± 1.5	28.5 ± 1.5	28.6 ± 1.5
91	35.1 ± 2.5	35.1 ± 2.7	35.3 ± 2.3	34.7 ± 2.4
357	41.1 ± 4.6	40.3 ± 4.3	40.9 ± 4.7	39.5 ± 3.7
721	38.2 ± 3.9	40.0 ± 4.0	39.3 ± 5.0	39.3 ± 3.1
Body Weight (g) of Female Mice				
Days	Control	Low Dose	Mid Dose	High Dose
-1	24.5 ± 1.2	24.5 ± 1.2	24.6 ± 1.2	24.6 ± 1.2
91	30.4 ± 2.5	30.2 ± 2.5	30.3 ± 2.2	29.7 ± 2.3
357	35.9 ± 4.8	36.0 ± 4.6	34.9 ± 3.7	34.0 ± 3.4
721	36.3 ± 4.1	36.3 ± 4.6	37.0 ± 3.4	35.9 ± 3.4

Food Consumption (g/animal/day) in Male Mice				
Weeks	Control	Low Dose	Mid Dose	High Dose
1	6.7 ± 0.9	7.0 ± 1.7	4.1 ± 2.7	6.9 ± 1.2
13	5.4 ± 0.6	5.8 ± 1.0	5.6 ± 0.9	5.4 ± 1.0
51	4.6 ± 0.8	4.9 ± 0.7	4.7 ± 0.6	4.6 ± 0.7
103	5.2 ± 0.7	5.4 ± 0.7	5.4 ± 0.8	5.4 ± 0.7
Food Consumption (g/animal/day) in Female Mice				
Weeks	Control	Low Dose	Mid Dose	High Dose
1	6.6 ± 1.0	6.9 ± 1.0	6.7 ± 1.1	6.9 ± 1.2
13	6.2 ± 1.7	6.8 ± 1.8	6.6 ± 1.7	6.4 ± 1.7
51	5.3 ± 1.2	5.2 ± 1.1	5.4 ± 1.4	5.5 ± 1.3
103	5.6 ± 0.8	5.4 ± 1.0	5.7 ± 0.7	5.9 ± 1.0

6. Plasma Levels of the Drug (MDL 73,147) and its Metabolite (MDL 74,156) (Report # K-95-0434-D): Levels of the parent drug (MDL 73,147) were below detection limit in both sexes. The levels of the main metabolite (MDL 74,156) increased with increasing dosages and there were no sex differences. Based on AUC values, high dose treated mice were exposed to 2.3-3.8 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]).

MDL 74,156: Mean $AUC_{0\text{-}24\ hr}$ (ng.hr/ml) Values				
Days	Sex (M/F)	75 mg/kg/day	150 mg/kg/day	300 mg/kg/day
92	M	1,948	4,784	6,164
	F	1,875	3,379	6,441
365	M	2,382	6,196	11,850
	F	2,151	4,716	7,272

7. Gross Pathology: No treatment related effects were seen, except increased incidences of liver nodules in males (control = 5/31, low dose = 11/25, mid dose = 21/35 and high dose = 20/31) and uterine "nodules/enlargement" in females (control = 1/20, low dose = 10/27, mid dose = 8/22 and high dose = 16/34) were seen.

8. Organ Weights: Dose related increase in liver weights were seen in treated mice (absolute weights: 35%, 50% and 81% in males 12%, 26% and 25% in low, mid and high dose respectively; corresponding increases in relative liver weights were 27%, 41% and 74% in males and 10%, 16% and 25% in females respectively).

9. Histopathology:

Non-neoplastic Findings: Only in treated males increased incidences of altered eosinophilic foci in the liver, hepatocellular hypertrophy and hepatocellular single cell degeneration/necrosis were seen. The incidences were as follows:

Non-neoplastic Findings in Liver

Findings	Sex (M/F)	Control	75 mg/kg/day	150 mg/kg/day	300 mg/kg/day	Exact p-value
Centrolobular hypertrophy	M	0/55	2/55	7/55	24/55	0.0000
	F	0/53	1/54	1/55	2/55	0.2382
Single cell degeneration/necrosis (very slight-moderate)	M	1/55	3/55	17/55	17/55	0.0000
	F	2/53	0/54	1/55	4/55	0.2503
Eosinophilic foci	M	2/55	8/55	13/55	14/55	0.0010
	F	0/53	1/54	0/55	0/55	0.7465

Neoplastic Findings:

In males increased incidences of hepatocellular adenomas (control = 1.8%, low dose = 7.3%, mid dose = 18.2% and high dose = 23.6%; p = 0.0001; Peto trend test [published historical control incidence rate = 18.6%, range) and hepatocellular carcinomas (control = 12.7%, low dose = 12.7%, mid dose = 27.3% and high dose = 23.6%; p = 0.0512; Peto trend test [published historical incidence rate = 13.05%, range were seen. Increase in the incidences of hepatocellular adenomas reached to statistical significance only in one sex (males) while increase in the incidences of hepatocellular carcinoma was not statistically significant. It should also be noted that incidence rate of hepatocellular adenoma in high dose treated male mice was within range of historical incidence rate. If one analyzes the incidence of any liver lesion (i.e. adenomas and/or carcinomas) then the result is statistically significant in males (control = 14.5%, low dose = 20.0%, mid dose = 45.4% and high dose = 47.3%; 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. In addition, increased incidence of polypoid adenoma in uterus were seen in treated females (control = 1.8%, low dose = 5.4%, mid dose = 9.1% and high dose = 10.9%) which was not significant statistically
(p = 0.0730; Peto trend test).

