CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 020623

PHARMACOLOGY REVIEW(S)

Ahmad

NDA 20-623 REVIEW # 1

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

JUL -5 1996

Sponsor and Address: Hoechst Marion Roussel, Inc.
Kansas City, Missouri 64134

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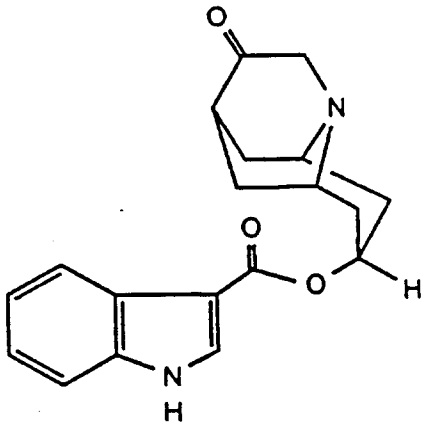
Date of HFD-180 Receipt: October 2, 1995

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
(Original Summary)

Drug: Dolasetron mesylate/MDL 73,147/ANZEMET Tablets

Chemical Name: (2α, 6α, 8α, 9aß)-Octahydro-3-oxo-2, 6-methano-2H-quinolizin-8-yl-1H-indol-3-carboxylate monomethane sulfonate, monohydrate.



$\cdot CH_3SO_3H \cdot H_2O$

$C_{19}H_{20}N_2O_3 . CH_3SO_3H . H_{20}$ MW 438.50

Formulation: Each tablet contains 25, 50, 100 or 200 mg of dolasetron mesylate monohydrate along with inactive ingredients such as carnauba wax, croscarmellose sodium, hydroxy-propyl methylcellulose, lactose, magnesium stearate, polyethylene glycol, polysorbate 80, pregelatinized starch, synthetic red iron oxide, titanium dioxide, white wax, lecithin, synthetic black iron oxide

Category: Serotonin 5-HT_3 receptor antagonist

Proposed Marketing Indication: Anzemet is indicated for the prevention of cancer chemotherapy-induced nausea and vomiting and prevention of postoperative nausea and vomiting.

Dose: The recommended oral adult dose of Anzemet is one 200 mg tablet given within 1 hr prior to chemotherapy for the prevention of cancer chemotherapy-induced nausea and vomiting. The recommended oral dose in pediatric patients (2-17 years old) is 2.4 mg/kg given within 1 hr prior to chemotherapy.

For the prevention of postoperative nausea and vomiting, the recommended oral adult dose of Anzemet is one 50 mg tablet given 2 hr prior to surgery. The recommended oral dose in pediatric patients (2-17 years old) is 1.2 mg/kg given within 1 hr prior to surgery.

PRECLINICAL STUDIES AND TESTING LABORATORIES

Type of Study	Study #	Drug Lot #	Testing Laboratories	Page #
Pharmacology				
Absorption: Rat, rabbit, dog and monkey				5 14
Distribution: Rat				20
Metabolism: Rat, rabbit, dog and monkey				23
Excretion: Rat, rabbit dog and monkey				
ACUTE TOXICITY				29
Mouse				
Oral	C-88-0017-T	15	DPT	
I.V.	C-88-0012-T	07	DPT	
Rat				
Oral	C-88-0017-T	15	DPT	
I.V.	C-88-0012-T	07	DPT	
Dog				
Oral	C-88-0020-T	15	DPT	
I.V.	C-88-0013-T	07	DPT	
Monkey				
Oral	C-88-0020-T	15	DPT	
I.V.	C-88-0013-T	07	DPT	
Subacute/Subchronic/ Chronic Toxicity:				
Rat				
1-Month (I.V.)	I-92-0148-T	8611093	DIC	31
1-Month (gavage)	C-91-0071-T	C-46711	DPT	34
3-Month (gavage)	C-89-0017-T			36
1-Year (gavage)	I-93-0020-T	C-47320, C-47341, C-47342, R-47344, C-48351, R-48613 and C-48616	DCT	37
Dog				
1-Month (I.V.)	C-88-0014-T	06	DPT	41
1-Year (capsule)	I-92-0168-T	C-46711, C-47320, C-47342, C-47344, C-48351, C-48616 and R-48613	DCT	43
Monkey				
1-Month (I.V.)	C-88-0015-T	06	DPT	45
3-Month (gavage)	C-89-0020-T	23	DPT	47

DPT = Department of Pathology and Toxicology
Merrell Dow Research Institute
Merrell Dow Pharmaceuticals, Inc.,
Cincinnati, Ohio

DCT = Department of Toxicology
Cincinnati Center
Marion Merrell Dow Inc.,
Kansas City, MO

DIC = Department of Drug Safety
Indianapolis Center
Marion Merrell Dow Inc.,
Kansas City, MO

HES = Health Environmental Sciences
The Dow Chemical Co.
Indianapolis, IN

HEST = Health Environmental Sciences
The Dow Chemical Co.
Freeport, TX

Sponsor has also submitted 2-week S.C. toxicity study (report # I-90-0042-T) of MDL 62,198 (antimicrobial agent for topical use) and 1-month S.C. toxicity study (report # TT-90-172-T) of MDL 62,873 (a semisynthetic glycopeptide antibiotic to treat staphylococcal infections) in this submission. These studies are not relevant to the present application (report # I-90-0042-T) and will not be reviewed here.

PHARMACOLOGY:

PRIMARY ACTIVITY

1. Anti-Emetic Activity:

Ferrets: MDL 73,147 given 30 min before cisplatin dose or 0.05-0.5 i.v. dose given 30 min prior to and 45 min following 10 mg/kg i.v. dose of cisplatin) significantly reduced the number of cisplatin-induced vomiting and delayed the onset of the first vomiting episode. A dose of 0.5 mg/kg (i.v.) administered twice completely inhibited vomiting induced by cisplatin in the ferret while a single oral dose was less effective

In above experiment MDL 73,147EF (dolasetron mesylate salt) and MDL 73,147A (dolasetron hydrochloride salt) were equal in potency.

MDL 74,156 (the main metabolite of MDL 73,147) was also pharmacologically achieved i.e., MDL 74,156 (0.05-0.5 mg/kg i.v. given 30 minutes before and 45 min after the administration of 10 mg/kg (i.v.) dose of cisplatin) significantly reduced the number of cisplatin-induced vomiting and delayed the onset of the first vomiting episode (inhibition of cisplatin induced vomiting ranged 25-80%). Sponsor did not analyze the relative antiemetic effects of MDL 73,147 and MDL 74,156 in this model.

Dogs: MDL 73,147 (0.1, 0.3 or 1 mg/kg i.v. given 30 min prior to 3 mg/kg/ i.v. dose of cisplatin) significantly reduced the number of cisplatin-induced vomiting and delayed the onset of the first vomiting episode. Antiemetic effect of MDL 73,147 in dogs were similar to that seen with other 5-HT_3 antagonists (ICS 205-930 [tropisetron], ondansetron and zacopride). Sponsor did not report ED_{50} values of various 5-HT_3 antagonists.

2. Antagonism at 5-HT_3 Receptors:

a. Effect on Bezold-Jarisch Reflex (BJR):

In anesthetized rats, MDL 73,147 and its metabolites (MDL 73,405, MDL 102,382 and MDL 73,492) at 1 mg/kg i.v. dose level almost completely inhibited BJR. Furthermore, MDL 73,147 and its active metabolite MDL 73,405 had a duration of action exceeding 5 hr after the i.v. dose. Similar results were seen when MDL 73,147 was given via oral route. Peak inhibition of BJR were comparable when dolasetron, ondansetron, granisetron or tropisetron (1 mg/kg) were given via oral route to anesthetized rats, however, the duration of action was significantly lower in dolasetron and tropisetron treated rats than ondansetron or granisetron treated rats.

Inhibition of BJR in Anesthetized Rats				
	Dose (1 mg/kg,I.V.)		Dose (1 mg/kg, Oral)	
Compound	Peak Inhibition (%)	Inhibition After 5 hr (%)	Peak Inhibition (%)	Inhibition After 5 hr (%)
MDL 73,147EF	90 ± 3	77 ± 4	83 ± 3	60 ± 10
MDL 73,405 ([+]-enantiomer of MDL 74,156)	87 ± 5	83 ± 7	--	--
MDL 102,382 (5'-OH MDL 74,156)	91 ± 2	57 ± 15	--	--
MDL 73,492 (6'-OH MDL 74,156)	95 ± 1	44 ± 10	--	--
Ondansetron	91 ± 2	41 ± 13	77 ± 7	10 ± 4
Granisetron	--	--	81 ± 3	35 ± 15
Tropisetron	--	--	85 ± 1	68 ± 6

b. Binding to 5-HT_3 Receptors: Binding affinity and selectivity of MDL 73,147 were assessed in conventional radioligand binding assays. MDL 73,147 and its main metabolite (MDL 74,156) binds selectively to 5-HT_3 receptors (Ki=0.8-20 nM) and the affinity of metabolites (MDL 74,156) was greater than the parent drug.

Both compounds (MDL 73,147 and MDL 74,156) at a concentration of 10 mcM had no significant affinity for 5-HT_{1A}, 5-HT_{1C}, 5-HT_{1D} and 5-HT_2 receptors, α_1 and α_2 adrenoceptors, dopamine D_2-receptors,

dopamine D_3-receptors, histamine H_1 receptors, muscarinic m_1-, m_2-, m_3, m_4- and m_5- receptors, neurokinin NK_1 receptors and endothelial ET_A and ET_B receptors in conventional radioligand binding assays.

In isolated perfused rabbit heart, MDL 73,147 and MDL 74,156 inhibited the positive chronotropic effect of 5-HT (PA_2: 9.8 and 9.4 for MDL 73,147 and MDL 74,156 respectively.

In neuroblastoma cells, MDL 73,147 and MDL 74,156 inhibited the activation of 5-HT_3 receptor by 5-HT with IC_{50} values of 3.8 nM and 0.1 nM respectively. In this experiment 1 nM of MDL 74,156 was equipotent with 1 nM of tropisetron (5-HT_3 receptor antagonist: a comparator).

MDL 73,147, MDL 74,156 and tropisetron blocked rat brain 5-HT_4 receptors with Ki values of 330 nM, 43 nM and 101 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, MDL 74,156 and tropisetron had no significant agonist activity and only weak antagonist activity were seen (IC_{50}: 26, 15 and 9.8 μM for MDL 73,147, MDL 74,156 and tropisetron respectively).

MDL 73,147 metabolites [(+) and (-)-enantiomers of MDL 74,156 and 6'-OH MDL 74,156] showed significant affinity with benzodiazepine receptors (Ki=131, 25 and 198 nM respectively). However, in an in-vivo experiment (assessing motor coordination of mice on the rotarod) MDL 73,147 (20 mg/kg i.p.) had no effect on rotarod time and did not mimic the sedative actions of chlordiazepoxide nor MDL 73,147 (10 mg/kg i.p.) reverse the action of chlordiazepoxide. Hence, MDL 73,147 did not exhibit benzodiazepine agonist or antagonist activity in vivo.

SECONDARY ACTIVITY

1. <u>Effects on Central Nervous System</u>:

a. <u>Effects on Dopamine Neurons in Rats</u>: In rat, MDL 73,147EF (a single dose of 0.5 mg/kg, i.v.) had no significant effect on the firing rate of neurons in the A9 or A10 regions of the rat mid-brain. Thus, acute administration of MDL 73,147EF is void of any extrapyramidal effects. However, chronic administration of MDL 73,147EF (5.0 mg/kg i.p. X 21 days), haloperidol (0.5 mg/kg, i.p. X 21 days) significantly decreased the number of actively firing neurons in A9 and A10 regions of the rat brain. In another experiment, chronic administration of MDL 73,147EF (1 or 3 mg/kg, s.c. twice a day for 14 days) or ondansetron (0.1 mg/kg, s.c., twice a day for 14 days) significantly decreased

dopaminergic activity in rat brain (as indicated by significant decreases in levels of dopamine and its metabolites in the frontal cortex and in the striatum and lack of change in dopamine turnover [data presented graphically]). Hence, chronic treatment with MDL 73,147 or ondansetron down regulated the activity of specific dopaminergic systems in the rat brain.

b. Effect of MDL 73,147EF on Latent Inhibition in Rat: MDL 73,147EF and haloperidol (0.1 mg/kg, s.c.) both significantly potentiated the "latent inhibition (a process of screening out irrelevant stimuli which is deficient in acute schizophrenia)" in rat.

2. Effect on Cardiovascular System:

a. Conscious Normotensive Rats: In this experiment, rats were given cumulative i.v. doses of MDL 73,147A (1, 3, 9, 27 and 54 mg/kg [drug was infused over 2 min period at interval of 15 or 30 min]). Transient (<5 min) non-dose related increases in mean arterial blood pressure and heart rate were seen at ≤27 ng/kg. At cumulative dose of 54 mg/kg, no effect on blood pressure was evident, while heart rate was transiently decreased (data presented graphically). In a second experiment, a single i.v. dose (50 mg/kg) significantly increased heart rate and mean arterial blood pressure in conscious normotensive rats, and these effects were transient (lasted for about 5 min).

b. Effect of I.V. MDL 73,147A on Cardiovascular Activity of an M_1 Muscarinic Receptor Agonist in Rats: In anesthetized pithed acutely adrenalectomized rats, an i.v. dose up to 20 mg/kg of MDL 73,147A had no significant effect on the increase in blood pressure induced by McN-A-343 (60 μg/kg, i.v.; muscarinic receptor agonist). Hence, MDL 73,147 had no ganglion blocking activity.

c. Conscious Normotensive Dogs: MDL 73,147EF (4 mg/kg/day, i.v. for 5 days) had no significant effect on blood pressure and heart rate during the first 4 days of treatment. However, on 5th day of treatment arterial blood pressure was increased by 6 ± 0.3 mmHg during the first 14 hr after drug administration (data were collected every 15 min during study period).

In conscious dogs, oral administration of MDL 73,147EF (10 mg/kg/day for 4 days) did not significantly affect arterial blood pressure but heart rate was significantly increased (about 16 bpm) at 3 and 4 hours after drug administration. All ECG (P.R and T waves amplitudes, PR and QRS intervals) values remained within normal range.

d. Anesthetized Normotensive Dogs: In anesthetized dogs, intravenous doses of 0.5 and 1 mg/kg of MDL 73,147EF had no effect on heart rate or blood pressure. MDL 73,147EF at 2 and 4 mg/kg (i.v.) produced transient (<90 sec) reduction in blood pressure (7-11%) without affecting heart rate. Lead II ECG was not affected with any of the test dose (0.5-4 mg/kg, i.v.).