Neoplastic Findings in Mice

Site/Type	Sex (M/F)	Dose (mg/kg/day)				p-value* Trend Test
		Control	75	150	300	
Liver/hepatocellular Adenoma	M	1/55 (1.8)	4/55 (7.3)	10/55 (18.2)	13/55 (23.6)	0.0001
	F	0/55 (0)	4/55 (7.3)	1/55 (1.8)	3/55 (5.4)	
Hepatocellular Carcinoma	M	7/55 (12.7)	7/55 (12.7)	15/55 (27.3)	13/55 (23.6)	0.0512
	F	1/55 (1.8)	1/55 (1.8)	0/55 (0)	2/55 (3.6)	
Hepatocellular adenoma &/or carcinoma	M	8/55 (14.5)	11/55 (20.0)	25/55 (45.4)	26/55 (47.3)	0.0000
	F	1/55 (1.8)	5/55 (9.1)	1/55 (1.8)	5/55 (9.1)	
Uterus/polyp (adenomas)	F	1/55 (1.8)	3/55 (5.4)	5/55 (9.1)	6/55 (10.9)	0.0730

( ) = Number in ( ) represent %.
* = p values were calculated by Dr. Chen (HFD-715).

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 hepato-cellular 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%, 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 2.3 - 3.8 fold higher levels of MDL 74,156 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]). 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.

3-Month Dietary Toxicity Study in Rats
(Report # I-93-0039-T)

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

Study Started: December 3, 1991

Study Completed: July 9, 1993

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

Animals: Male and female Sprague Dawley rats [Crl:CD(SD)BR (VAF Plus): weeks old, g].

Drug Batch No.: C-49319

Methods: Groups of rats (22/sex/group) were given MDL 73,147EF via diet at daily doses of 0, 100, 250, 500, 1000 and 2000 mg/kg/day for 3 months. All animals were observed daily for clinical signs and mortality. Body weights and food intakes were recorded weekly. Ophthalmoscopic examinations were performed at pre-test and at the end of treatment period. Just before sacrifice blood samples were collected by cardiac puncture for hematological and serum chemistry tests. Twelve rats/sex/group were used for toxicokinetic studies. Blood samples were collected at 5 hr before the end of light cycle (3:00 p.m.) and 1 hr after the start of dark cycle (9:00 p.m.) on days 30 and 92 of the study for monitoring drug levels in plasma. At the end of study period all surviving rats were sacrificed and subjected to complete necropsy and histopathological examinations were limited to control and high dose groups. However, kidney, ureters and urinary bladders were examined microscopically from lower dose groups also.

Results:

1. Observed Effects: Brown urine and brown nasal discharge was seen in some rats treated with 500 mg/kg/day and higher dose levels.

2. Mortality: One out of 10 males and 2 out of 10 females treated with 1000 mg/kg/day died during study period. All rats treated with 2000 mg/kg/day died between days 9 and 25 of the study. All these deaths were treatment related. Histological examinations revealed urinary tract lesions, congestion/hemorrhage of urinary bladder and degeneration of proximal tubules in the kidneys in most of the rats.

3. Body Weight/Food Consumption/Water Consumption: At the end of treatment period, in males, body weight gains were reduced by 2.1%, 8.5% and 18.7% at 100, 250 and 500 mg/kg/day respectively (rats treated with 1000 mg/kg/day lost 4.4% of their initial weights), when compared to control values. In females, body weight gains were reduced by 7.6% and 14.9% at 250 and 500 mg/kg/day dose levels respectively (in female rats treated with 100 mg/kg, body weight gains were increased by 3.4% while female rats treated with 1000 mg/kg/day lost 8.4% of their initial weights), when compared to control values. Throughout the study period, food intakes were significantly reduced in rats treated with 1000 mg/kg/day (males: 34% and females: 18%).

4. Achieved Doses: Based on food consumption data, achieved doses were 74, 178, 352, 669 and 368 in males and 85, 202, 413, 743 and 775 mg/kg/day for females at 100, 250, 500, 1000 and 2000 mg/kg/day theoretical dose levels respectively.

5. Hematology/Coagulation/Bone Marrow: Decreases in red blood cells (14%), hemoglobin (16%) and hematocrit (17%) were seen in males treated with 1000 mg/kg/day. Additionally, reticulocyte counts were increased dose dependently in treated males (control: 3.2%, 100 mg/kg/day = 3.5%, 250 mg/kg/day: 4.3% 500 mg/kg/day: 3.9% and 1000 mg/kg/day: 10.8%).