In another experiment, anesthetized dogs were given escalating i.v. doses (1, 5, 12.5 and 12.5 mg/kg; each dose was infused over 0.5-2 min and there were 15-30 min intervals between each dose level) of MDL 73,147EF and various cardiovascular parameters (heart rate, blood pressure (BP), end diastolic BP, left ventricular BP, dP/dt max, QRS interval, RR interval and PQ interval) were monitored. A cumulative dose of 6 mg/kg had no effect in any of the above mentioned parameters except PQ intervals was significantly prolonged. However, a cumulative i.v. dose of 18.5 mg/kg reduced significantly left ventricular dP/dt max and significantly increased PQ intervals. A cumulative i.v. dose of 31 mg/kg significantly decreased left ventricular BP, dP/dt max, systemic BP and heart rate, and also significantly increased PQ and RR intervals (data presented graphically).

e. Effects of I.V. MDL 73,147EF and Ondansetron on ECG in Conscious Dogs: In this experiment conscious dogs were given cumulative i.v. doses of (0.1, 0.3, 1.0, 3.0 and 30 mg/kg) MDL 73,147EF or ondansetron (0.05, 0.2, 0.7, 2.2, 7.2 and 15 mg/kg) and control animals received vehicle in similar fashion. Successive doses of drug or vehicle were given every 20 minutes. ECG recording indicated that MDL 73,147EF significantly increased PR interval at cumulative doses of 3 to 30 mg/kg (increase in PR interval: 9 m sec at 3 mg/kg, 17 m sec at 10 mg/kg and 47 m sec at 30 mg/kg) while a 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 increased QTc interval (MDL: 47 m sec, ondansetron: 35 m sec) compared to baseline.

f. Effect of MDL 73,147EF and MDL 74,156 (main metabolite) on Isolated, Perfused Guinea Pig Hearts: In perfused guinea pig hearts, MDL 73,147EF (drug), MDL 74,156 (main metabolite), MDL 73,902 (epimer of MDL 73,147EF, 300-fold less potent as 5-HT_3 antagonist as MDL 73,147EF), MDL 72,222 and MDL 73,148 (tropisetron) at 1 μM increased the absolute refractory period by 29%, 17%, 45%, 21% and 17% respectively. Hence, these compounds have antiarrhythmic properties, however, there is no correlation between 5-HT_3 antagonistic potencies and antiarrhythmic activities of various 5-HT_3 antagonists (see MDL 73,147EF vs MDL 73,902).

g. Effect of MDL 73,147EF, MDL 102,382 and MDL 73,492 (the 5' and 6' Hydroxy metabolite of MDL 74,156 on Action Potential in Guinea Pig Papillary Muscle: In guinea pig papillary muscle the action potential duration was unaffected by MDL 73,147EF (10 μM), however, V_{max} (dV/dt of action potential) was reduced by 33 - 42% (class I antiarrhythmic agent). Metabolite MDL 102,382 and MDL 73,492 had no significant effect on guinea pig papillary muscle action potential. The comparator, ondansetron (10 μM) had no significant effect on V_{max} but it increased the duration of action potential by 23% (class III antiarrhythmic agent). Similar results (i.e. inhibition of V_{max}) were seen when MDL 73,147EF was tested in dog Purkinje fibers.

h. Effect of MDL 73,405 [(+)-enantiomer of MDL 74,156; the Primary Metabolite of Dolasetron] and MDL 73,349 [(-)-enantiomer of MDL 74,156, the Minor Metabolite of Dolasetron] on Action Potential in Guinea Pig Papillary Muscle: The dV/dt of action potential (V_{max}) was inhibited by about 18% and 50% in MDL 73,405 and MDL 73,349 (10 μM) treated system. Both of these metabolites indicated class I_c type antiarrhythmic properties.

i. Effect of MDL 73,147EF, MDL 74,156 (racemic mixture) and MDL 73,405 [(+)-enantiomer of MDL 74,156] and Flecainide (class I_c antiarrhythmic agent) on α-Subunit of Human Heart Sodium Channel Expressed in Xenopus Oocytes: MDL 73,147EF, MDL 74,156 and MDL 73,405 blocked sodium channel (as measured by measuring amplitude of sodium current) with K_d 50 values of 1.1, 0.567 and 1.2 mM respectively. The positive control (flecainide) reduced sodium current in this experiment with a K_d 50 value of 65 μM. At $\leq$10 μM, MDL 73,147EF, MDL 74,156 and MDL 73,405 induced no phasic block of sodium current while flecainide reduced sodium current by 21%. In this in vitro experiment, significant sodium channel blocking activity was only observed at concentration >10 μM of MDL 73,147EF or its metabolites.

3. Miscellaneous Pharmacology:

a. Effect on Body Temperature: In mice, 10 mg/kg (s.c.) of MDL 73,147A had no significant effect on body temperature, while 100 mg/kg (s.c.) reduced the body temperature by 3°C; by 180 min post-dosing body temperature returned to normal.

b. Effect on Behavior in Mice: MDL 73,147A produced minimal changes in behavior of mice at doses up to 100 mg/kg (i.p or s.c.).

c. Antinociceptive Activity in Mice: MDL 73,147A (100 mg/kg, i.p.) increased slightly, but significantly the time to the first sign of discomfort in "Hot Plate" test for analgesia in mice. MDL 73,147A (1, 10 and 100 mg/kg) had no effect on analgesic response to morphine (16 mg/kg) in mice.

d. Anti-inflammatory Activity: MDL 73,147A (5 and 50 mg/kg, i.p.) had no significant effect on carrageenan induced edema in rat paw.

e. Local Anesthesia: In the guinea pig wheal test for local anesthesia, MDL 73,147A and lidocaine were equipotent (EC_{50} = 2.3 mM).

f. Effect on Gastric Emptying in Mice: Both MDL 73,147A (5 mg/kg) and metoclopramide (5 mg/kg) significantly enhanced gastric emptying in mice.

g. Effect of MDL 73,147EF and MDL 74,156 on Synaptic Transmission in Rat Isolated Sympathetic Ganglia: MDL 73,147EF and MDL 74,156 had no significant effect on synaptic transmission in rat isolated sympathetic ganglia. However, at 30 μM of MDL 74,156 synaptic transmission was inhibited by 27%.

h. Effect of MDL 73,147A on Monoamine Oxidase (MAO) Activity: In vitro, MDL 73,147A inhibited MAO-A and MAO-B activities in rat brain (IC_{50}: 50 and 407 μM respectively).

i. Effect of MDL 73,147EF on Experimentally-Induced Cardiac Arrhythmias in Rats and Dogs: In anesthetized rats, MDL 73,147EF (5, 12.5 and 25 mg/kg, i.p. 15 min before ligation of left coronary artery) dose dependently protected (i.e. reduced the incidence, number and duration of ectopic beats, and duration and incidence of ventricular fibrillation) against ischemia induced arrhythmias.

In normotensive anesthetized dogs, MDL 73,147EF or MDL 73,902 (epimer of MDL 73,147: both dose dependently prolonged the time of onset of ventricular hyperautomaticity (induced by direct application of constant current to heart). It should be noted that in this experiment MDL 73,902 was about 6 times more potent than MDL 73,147 while MDL 73,902 has an affinity about 300-fold less then MDL 73,147 for 5-HT_3 receptor. Hence, observed antiarrhythmic activity the drug (MDL 73,147) is not related to 5-HT_3 receptor blockade.

In renal hypertensive dog model, MDL 73,147EF significantly "reduced ventricular premature contractions (PVCs) and increased the threshold of ventricular fibrillation" (data were not presented).

4. Interaction With Other Drugs:

a. Cisplatin:

L1210 Leukemia Model: In this model male DBA_2 mice were given a single dose of cisplatin (2, 4, 6, 8, 10 or 12 mg/kg, i.p.) with or without 5 mg/kg of MDL 73,147EF (i.v.) on days 1, 5 and 9 following tumor (L1210 leukemia) inoculation. The maximum increase in life span was seen at 6 - 8 mg/kg cisplatin (% increase in life span over control = , and similar result was seen in the presence of MDL 73,147EF.

ADJ/PC6 Plasmacytoma Model: In this model ADJ/PC6 plasmacytoma mice were given a single dose of cisplatin (0.25, 0.5, 1, 2, 4, 8 and 16 mg/kg, i.p. or i.v.) with or without 5 mg/kg of MDL 73,147EF (i.p. or i.v.) on day 20 after tumor implantation. Ten days after drug treatment mice were sacrificed and tumor weights were determined. Irrespective of route of administration, MDL 73,147EF had no significant effect on cisplatin antitumor potency nor on its toxicities.

HX/110 Human Ovarian Tumor Xenograft Model: In this model female BalbC nude mice were implanted subcutaneously with tumor fragments. Mice were treated with cisplatin (2, 4 and 6 mg/kg, i.p.) with or without 5 mg/kg of MDL 73,147EF (i.v.) once a week for 4 weeks beginning 6 weeks after tumor implantation. In this experiment MDL 73,147EF had no significant effect on cisplatin efficacy (data presented graphically).

b. Hexobarbital and Thiopental-Induced Sleep in Mice: MDL 73,147EF (10 mg/kg, i.p. or p.o.) had no significant effect on hexobarbital or thiopental induced sleep time in mice.

c. Effects on Volatile Anesthetics (Halothane, Isoflurane and Enflurane) Induced Sleep in Mice: MDL 73,147EF (10 mg/kg, i.p.) had no significant effect on halothane, isoflurane or enflurane induced sleep time in mice.

d. Effects on the Activity of Neuromuscular Blocking Agents: MDL 73,147EF or MDL 73,405 had no significant effect on the neuromuscular blockade produced by D-tubocurarine in rat phrenic nerve-hemidiaphragm. Similar results were seen when neuromuscular blockade was produced by atracurium besylate (non-depolarizing neuromuscular blocker).

e. Effect of MDL 73,147EF and its Metabolites on Acetylcholinesterase and Butyrylcholinesterase Activity In Vitro: MDL 73,147EF and its metabolites (MDL 73,405, MDL 73,349, MDL 102,382 and MDL 73,492) at 100 μM had no significant effect on electric eel acetylcholinesterase or human erythrocyte acetylcholinesterase. However, human butyrylcholinesterase was weekly inhibited by the metabolites of dolasetron (IC_{50}: MDL 73,147EF (parent drug) = >100 μM, MDL 73,492 = 25 μM, MDL 102,382 = 7 μM, MDL 73,405 = 4 μM and MDL 73,349 [the (-)-enantiomer of MDL 74,156, a minor metabolite in human] = 0.25 μM).

f. Effect on Acetylcholine-Induced Contraction in Isolated Fuinea Pig Trachea: MDL 73,147EF (1 nM - 100 μM) had no significant effect on acetylcholine-induced contractions of guinea pig atria. Furthermore, MDL 73,147EF did not alter anticholinergic effects of atropine (10 nM) in this experiment.

g. Interaction with Histamine H_2 Antagonist: In isolated guinea pig atria, histamine dose-dependently increased heart rate. Ranitidine (histamine H_2 receptor antagonist: 1 μM) decreased heart rate by 13% and MDL 73,147EM (1 μM) by 10%, however, ranitidine plus histamine inhibited by about 23%. Data are not very impressive, nevertheless, MDL 73,147EF may slightly attenuate the positive chronotropic effect of histamine, but had no significant effect on histamine H_2 receptor blocking activity of ranitidine.

ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION (ADME):

Absorption, distribution, metabolism and excretion studies have been conducted in rat, rabbit, dog and monkey.

Rat:

Pharmacokinetics of MDL 73,147 and its Metabolite MDL 74,156 in Male Rats Following Oral and I.V. Dose of MDL 73,147EF (Report # K-93-005-D)

Methods: Male Sprague Dawley rats were given a single oral (gavage: 30 mg/kg) or I.V. (15 mg/kg) dose of MDL 73,147EF. Blood samples were collected by cardiac puncture at 0.083, 0.167, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 18 and 24 hours after drug administration (3 rats/time point were used) for measuring MDL 73,147 (the drug) and its main metabolite (MDL 74,156) according to

Various pharmacokinetic parameters were also calculated.

Results: The bioavailability of a 30 mg/kg oral dose was only 3.5% (may be due to extensive first pass effect). The mean residence times of MDL 73,147 (p.o. = 0.87 hr and i.v. = 0.10 hr) were significantly lower than those of MDL 74,156 (p.o. = 3.6 hr and i.v. = 2.5 hr). The drug (MDL 73,147) was not widely distributed to tissues (Vd = 1.1 L/kg) while MDL 74,156 was widely distributed (Vd = 27.0 L/kg, assuming 100% conversion of MDL 73,147 to MDL 74,156). The systemic plasma clearance were 189 ml/min/kg and 180 ml/min/kg for MDL 73,147 and MDL 74,156 respectively. It should be noted that systemic clearance for both drugs and its main metabolite were significantly larger than hepatic plasma flow in rats (33 ml/min/kg; blood flow = 55.2 ml/min/kg, hematocrit = 0.4 [Pharma. Res. 10 (7):1093-1096, 1993]), which suggests extensive extra-hepatic clearance. The plasma $t_{½}$ values were 0.52 and 0.11 hr for oral and I.V. dose respectively and corresponding $t_{½}$ values for MDL 74,156 were 3.7 and 2.4 hr respectively.

Table 5–133. Pharmacokinetic Parameters Characterizing the Plasma Concentration Time Profile of MDL 73,147 (Free Base) and MDL 74,156 in Male Sprague-Dawley Rats Administered a 30 mg/kg Oral or a 15 mg/kg IV Dose of MDL 73,147EF.				
Parameter	*30 mg/kg oral dose (22.2 mg/kg free base)*		*15 mg/kg iv dose (11.1 mg/kg free base)*	
	MDL 73,147	*MDL 74,156*	*MDL 73,147*	*MDL 74,156*
Bioavailability %	3.5	(61.6)*	–	–
CL_s (mL/min/kg)	–	–	189	(180)*
CL_o (mL/min/kg)	5402	–	–	–
V_{ss} (L/kg)	–	–	1.1	(27.0)*
$t_{1/2}$ (h)	0.52	3.7	0.11	2.4
MRT_B (h)	0.87	–	0.10	–
MRT_{BM} (h)	–	3.6	–	2.5
MAT (h)	0.77	–	–	–
$AUC_{(0-\infty)}$ (ng•h/mL)	68.9	1274.7	978	1035
AUMC (ng•h^2/mL)	60.1	5393	96.7	2679
C_{max} (obs) (ng/mL)	115	579	4156	669
t_{max} (obs) (h)	0.167	0.5	0.083	0.25
λ_z (h^{-1})	1.3	0.19	6.35	0.29
* Values calculated assuming 100% conversion of MDL 73,147 to MDL 74,156.				

Cls = Intravenous clearance
Clo = Oral clearance
V_{ss} = Steady state volume of distribution
MRT_B = Mean Residence time in the body
MRT_{BM} = Mean residence time of metabolite in the body
MAT = Mean absorption time

Sponsor's Table 5-133, Vol. 1.23, Page 296

The Absorption, Metabolism and Excretion of Oral and I.V ^{14}C-MDL 73,147EF in Female SD Rats
(Report # I-89-0015-D)

Methods: Four female Sprague Dawley rats were given a single oral (gavage) dose (10 mg/kg or 300 mg/kg) or I.V. dose (10 mg/kg) of ^{14}C-MDL 73,147. The volume of administration was fixed at 3 ml/kg. Urine samples were collected at 6, 12, 24, 38, 72, 96 and 120 hours after drug administration and feces were collected every 24 hr for up to 120 hr post-dosing. Levels of radioactivity were measured in each sample by . Levels of parent drug (MDL 73,147) and its main metabolite (MDL 74,156) were measured

Results: Irrespective of doses and route of administration, about of the administered radioactivity were excreted n urine and feces over a 120 hr period, and most of the excretion occurred within the first 24 hr of administration. Based on urinary excretion data after oral and I.V. doses, it is evident that oral dose of ^{14}C-MDL 73,147 (10 mg or 300 mg/kg) was absorbed completely and metabolized rapidly in female rats. Irrespective of route of administration, no parent drug was seen in urine or feces samples. Five and 3 radioactive peaks were seen in urine and fecal samples respectively. Both in urine and feces, the major radioactive peak represented metabolite MDL 74,156.