6. Blood Chemistry/Urinalysis: In serum of 1000 mg/kg/day treated rats, increased urea (males: 50% and females: 44%) and phosphorous (males: 14% and females: 67%) and decreased creatinine (males: 8% and females: 25%), total protein (males: 15% and females: 16%) and albumin (males: 10% and females: 67%) were seen. Additionally, serum potassium and phosphorus levels were increased dose dependently in treated females (potassium: and phosphorus:). At 1000 mg/kg/day, significant increases in alkaline phosphatase (males: 137% and females: 206%) and alanine aminotransferase severe (males: 103% and females: 85%) were also seen. Hematuria was seen in rats treated with ≥500 mg/kg/day dose levels. Slight hematuria was also seen in 1 out of 10 males each in 100 and 250 mg/kg/day treated groups.

7. Organ Weights: Generalized decreases in absolute weights of various organs and increases in their relative weights were due to treatment related decreases in body weight gain at ≥500 mg/kg/day dose levels.

8. Gross Pathology: One out of 9 males treated with 1000 mg/kg/day had dark urinary bladder content.

9. Histopathology: Treatment related findings were seen in kidneys, ureters and testes.

		Dose (mg/kg/day)					
Findings	Sex (M/F)	Control	100	250	500	1000	2000
Kidneys:							
Hyperplasia of papillary epithelium	M	0/10	0/10	0/10	0/10	7/9	---
	F	0/10	0/10	0/10	0/10	2/8	---
Hyperplasia of pelvic epithelium	M	0/10	0/10	0/10	0/10	3/9	---
	F	0/10	0/10	0/10	0/10	1/8	---
Ureter:							
Inflammation: Mucosa or submucosa	M	0/9	0/9	0/10	1/10	4/9	---
	F	0/9	0/8	0/10	0/9	0/7	---
Testes:							
Degeneration (tubule)	M	0/10	ND	ND	ND	5/9	---

ND = Not done
-- = All animals died

10. Plasma Levels of the Drug (MDL 73,147) and its Metabolite (MDL 74,156) (Report # K-93-0393-D): Levels of MDL 73,147 in females on days 30 and 92 were significantly higher (3-25 fold) in females than males, while levels of its metabolite (MDL 74,156) were comparable in males and females. Levels of parent drug and its metabolite (MDL 74,156) remained relatively constant after 30 and 92 days of dietary regimens (sponsor reported levels at 3 p.m. and 9 p.m. and AUC values were not reported).

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.

APPEARS THIS WAY
ON ORIGINAL

FDA CDER CARCINOGENICITY ASSESSMENT COMMITTEE (CAC/CAC-EC) RODENT CARCINOGENICITY FACTSHEET

NDA: 20-623 DRUG CODE #:
CAS #: DATE:
DIVISION(s): HFD-180
DRUG NAME(s): Anzemet (Dolasetron)
SPONSOR: Hoechst Marion Roussel, Inc.
LABORATORY: Health and Environmental Sciences
The Dow Chemical Co.
Indianapolis, IN 46268-0470

P/T REVIEWER(s): Tanveer Ahmad, Ph.D.
P/T REVIEW DATE: May 24, 1996
CARCINOGENICITY STUDY REPORT DATE: 8-2-95

THERAPEUTIC CATEGORY: Prevention of cancer chemotherapy-induced nausea and vomiting and prevention of post-operative nausea and vomiting.

PHARMACOLOGICAL/CHEMICAL CLASSIFICATION: 5-HT_3 receptor antagonist
PRIOR FDA DOSE CONCURRENCE (Div./CAC)? (Y/N; Date): No

MUTAGENIC/GENOTOXIC (Y/N/equivocal/na; assay): Negative 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.

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

RAT STUDY DURATION (weeks): 104
STUDY STARTING DATE: September 11, 1992
STUDY ENDING DATE: August 2, 1995
RAT STRAIN: Crl:CD(SD)BR Sprague Dawley
ROUTE: Diet
DOSING COMMENTS:

No. Rats in Control1 (C1): 75 Control2 (C2): 75
Low Dose (LD): 75 Middle Dose (MD): 75
High Dose (HD): 75 High Dose2 HD2): 75
RAT DOSE LEVELS (mg/kg/day)

Rat Low Dose: 25 (m) & 50 (f) Rat Middle Dose: 75 (m) & 150 (f)
Rat High Dose: 150 (m) & 300 (f) Rat High Dose2: 300 (m)* & 600 (f)*

*Dose adjusted during study. Due to severe toxicity seen in "rat high dose 2", all animals were killed on day 176 of the study. These rats were not used in any analysis.

Basis for Doses Selected (MTD; AUC ratio; saturation; maximum feasible): MTD

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

RAT TUMOR FINDINGS: None

RAT STUDY COMMENTS: 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.