Dog:

Pharmacokinetics of MDL 73,147EF and its Metabolite MDL 74,156 in Male Beagle Dogs
(Report # K-93-0803-D)

Methods: Male beagle dogs (n=3) were given a single I.V. dose of 6 mg/kg of ^{14}C-MDL 73,147EF. These dogs were also given a single oral (gavage) dose of 6 mg/kg of ^{14}C-MDL 73,147EF after a one week of wash-out period. Blood samples were collected at -0.5, 0.083, 0.167, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 18, 24, 36 and 48 hours after drug administration. Levels of radioactivity were measured in each sample Drug and its metabolites were measured

Results: The bioavailability of a 6 mg/kg oral dose was 13.2% (may be due to extensive first pass effect). The systemic clearance of MDL 73,147 was 187 ml/min/kg, which is more than 7 times the hepatic plasma flow in dogs [~25 ml/min/kg; Gombar, C.T. et al; Drug Metab. and Disp. 14(5), 540-548, 1986], which suggests extra-hepatic clearance. The systemic clearance of

MDL 74,156 was comparable to hepatic plasma flow after oral or I.V. dose. The drug was not widely distributed (Vd=1.2 L/kg) while MDL 74,156 was widely distributed (Vd=8.4 L/kg). The mean residence times in the body were 0.11 hr for MDL 73,147 and were 7.1 hr for MDL 74,156. Irrespective of route of administration $t_{½}$ values were 0.1 and 5.1 hr for MDL 73,147 and MDL 74,156 respectively.

Table 5–134. Average Pharmacokinetic Parameters of MDL 73,147 (Free Base) and MDL 74,156 in Male Beagle Dogs Administered a 6 mg/kg Oral or a 6 mg/kg IV Dose of MDL 73,147EF.

Parameter	*MDL 73,147*		*MDL 74,156*	
	6 mg/kg iv	6 mg/kg po	6 mg/kg iv	6 mg/kg po
Bioavailability (%)	–	13.2 ± 4.8	–	79*
AUCpo/AUCiv (%)	–	–	–	78.8 ± 5.3
CLS (mL/min/kg)	187 ± 20	–	19.6 ± 2.9	–
CLpo (mL/min/kg)	–	1517 ± 609	–	24.5 ± 4.9
Vss (L/kg)	1.2 ± 0.4	–	8.4 ± 0.3	–
t1/2 (h)	0.10 ± 0.03	0.14 ± 0.06	5.1 ± 0.5	5.1 ± 0.6
MRTB (h)	0.11 ± 0.03	0.30 ± 0.12	7.1 ± 0.9	6.7 ± 0.8
MRTT (h)	0.19 ± 0.003	–	–	–
MRTC (h)	0.086 ± 0.030	–	–	–
MAT (h)	–	0.19 ± 0.13	–	–
AUC(0-∞) (ng•h/mL)	401 ± 44	53.0 ± 19.0	3883 ± 596	3071 ± 609
AUMC (ng•h2/mL)	42.3 ± 9.5	15.4 ± 6.9	28485 ± 7891	21901 ± 6825
Cmax (obs) (ng/mL)	1536 ± 138	212 ± 137	677 ± 61	617 ± 104
tmax (obs) (h)	0.083	0.083-0.167	0.167-0.5	0.25-0.5
ka (h −1)	–	6.81 ± 3.68	–	–
λz (h −1)	6.98 ± 1.74	5.53 ± 2.07	0.137 ± 0.138	0.138 ± 0.015

* Calculated absolute bioavailability of MDL 74,156.

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The Absorption, Metabolism and Excretion of Oral and I.V. ^{14}C-MDL 73,147EF in Beagle Dogs
(Report # I-88-0020-D)

Methods: Three male Beagle dogs were given a single oral (gavage) dose (6 mg/kg) or a single I.V. dose (1 or 6 mg/kg) of ^{14}C-MDL 73,147EF. The volumes of administration were fixed at 0.1 ml/kg and 0.6 ml/kg for 1 and 6 mg/kg dose levels respectively. Blood samples were collected from the jugular vein at pre-dose, 0.017 (i.v.), 0.083, 0.25, 0.5, 0.75 (6 mg/kg i.v.), 1, 1.25 (6 mg/kg i.v.), 1.50 (6 mg/kg i.v.), 1.75 (6 mg/kg i.v.), 2, 2.5 (6 mg/kg/i.v.), 3, 5, 8, 12, 18, 24, 36, 48, 72, 96 and 120 hours after drug administration. Urine samples were collected at 6, 12, 18, 24, 36, 48, 72, 96 and 120 hr after drug administration and feces were collected every 24 hr for up to 120 hr post-dosing. Levels of radioactivity were measured in each sample by LSC methods. Additionally, levels of the parent drug and its metabolite MDL 28,577 (=MDL 74,156) were also measured in plasma samples by methods. profile of ^{14}C metabolite in urine and feces were also monitored.

Results: Irrespective of route of administration, no MDL 73,147 (parent drug) was seen in urine or feces samples. About and of administered radioactivity were excreted in urine and feces over 120 hr period and most of the ^{14}C excretion occurred within the first 24 hrs of administration. Based on urinary excretion data after oral and I.V. doses, about of the administered dose was absorbed. More than seven radioactive metabolites were seen in urine and fecal samples. About of the dose represented MDL 74,156 (metabolite) in urine sample and of the dose as hydroxylated MDL 74,156 in feces.

After I.V. dose, the level of parent drug (MDL 73,147) declined rapidly, such that at 45 min. after drug administration it was below detection limit (10 ng/ml). However, C_{max} of the major metabolite MDL 74,156 was seen at 6 min. after I.V. dose and levels remained above the detection limit for at least 12 hours.

Rabbit:

The Absorption, Metabolism and Excretion of Oral and I.V. ^{14}C-MDL 73,147EF in Female Rabbits
(Report # I-89-0017-D)

Methods: Four female New Zealand white rabbits were given a single I.V. dose (10 mg/kg) or a single oral (gavage) dose (10, 50 or 150 mg/kg) of ^{14}C-MDL 73,147EF. The I.V. Dose volume was

1.5 ml/kg and the oral dose volume was 3 ml/kg. Urine samples were collected at 6, 12, 24, 48, 72, 96 and 120 hours after drug administration and feces were collected every 24 hr for up to 120 hours post-dosing. Levels of radioactivity were measured in each sample

Results:

Percent of ^{14}C Dose Excreted in Rabbits (n=4)				
	10 mg/kg (I.V.)	10 mg/kg (P.O.)	50 mg/kg (P.O.)	150 mg/kg (P.O.)
Urine (0-120 hr)	58.1 ± 5.9	23.8 ± 6.0	44.3 ± 5.4	49.1 ± 5.4
Feces (0-120 hr)	35.0 ± 4.9	65.7 ± 5.5	48.8 ± 7.3	39.8 ± 4.5
Cage Wash	1.9 ± 1.8	1.0 ± 0.6	2.7 ± 2.0	2.6 ± 2.0
Total	95.0 ± 0.6	90.5 ± 1.8	95.8 ± 5.3	91.5 ± 3.6

Irrespective of dose and route of administration, about 24 - 58% and 35 - 66% of the administered radioactivity were excreted in urine and feces respectively over 120 hr period and most of the excretion occurred during the first 48 hr period. Irrespective of route of administration, no parent drug was seen in urine and feces sample. A total of 6 ^{14}C peaks in urine and 2 ^{14}C peaks in feces were seen upon analysis of excreta. Two of the peaks were identified as metabolite MDL 74,156 and hydroxylated MDL 74,156 in urine and feces. Based on urinary excretion data after oral and I.V. doses, rabbits absorbed about 75% and 85% of the 50 and 150 mg/kg oral doses of ^{14}C-MDL 73,147EF respectively. In contrast only 41% of the 10 mg/kg oral dose level was absorbed in rabbit when urinary excretion data were used for calculation (this could be related to dose dependent elimination from plasma).

Monkey:

The Absorption, Metabolism and Excretion of Oral and I.V. ^{14}C-MDL 73,147EF in Cynomolgus Monkeys
(Report # I-89-0013-D)

Methods: Four cynomolgus monkeys were given a single I.V. dose (5 mg/kg, 0.5 ml/kg) of ^{14}C-MDL 73,147EF. These monkeys were also given a single oral (gavage) dose of 5 mg/kg (3 ml/kg) of ^{14}C-MDL 73,147EF after a week of wash-out period. Blood samples were collected from jugular vein at pre-dose, 0.083 (i.v.), 0.25, 0.5,

1, 2, 3, 5, 8, 12, 18 and 24 hours after drug administration. Urine and feces samples were collected every 24 hr for up to 120 hours after drug administration. Levels of radioactivity were measured in each sample Levels of parent drug (MDL 73,147EF) and its main metabolite (MDL 74,156) were measured by

Results: About 51% and 44% of administered radioactivity were excreted in urine and feces over 120 hr period after I.V. dose of 5 mg/kg, while 36% and 56% of the administered radioactivity were excreted in urine and feces over 120 hr period after a oral dose of 50 mg/kg. Plasma levels of MDL 73,147 declined very rapidly ($t_{½}$ = 0.15 hr) after i.v. dose. Plasma levels of the major metabolite (MDL 74,156) declined slowly and its $t_{½}$ values were 4.2 and 5.5 hr after I.V. and oral dose respectively. No parent drug was seen in urine and feces samples. Metabolites such as MDL 74,156 and its monohydroxylated derivative were mainly seen in urine and feces samples.

Pharmacokinetic Parameters in Monkeys				
	MDL 73,147 (Drug)		MDL 74,156 (Metabolite)	
Parameters	I.V. (5 mg/kg)	Oral (50 mg/kg)	I.V. (5 mg/kg)	Oral (50 mg/kg)
Bioavailability (%)	--	9	--	
C_{max} (ng/ml)	530.7	25.0	755	592
t_{max} (hr)	0.083		0.083	2.1
$AUC_{0-\infty}$ (ng.hr/ml)	172.5	ND	1097.5	3900
$t_{½}$ (hr)	0.15	ND	4.22	5.5

DISTRIBUTION:

The Absorption, Distribution and Elimination of Radioactivity in Sprague-Dawley and Long Evans Rats
(Report # I-89-0018-D)

Methods: Male Sprague-Dawley (SD) rats (n=30) and male Long-Evans (LE) rats (n=30) were given single oral (gavage) or I.V. dose (1 mg/kg) of ^{14}C-MDL 73,147. Urine and feces samples were collected at pre-dose and at every 24 hr after drug administration for up to 96 hours. Additionally, 3 rats/time point (time points: 0.5, 1, 2, 6, 12, 24, 48, 72 and 96 hours post-dose) were sacrificed, blood and various tissues were collected to determine tissue distribution. Levels of radioactivity in various samples were measured by

Results: Based on urinary excretion data (oral vs I.V.), about 56% of administered oral radioactivity was absorbed (this is contradictory to the finding of report # I-89-0015-D: 100% absorption) and distributed throughout the body. Radioactivity levels in liver, kidney, stomach and small intestine were higher than that seen in plasma (SD and LE rats). 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 and eliminated very slowly ($t_{½}$=130 hrs). In both SD and LE rats, about 21% of the oral dose was excreted in urine while 70% of the oral dose was eliminated in the feces over 96 hr period. After I.V. dose, in SD rats, about 37% and 54% of the administered dose were eliminated in urine and feces respectively.

TABLE VI. TISSUE TO PLASMA ^{14}C CONCENTRATION RATIOS FOR TISSUES OF MALE SPRAGUE-DAWLEY AND LONG-EVANS (LE) RATS GIVEN SINGLE 1 MG/KG ORAL DOSES OF [^{14}C]MDL 73,147EP IN AQUEOUS SOLUTION.

		TISSUE/PLASMA ^{14}C CONC. RATIO								
TISSUE	STATISTIC	0.5 H	1 H	2 H	6 H	12 H	24 H	48 H	72 H	96 H
EYES	MEAN	0.21	0.311	0.436	1.8	4.8[a]	BLQ[b]	BLQ	BLQ	BLQ
	SD	0.03	0.062	0.095	0.2					
EYES (LE RAT)	MEAN	0.709	0.726	1.65	6.4	29	43[c]	BLQ	BLQ	BLQ
	SD	0.861	0.313	0.19	1.38	6				
SKIN	MEAN	0.796	1.19	1.41	2.8	3.6[c]	BLQ	BLQ	BLQ	BLQ
	SD	0.062	0.25	0.51	0.1					
SKIN (LE RAT)	MEAN	0.799	1.09	1.38	2.44	3.6[c]	13[c]	BLQ	BLQ	BLQ
	SD	0.135	0.26	0.84	0.52					
ADRENALS	MEAN	1.87	4.00	3.79	11.2	16[c]	BLQ	BLQ	BLQ	BLQ
	SD	0.41	2.79	1.22	1.1					
BONE MARROW	MEAN	0.813	1.09	1.35	3.2[c]	BLQ	BLQ	BLQ	BLQ	BLQ
	SD	0.106	0.17	0.39						
BRAIN	MEAN	0.068[a]	0.13[c]	0.14	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
	SD			0.02						
ERYTHROCYTES	MEAN	0.623	0.777	0.737	1.3	BLQ	BLQ	BLQ	BLQ	BLQ
	SD	0.037	0.050	0.112	0.3					
HEART	MEAN	0.949	1.20	1.39	4.23	4.3[c]	BLQ	BLQ	BLQ	BLQ
	SD	0.231	0.31	0.31	0.48					
KIDNEY	MEAN	9.718	11.80	8.547	18.4	33[c]	BLQ	BLQ	BLQ	BLQ
	SD	1.656	1.78	1.923	2.6					
LIVER	MEAN	64.75	61.47	40.54	92.6	240[c]	BLQ	BLQ	BLQ	BLQ
	SD	6.35	30.13	4.58	18.8					
LUNG	MEAN	1.442	1.771	2.176	9.67	16[c]	BLQ	BLQ	BLQ	BLQ
	SD	0.189	0.352	0.613	0.96					
PANCREAS	MEAN	1.61	1.87	3.309	14.8	9.1[c]	BLQ	BLQ	BLQ	BLQ
	SD	0.46	0.34	0.919	5.2					
PERIRENAL FAT	MEAN	0.23	0.339	0.396	1.2	BLQ	BLQ	BLQ	BLQ	BLQ
	SD	0.03	0.102	0.069	0.2					
SKELETAL MUSCLE	MEAN	0.521	0.623	0.627	4.03	7.6[c]	BLQ	BLQ	BLQ	BLQ
	SD	0.231	0.113	0.283	0.91					
SMALL INTESTINE	MEAN	33.92	32.42	30.86	57.9	14[c]	BLQ	BLQ	BLQ	BLQ
	SD	2.04	0.10	5.38	10.8					
SPLEEN	MEAN	1.16	1.23	1.41	4.41	5.8[c]	BLQ	BLQ	BLQ	BLQ
	SD	0.16	0.28	0.22	0.69					
STOMACH	MEAN	71.51	56.23	26.46	20.9	8.1[c]	BLQ	BLQ	BLQ	BLQ
	SD	10.92	31.06	3.83	21.0					
TESTES	MEAN	0.224	0.28	0.438	4.38	5.0[c]	BLQ	BLQ	BLQ	BLQ
	SD	0.056	0.05	0.119	1.12					
THYROID	MEAN	1.6	1.8	1.9	6.6[c]	BLQ	BLQ	BLQ	BLQ	BLQ
	SD	0.2	0.4	0.5						

[a] Concentrations were below limit of quantitation for two rats (n=1).
[b] BLQ = Below Limit of Quantitation. Limit of quantitation = twice background dpm.
[c] Concentration was below limit of quantitation for one rat (n=2).