COVERSHEET FOR CARCINOGENICITY STUDY IN RATS

1. No. of Studies: K-95-0572-T

2. Name of Laboratory: Health and Environment Sciences
The Dow Chemical Co.

3. Strain: Crl:CD (SD) BR Sprague Dawley Rats

4. No./sex/group: 75

5. Doses (O, 0, L, M, H): 0, 0, 25 (male), 50 (female), 75 (male), 150 (male and female) and 300 (female) mg/kg/day.

6. Basis for dose selection stated: Yes

7. Interim sacrifice: No

8. Total duration (weeks): 104

9. Week/site for first tumor:

	Male	Female
0	22/Malignant lymphosarcoma (spleen)	48/Benign adenoma (pituitary)
0	57/Benign adenoma (pituitary)	22/Malignant lymphosarcoma (marrow)
L	60/Benign adenoma (pituitary)	37/Benign adenoma (pituitary)
M	25/Malignant fibrous histiocytoma (jaw)	40/Malignant adenocarcinoma (mammary gland)
H	53/Benign adenoma (pituitary)	63/Benign fibroadenoma (mammary gland)

10. No. alive at termination:

	Male	% Survival	Female	% Survival
0	24/75	32	27/75	36
O	21/75	28	27/75	36
L	18/75	24	26/75	35
M	28/75	37	24/75	32
H	27/75	36	41/75	55

11. Statistical Methods Used: Tumor data were analyzed according to Peto etal (IARC Supplement 2, 1980).

12. Attach tumor and non-tumor data for each tissue (i.e., benign; malignant; hyperplastic): See Appendix 2.

Two-Year Dietary Carcinogenicity Study in Rats
(Report # K-95-0572-T)

Testing Laboratories: Health and Environmental Sciences
The Dow Chemical Co.,
Indianapolis, IN 46268-0470

Study Started: September 11, 1992

Study Completed: August 2, 1995

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

Test Species: Male and Female Crl:CD(SD)BR Sprague Dawley Rats (94-132 g, age not given).

Route of Administration: Via diet.

Dose Levels: 0, 25, 75, 150 and 300 mg/kg/day for males and 0, 50, 150, 300 and 600 mg/kg/day for females.

Drug Batch No.: 3601, 3602, 69550 and 69551.

Methods: In this study dose selection was based on 3-month oral (dietary) toxicity study (report # I-93-0039-T) in rats, in which maximum tolerated dose (MTD) was 250 mg/kg/day. However, sponsor selected 300 mg/kg/day for males and 600 mg/kg/day for females as the top dose in the main carcinogenicity study in rats, and remaining dose levels were 0, 75 and 150 mg/kg/day in males and 0, 150 and 300 mg/kg/day in females respectively. MDL 73,147EF was administered via diet and control animals received unmedicated diet. The duration of treatment was 2-years and there were 75 rats/sex/group. 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) each containing 75 rats/group and dosed for 2-years (day 176 was designated as study day 1 for these groups). All animals were observed daily for clinical signs and mortality. Body weights and food consumptions were recorded weekly during the first three months, bi-weekly during the second three months and every 4 weeks thereafter. Just before sacrifice blood samples were collected by cardiac puncture for hematological tests. Urine samples were also collected for urinalysis. Sponsor did not provide summary results of hematological tests and urinalysis. Twelve rats/sex in control

groups and 30 rats/sex in treatment groups were also included and used for toxicokinetic study. Blood samples from these animals were collected at the end of 3-month and 12-month of treatment and time of collections were 8:00 p.m., 2:00 a.m., 8:00 a.m. and 2:00 p.m. (3 rats/sex/group/time point were used) for measuring MDL 73,147EF and MDL 74,156 levels. All surviving rats were sacrificed at the end of treatment period and subjected to complete necropsy and histopathological examinations. Tumor data were analyzed according to Peto etal (IARC Supplement 2, 1980).

Results:

1. Achieved Doses: The mean intakes of MDL 73,147 were 24 (range:), 70 (range: and 144 (range: for males and 47 (range: 142 (range: and 293 for females at low (25 mg/kg/day in males and 50 mg/kg/day in females), mid (75 mg/kg/day in males and 150 mg/kg/day in females) and high dose (150 mg/kg/day in males and 300 mg/kg/day in females) respectively.

2. Observed Effects: Increased incidences of hematuria was seen in rats treated with ≥75 mg/kg/day, and the effect was dose related (males: control = 5.4%, low dose = 14.7%, mid dose = 25.3% and high dose = 89.3%; females: control = 2.7%, low dose = 8.0%, mid dose = 28.4% and high dose = 86.7%).

3. Mortality: Treatment had no significant effect on intercurrent mortality rates. Survival rates at the end of treatment period were comparable in all groups.

INTERCURRENT MORTALITY RATES						
Male Rats						
Weeks	**Control 1**	**%**	**Control 2***	**%**	**Mid[2] Dose**	**%**
0 - 53	5/75	7	1/75	1	4/75	5
54 - 78	13/70	19	14/74	19	15/71	21
79 - 105	33/57	58	39/60	65	28/56	50
Terminal	24	--	21	--	28	--
Survival rate	--	32	--	28	--	37
Female Rats						
0 - 51	3/75	4	2/75	3	4/75	5
52 - 78	9/72	12	15/73	20	12/71	17
79 - 105	36/63	57	31/58	53	35/59	59
Terminal	27	--	27	--	24	--
Survival rate	--	36	--	36	--	32

* = Groups added on day 176 of the study and dosed for 2-years.
[1] = 25 mg/kg/day in males and 50 mg/kg/day in females.
[2] = 75 mg/kg/day in males and 150 mg/kg/day in females.
[3] = 150 mg/kg/day in males and 300 mg/kg/day in females.

4. Body Weight/Food Consumption/Water Consumption:

Body Weight (g) of Male Rats			
Days	**Control 1**	**Control 2**	**Mid[2] Dose**
-1	250 ± 11	266 ± 12	251 ± 11
91	597 ± 52	614 ± 46	581 ± 45
364	796 ± 89	845 ± 97	747 ± 80
728	793 ± 134	803 ± 147	687 ± 122

Body Weight (g)			
Days	**Control 1**	**Control 2***	**Mid[2] Dose**
-1	189 ± 10	193 ± 13	189 ± 10
91	324 ± 27	332 ± 30	304 ± 25
364	428 ± 53	456 ± 73	377 ± 46
728	493 ± 116	544 ± 157	422 ± 81

* = Groups added on day 176 of the study and dosed for 2-years.
[1] = 25 mg/kg/day in males and 50 mg/kg/day in females.
[2] = 75 mg/kg/day in males and 150 mg/kg/day in females.
[3] = 150 mg/kg/day in males and 300 mg/kg/day in females.

Body weight gains in mid and high dose treated rats were compared with control 1 values, while body weight gains in low dose rats were compared with control 2 values. At the end of 91 days of treatment, body weight gains in males were reduced by 3%, 3.3% and 5.4% at low, mid and high dose respectively, while in females corresponding values were 0%, 5.8% and 13.4% respectively. 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 levels respectively and corresponding values in females were 15%, 15% and 27% respectively. Overall food intakes were not affected by the treatment.

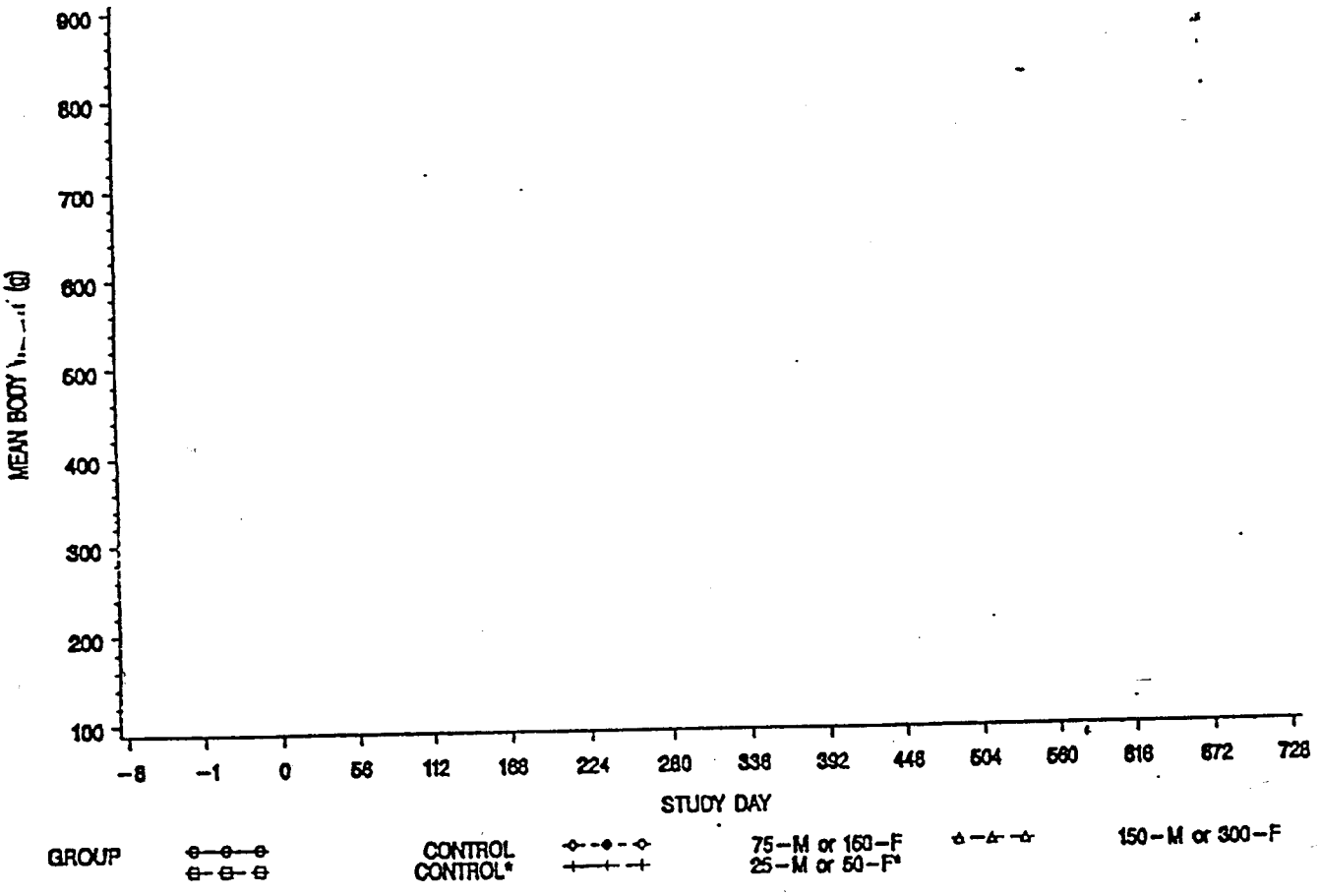
Food Consumption (g/animal/day) in Male Rats					
Weeks	Control 1	Control 2*	Low[1] Dose*	Mid[2] Dose	High[3] Dose
1	24 ± 5	28 ± 2		27 ± 2	
13	26 ± 4	26 ± 2		26 ± 4	
52	29 ± 3	30 ± 4		28 ± 4	
104	27 ± 5	30 ± 5		26 ± 6	
Food Consumption (g/animal/day) in Female Rats					
Weeks	Control 1	Control 2*		Mid[2] Dose	
1	18 ± 3	19 ± 2		18 ± 2	
13	17 ± 2	19 ± 2		16 ± 2	
52	21 ± 3	23 ± 4		21 ± 3	
104	22 ± 8	28 ± 9		24 ± 7	