METABOLISM:

Metabolism of MDL 73,147EF in Rats and Dogs
(Report # K-93-0802-D)

Methods: Male rats (n=3) were given a single I.V. dose (10 mg/kg) of ^{14}C-MDL 73,147EF, and dogs (n=2) were given a single I.V. dose of 6 mg/kg of ^{14}C-MDL 73,147EF. Urine samples were collected during -24-0, 0-2, 2-6, 6-12, 12-24 and 24-48 hours after drug administration and fecal samples were collected during -24-0, 0-12, 12-24 and 24-48 hours after drug administration. Levels of radioactivity were measured in each sample and levels of drug and its metabolites (MDL 74,156, MDL 102,382 [5'-OH MDL 74,156] and MDL 73,492 [6'-OH MDL 74,156]) were monitored by

Results: During the first 24 hr, about 38% of the administered radioactivity was excreted in urine of rats and dogs. Most of the urinary radioactivity represented metabolites of MDL 73,147. MDL 74,156 was the major metabolite in both rat and dog urine (20% and 24% of dose in rats and dogs respectively). N-oxide metabolite of MDL 74,156 was also seen in rat (1.4% of dose) and dog (1.9% of dose) urine, but not in feces. 5'-OH and 6'-OH MDL 74,156 were mainly seen in feces of rats and dogs (rats metabolized MDL 74,156 primarily to its 5'OH while dogs metabolized it primarily to its 6'-OH). In addition, two radioactive peaks were seen in urine samples (dogs only) and 5 radioactive peaks were seen in fecal samples (4/5 unknown peaks were seen in rats and 2/5 unknown peaks were seen in dogs). One of the unknown peak in rat and dog feces was later identified as 7'-OH MDL 74,156 (dogs=11.2% of the dose and rats=1.8% of the dose) (report # K-94-0055-D). No parent drug was seen in urine or feces samples. Thus, in both species (rats and dogs) MDL 73,147 mainly metabolizes to MDL 74,156, and then to N-oxide, 5'-OH, 6'-OH and 7'-OH of MDL 74,156 and corresponding conjugates (for detail metabolic pathway, see Fig. 1). Similar results were seen in study # I-88-0022-D (sponsor indicated metabolite MDL 74,156 as MDL 28,577).

APPEARS THIS WAY
ON ORIGINAL

Figure 3: **MDL 73,147**

MDL 73,147
(Dolasetron)

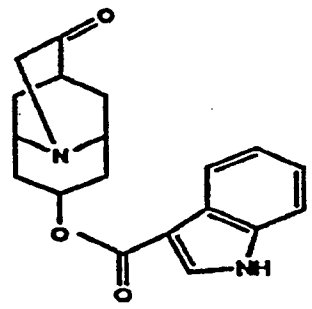

Fig. 1, Sponsor's Fig 3, Vol. 1.57, Page 295

Percent of Administered Dose				
	Rat (10 mg/kg, I.V.)		Dog (6 mg/kg, I.V.)	
Metabolites	Urine	Feces	Urine	Feces
MDL 74,156	20.0 ± 2.8	16.0 ± 0.8	24.5 ± 7.6	10.2 ± 1.5
N-oxide MDL 74,156	1.9 ± 0.3	---	1.4 ± 0.1	---
5'-OH MDL 74,156	4.3 ± 1.2	22.4 ± 5.3	1.7 ± 0.8	2.9 ± 0.5
6'-OH MDL 74,156	2.4 ± 0.3	5.5 ± 0.9	2.1 ± 0.7	15.8 ± 3.5
Conjugates	8.6 ± 1.7	---	3.5 ± 3.0	---
Total ^{14}C	37.8 ± 6.2	66.1 ± 5.9	37.9 ± 0.3	42.5 ± 6.2

Note: Urinary excretion was measured over 24 hr period in rats and dogs. Fecal elimination was measured over 24 hr period in rats and over 48 hr period in dogs.

Metabolism of MDL 73,147EF in Rabbits
(Report # I-90-0017-DM)

Methods: Sixteen female New Zealand white rabbits were given a single I.V. dose (10 mg/kg) or a single oral (gavage) dose (10 or 150 mg/kg) of ^{14}C-MDL 73,147EF. Urine and fecal samples were collected every 24 hr for up to 72 hr post-dosing. Levels of radioactivity were measured in each sample , and levels of drug and its metabolites were monitored by

Results: Irrespective of route of administration about and of the administered radioactivity were eliminated in urine and feces over 72 hr period. No parent drug was seen in urine and fecal samples. MDL 74,156, 5'-OH and 6'-OH MDL 74,156 and their conjugates were identified in urine and fecal samples of rabbits (sponsor did not clearly indicate amounts of various metabolites) (for structure see Fig. 1).

Metabolism of MDL 73,147 in Monkeys
(Report # I-89-0016-D)

Methods: Four cynomolgus monkeys were given a single I.V. dose (5 mg/kg, 0.5 ml/kg) or a single oral dose (50 mg/kg, 3 ml/kg) of ^{14}C-MDL 73,147. Urine and feces samples were collected every 24 hr for up to 120 hours after drug administration. Levels of radioactivity were measured in each sample
Levels of parent drug and its metabolites were measured by

Results: No parent drug was seen in urine or feces samples. Major urinary metabolite was MDL 74,156 and the major fecal metabolite was 5'-OH MDL 74,156 (see Fig. 1). Two additional unidentified radioactive peaks were seen in urine and feces samples. Quantitation of various metabolites were not meaningful because they were represented as % of eluted radioactivity in each peak.

In Vitro Metabolism of MDL 73,147
(Report # S-88-0050-D and # I-88-0026-D)

Rat liver 10,000 g supernatant in the presence of NADPH generating system rapidly metabolized MDL 73,147 to MDL 74,156 and this reaction was not dependent on cytochrome P-450, since inhibition with CO had no significant effect on MDL 74,156 production.

Binding of MDL 73,147EF and its Metabolite MDL 74,156 to Rat, Dog, Monkey and Human Plasma Protein
(Report # I-90-0016-D)

Rat, dog, monkey and human plasma samples were incubated with 50 - 5000 ng/ml of MDL 73,147EF or MDL 74,156. About of the drug (MDL 73,147EF) were bound to rat, dog, monkey and human plasma protein, while binding of MDL 74,156 in all 4 species.

Hepatic Enzyme Induction Potential of MDL 73,147 in Sprague Dawley Rats After Oral Administration
(Report # K-94-0286-D)

Methods: Groups of rats (5/sex/group) were given daily oral doses of 0 (vehicle: water), 50, 150 and 300 mg/kg/day of MDL 73,147 for 7 days. A positive control group was also included which received 75 mg/kg/day of phenobarbital (PB) for 7 days. The volume of administration was fixed at 3 ml/kg. At the end of treatment period all rats were sacrificed, enzyme induction potential was evaluated by measuring liver weights, microsomal protein, cytochrome P-450 levels and activities of aminopyrine-N-demethylase (APND: non-specific i.e. catalyzed by several P-450 isozymes), ethoxyresorufin-O-deethylase (ERDD: P-450 1A 1/2) and pentoxyresorufin-O-deethylase (PROD: P-450 2B 1/2).

Results: MDL 73,147 treatment had no significant effect on liver weights and levels, microsomal protein and cytochrome P-450, while PB increased liver weights by and levels of

microsomal protein and cytochrome P-450 by respectively, when compared to control values. In PB treated rats, activities of APND, EROD and PROD were increased by 232%, 306% and 4792% in males and 143%, 55% and 2593% in females respectively, while corresponding values in 300 mg/kg/day of MDL 73,147 treated rats were 26%, 179% and 220% in males and 6%, 36% and 50% in females respectively.