* = Groups added on day 176 of the study and dosed for 2-years.
[1] = 25 mg/kg/day in males and 50 mg/kg/day in females.
[2] = 75 mg/kg/day in males and 150 mg/kg/day in females.
[3] = 150 mg/kg/day in males and 300 mg/kg/day in females.

FIGURE 2

MDL 73,147EF: TWO-YEAR DIETARY CARCINOGENICITY STUDY IN SPRAGUE-DAWLEY RATS

Group Mean Body Weights - Females



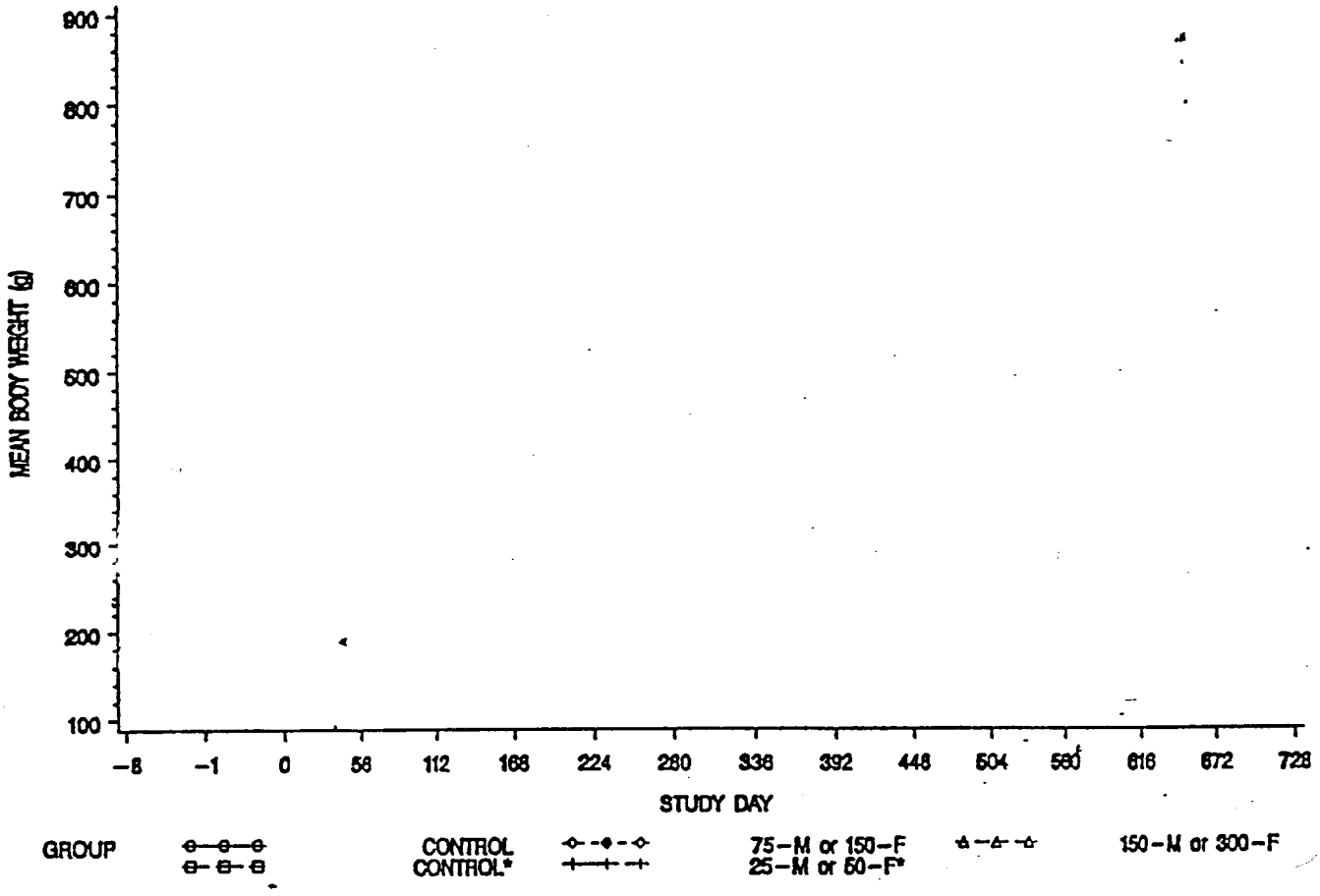
*Group Added on Study Day 176

Sponsor's Figure 2, Vol. 1.40, Page. 32

FIGURE 1

MDL 73,147EF: TWO-YEAR DIETARY CARCINOGENICITY STUDY IN SPRAGUE-DAWLEY RATS

Group Mean Body Weights - Males



*Group Added on Study Day 176

Sponsor's Figure 1, Vol. 1.40, Page 31

5. Plasma Levels of the Drug (MDL 73,147) and its Metabolite (MDL 74,156):

MDL 73,147: Mean $AUC_{0\text{-}24\ hr}$ (ng.hr/ml) Values				
Days	Sex (M/F)	Low[1] Dose	Mid[2] Dose	High[3] Dose
82	M		32	
	F		1950	
364	M		ND	
	F		1872	
MDL 74,156: Mean $AUC_{0\text{-}24\ hr}$ (ng.hr/ml) Values				
Days	Sex (M/F)	Low[1] Dose	Mid[2] Dose	
82	M		3947	
	F		10924	
364	M		5494	
	F		9894	

ND = Could not be determined.
[1] = 25 mg/kg/day in males and 50 mg/kg/day in females.
[2] = 75 mg/kg/day in males and 150 mg/kg/day in females.
[3] = 150 mg/kg/day in males and 300 mg/kg/day in females.

Levels of MDL 73,147 in male rats were mostly below detection limit. In females, levels of MDL 73,147 increased with increasing dosages. In both sexes the levels of MDL 74,156 increased with increasing dosages and there was no evidence of accumulation after repeat dosing (day 82 vs day 364).

6. Gross Pathology: No treatment related effects were seen.

7. Histopathology:

Non-neoplastic Findings: Increased incidences of thymus involution and cystic glandular hyperplasia in the mammary gland were seen in high dose treated female rats. The incidences were as follows:

Non-neoplastic Findings in Female Rats					
Site/Type	Control 1	Control* 2	Low[1] Dose	Mid[2] Dose	High[3] Dose
Thymus:					
Involution	15/26	16/26		17/23	
Mammary Gland:					
Cystic glandular hyperplasia	4/23	6/26		7/21	

* = Groups added on day 176 of the study and dosed for 2-years.
[1] = 50 mg/kg/day, [2] = 150 mg/kg/day and [3] = 300 mg/kg/day

Neoplastic Findings: No treatment related effects were seen.

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.

REPRODUCTIVE TOXICITY:

Oral Segment I. Fertility and General Reproductive Performance Study in Male Rats
(Report # I-93-0012-T)

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

Study Started: February 19, 1992

Study Completed: February 23, 1993

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

Animals: 10 weeks old Crl:CD BR VAF/Plus rats (males: g and females: g).

Drug Batch No.: -3564

Methods: Male rats (25/group) were given orally (gavage) 0 (water), 50, 200 and 400 mg/kg/day of MDL 73,147EF. The volume of administration was fixed at 3 ml/kg. Male rats were treated from 84 days prior to mating and throughout the mating phase (14 days). Each male was mated with 1 untreated female during the breading period. Rats were observed daily for mortality and toxic signs. Body weights and food/water consumptions were recorded weekly. At the end of breading period, one-half of males in each group were sacrificed and remaining males were sacrificed on day 119 of the study, testes and epididymides were weighed at necropsy. All mated females were sacrificed on day 20 of gestation, and was examined for the number of corpora lutea, the number of implants, the number of dead or resorbed fetuses and number of live fetuses. The live fetuses were weighed and sexed and examined externally.

Results: In males there were 12 deaths (control = 0/25, low dose = 1/25, mid dose = 2/25 and high dose = 9/25) during study period. Four of the 12 deaths were accidental, while remaining 8 deaths were most likely treatment related. In males, at the end of treatment period, body weight gains were reduced by 4.2%, 5.3% and 16.4% in low, mid and high dose respectively, when compared to the control values. No consistent effect on food intakes were seen in treated males. One out of 8 high dose treated male had dark urine in the bladder. Treatment had no effect on male breading performance (time to mating, copulation rate and fertility rate). Treatment had no significant effect on numbers of implantation, pre-implantation loss, litter size, sex ratios, and mean fetal weights. No treatment related abnormalities were observed in the fetuses upon external examinations. Hence 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).

Oral Segment I. Fertility and General Reproductive Performance Study in Female Rats
(Report # K-94-0548-T)

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

Study Started: March 25, 1992

Study Completed: July 29, 1994

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

Animals: 10 weeks old Crl:CD BR VAF/Plus rats (males: wt. not given and females: g).

Drug Batch No.: 3564

Methods: Groups of female rats (25/group) were given orally (gavage) 0 (water), 20, 60 and 100 mg/kg/day of MDL 73,147EF from 2 weeks prior to mating, throughout the mating phase (a maximum of 2 weeks), gestation, lactation and till they were sacrificed. The volume of administration was fixed at 3 ml/kg. In this study each female was mated with 1 untreated males during the mating period. Rats were observed daily for mortality and toxic signs. Body weights and food consumptions were recorded weekly. About

one-half of pregnant rats were sacrificed on day 20 of gestation, and was examined for the number of corpora lutea, the number of implants, the number of dead or resorbed fetuses and number of live fetuses. The live fetuses were weighed and sexed. Fetuses were eviscerated and two-third of fetuses were examined for skeletal major/minor abnormalities, the remaining fetuses were examined for visceral abnormalities and variations. The remaining dams were allowed to deliver spontaneously and F_1 offsprings were evaluated for postnatal development.

Results: Only in high dose group, salivation after drug administration was seen in 8 out of 25 female rats. One female from mid dose group was killed on day 4 of the study after receiving dosing injuries. Treatment had no significant effect on body weight gains and food intakes, except weight losses of 2.1% and 3.9% were seen during first week of gestation in control and low dose treated rats along with significant reductions in food intakes (about 60% less than the values seen during remainder of gestation period). This adverse effect on body weights and food intakes were related to malfunctioning of water bottle. However, body weight at the end of gestation period were comparable in all groups. The estrous cycle of the female rats revealed no differences between the control and treated groups. Precoital intervals, mating rates and pregnancy rates were comparable in all groups.

Parameters	Control	Low Dose	Mid Dose	High Dose
# of Female Pairs	25	25	24	25
# of Females Mated	24	23	24	25
Mating Rate (%)	96	92	100	100
# of Pregnant	23	20	22	22
Pregnancy Rate (%)	92	80	92	88

Dams Sacrificed at Day 20

No treatment related gross lesions were seen in female rats of F_0 generation. There were no significant changes in pregnancy parameters (mean numbers of corpora lutea, implants, resorption, live fetuses, litter size, sex ratios, and mean fetal weights). A total of 431 fetuses were examined for skeletal abnormalities and 213 fetuses were examined for visceral abnormalities. No treatment related major malformation was seen in fetuses.

Dams Sacrificed on Day 20 of Pregnancy				
Parameters	**Control**	**Low Dose**	**Mid Dose**	**High Dose**
# of Pregnant Dams	10	11	11	10
Mean # of Corpora Lutea/Dam	17.6 ± 3.5	16.1 ± 2.0	16.6 ± 2.5	18.0 ± 2.9
Mean # of Implants/Dam	16.6 ± 1.2	14.9 ± 1.6	16.3 ± 1.3	16.1 ± 1.9
Mean # of Live Fetuses/Dam	16.0 ± 1.6	14.4 ± 2.5	15.5 ± 1.9	15.5 ± 2.2
Mean # of Dead Fetuses/Dam	0	0	0	0
Early resorption (%)	0.6	0.5	0.7	0.6
Late resorption (%)	0	0	0	0
Mean Fetal Weight (g)	3.89 ± 0.28	3.83 ± 0.27	4.00 ± 0.23	3.92 ± 0.17
Sex Ratio (% females)	45	48	53	55

Dams Allowed to Deliver: Due to excessive mortality among F_1 pups during the first 2 days of postpartum (control = 61%, low dose = 74%, mid dose 78% and high dose 91%), sponsor terminated the second half of the experiment. The cause of deaths were related to India Ink which was used to tattoo the pups. Sponsor repeated the second part of the experiment (i.e. 13 females per group were treated as mentioned in the methods, and allowed to deliver spontaneously). The number of live/dead pups were recorded, and the live pups were weighed and sexed. Selected pups from each litter were reared by the dams until weaning. On day 21-23 of post partum all dams were sacrificed, necropsied and examined as mention above. During the nursing period the growth and differential of the pups were observed, and development parameters were assessed and reproductive performance of F_1 adult were also assessed. F_2 generation were examined for abnormalities and then killed on day 28 of post partum.

No significant differences in the gestation period between the groups were noted. The number of implantation sites, litter size, sex ratios, viability and pups weights throughout lactation period were not affected by the treatment. However, pre-natal losses were increased in treated females (control = 2.9%, low dose = 5.1%, mid dose = 12.3% and high dose = 14.3%). According to sponsor pre-natal loss in control is lower than the historical values (no historical data were submitted). It should be noted that pre-implantation loss and number of resorptions were not affected by the treatment in rats killed on gestation day 20. Postnatal development and differentiation were comparable in all groups. There was no significant effect on fertility test and mating performance test of F_1-generation rats. No treatment

related gross lesions were seen in rats of F_1 generation. Physical development were comparable in all groups, and no drug related gross lesion were seen in the F_2 pups at necropsy.

Dams Allowed to Deliver				
Parameters	**Control**	**Low Dose**	**Mid Dose**	**High Dose**
# of Pregnant Dams	12		10	
Gestation Length (days)	21.6		21.7	
# of Live Pups at day 0	191		143	
# of Live Pups at day 4	185		128	
Gestation Index	100.0		96.6	
Viability Index	96.9		97.2	
Lactation Index	100		100	

Gestation index = (# of live pups born/ total pups born) x 100
Viability Index = (# of live pups at day 4/ # of live pups on day 0) x 100
Lactation index = (# of live pups at day 21/ # of live pups at day 4) x 100

In conclusion, 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.

Morphologic Teratology Study of MDL 73,147EF in Rats
(Report # C-90-0035-T)

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

Dates Studies Started and Completed: October 29, 1989 and April 2, 1990

Test Species: Sprague-Dawley [Crl: CD(BR)] pregnant rats.

No. of Animals: 20 rats/group

Route of Administration: Oral (gavage)

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