TABLE 1: The effect of MDL 73,147 or PB on various parmaters of enzyme induction after oral administration to male and female rats for one week.‡

Parameters‡	Dose of MDL 73,147 or Phenobarbital (mg/kg/day)									
	0		50		150		300		PB (75)	
	M	F	M	F	M	F	M	F	M	F
Relative Liver Weight (% Body Weight)	4.914 ± 0.206	4.428 ± 0.275	4.873 ± 0.117	4.376 ± 0.042*	4.774 ± 0.113	4.311 ± 0.088*	4.667 ± 0.143	4.379 ± 0.108	6.039 ± 0.063	5.574 ± 0.081*
Microsomal Yield (mg/g liver)	15.53 ± 1.84	10.79 ± 3.24	14.01 ± 1.90	13.76 ± 0.49	13.46 ± 0.23	13.60 ± 0.81	15.54 ± 3.40	12.01 ± 2.02	18.37 ± 1.55	12.23 ± 1.80
P-450 (nmole/mg protein)	0.592 ± 0.031	0.289 ± 0.009*	0.448 ± 0.069	0.383 ± 0.025	0.658 ± 0.045	0.414 ± 0.027*	0.752 ± 0.072	0.401 ± 0.128	1.810 ± 0.056	0.759 ± 0.152*
APND (nmole/mg/min)	4.962 ± 0.062	3.123 ± 0.096*	4.184 ± 0.467	2.696 ± 0.071*	4.445 ± 0.477	3.088 ± 0.291*	6.265 ± 0.128	3.322 ± 0.492*	16.495 ± 0.652	7.602 ± 1.681*
EROD (nmole/mg/min)	0.0371 ± 0.013	0.0591 ± 0.019	0.0279 ± 0.006	0.0299 ± 0.001	0.0341 ± 0.005	0.0345 ± 0.002	0.1035 ± 0.031	0.0802 ± 0.028	0.1508 ± 0.039	0.0914 ± 0.027
PROD (nmole/mg/min)	0.0213 ± 0.007	0.0171 ± 0.005	0.0222 ± 0.003	0.0059 ±0.001*	0.0286 ± 0.004	0.0093 ±0.002*	0.0682 ± 0.006	0.0257 ±0.002*	1.0420 ± 0.227	0.4605 ±0.274*

‡ The parameters are reported as mean ± the standard error (SEM). N=3 for each sex for all parameters.

* Significantly different than the males (α=0.05).

Thus, MDL 73,147 is not a hepatic enzyme inducer. Phenobarbital a known hepatic enzyme inducer gave the expected results.

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 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.

TOXICOLOGY:

ACUTE TOXICITY:

Results of acute toxicity study in mice, rats, dogs and monkeys were 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:

Mice: The oral and iv LD_{50}s for combined sexes were 545 and 165 mg/kg, respectively. The most common symptoms noted were tremors and/or convulsions, depression, and death. Onset occurred within minutes of oral dosing and immediate with iv injection. The symptoms regressed within 5 or 6 hr or persisted until death. Most deaths occurred from CNS depression within 48 hr of oral dosing while with iv dosing, the deaths occurred within a few hours of dosing. Necropsy did not reveal any specific target organ toxicity.

Rats: The oral and iv LD_{50}s for combined sexes were 446 and 150 mg/kg, respectively. The most common symptoms noted were the same as those in mice and time to death was also similar. No data were available on the minimal doses that showed clinical symptoms, but the symptoms regressed within 5 hr or persisted until death with exception of a few rats in which clinical signs persisted for several days and the rats recovered fully. Most deaths occurred from CNS depression within 48 hr of oral dosing while with iv dosing, the deaths occurred within a few hours of dosing. Of those deaths that occurred in rats (61/80), most (56/80) occurred within 24 hr after dosing. Necropsy did not reveal any specific target organ toxicity.

Table 5.
Acute Toxicity of MDL in Rats and Mice

Animal/# /group	Route	Doses tested, mg/kg	LD_{50} mg/kg	Signs/Symptoms
Mice, 5	po	300-2,200	545	Tremors, convulsions, depression, and death; majority of deaths occurred within 48 hr of dosing
	iv	100-224	165	Tremors, convulsions, depression, unconsciousness and death within a few hours; but onset of symptoms was immediate.
Rats, 5	oral	300-2,200	446	Tremors, convulsions, depression, and death within 48 hr of dosing.
	iv	100-224	150	Tremors, convulsions, depression, unconsciousness, and death within a few hours; onset of symptoms was immediate.

Dogs

In the acute oral toxicity study with dogs (1/sex), single oral doses of MDL were given at 5, 10, or 30 mg/kg and the animals were observed for changes in clinical signs and body weights and also were given physical examinations. MDL produced emesis/retching at 10 and 30 mg/kg doses, but not at 5 mg/kg. In addition, soft stools, salivation occurred in one 30 mg/kg dog. Thus, the no-acute-oral-toxic dose for the dog was 5 mg/kg.

In the acute iv studies, the dogs were given MDL in single doses of 3, 4.5, 6, 20, or 30 mg/kg and the animals were observed as before. The lower doses of 3 and 4.5 mg/kg did not cause any systemic toxicity, but the higher doses caused emesis/retching, salivation, and tearing of eyes, particularly at 20 and 30 mg/kg doses. One male dog after 6 mg/kg also developed emesis and salivation within 43 min of dosing. At 30 mg/kg, tremors, chewing movements and panting were observed in one dog. Thus, 4.5 mg/kg was the no-acute-iv-toxic dose for the dog.

Monkeys

In the monkeys (1/sex), the animals were given single oral (by gavage) doses of MDL at 30, 60, 100 or 200 mg/kg and were observed similarly. No systemic toxicity was observed in any animal at any dose. In the monkeys that received MDL in single iv doses of 3, 6, 10, 20, or 30 mg/kg, generally there was no systemic toxicity except one monkey, that had received a 20 mg/kg dose, developed emesis.

Reviewer's Addendum:

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. After i.v. dose, the death occurred within few hours and after oral dose death occurred within 2 days. 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.

SUBACUTE/SUBCHRONIC/CHRONIC TOXICITY:

Rat:

1-Month I.V. Toxicity Study in Rats
(Report # I-92-0148-T)

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

Study Started: February 18, 1992

Study Completed: September 28, 1992

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

Animals: Male and female Sprague Dawley rats (old,).

Drug Batch No.: 8611093

Methods: In this study dose selection was based on 2-week i.v. dose ranging study (report # I-92-0149-T: 0, 30, 60 and 120 mg/kg/day) in rats, in which 120 mg/kg produced lethality. Decreased activity and convulsions were seen at 60 mg/kg/day. Based on these findings, sponsor selected 0, 15, 30 and 60 mg/kg/day for the present study. Groups of rats (18/sex/group) were given daily doses of 0 (vehicle: acetate buffered mannitol), 15, 30 and 60 mg/kg/day of MDL 73,147EF via i.v. route (into tail vein) for 1 month. The volume of administration was fixed at 2 ml/kg. Eight rats/sex/group were used for 2-week recovery period. All rats 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. Additionally, blood samples from 3 rats/sex/group were collected at 15 min and 6 hr after drug administration on day 1 and 28 of the study. Rats used for blood levels were not used for toxicological assessment. At the end of study period all surviving rats were sacrificed and subjected to complete necropsy and histopathological examinations.

Results:

1. **Observed Effects**: Lesions (discoloration, swelling, ulceration) at the injection site (tail) were seen in all rats treated with 30 mg/kg and higher dose levels. Tail discoloration was also seen in 1/18 low dose treated females. Additionally, decreased activity and convulsions were also seen in some of the high dose treated rats.

2. **Mortality**: One female from mid dose group died on day 28 of the study. The cause of death could not be determined. Additionally, 6 rats (3/sex) from high dose group were killed because of severe tail lesions.

3. **Body Weight/Food Consumption/Water Consumption**: At the end of treatment period body weight gains in treated males were decreased by 3.8%, 6.9% and 6.3% at low, mid and high dose levels respectively, when compared to control values. Females body weight gains were not affected by treatment. Treatment had no significant effect on food consumptions in rats (both sexes).

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**: No treatment related effects were seen.

7. **Organ Weights**: Only relative spleen weight was increased by 15% in high dose treated males, compared to control values.

8. **Gross Pathology**: Dose dependent increase incidence of tail lesions (injection site) were seen in treated rats.

9. **Histopathology**: Lesions such as inflammation, hemorrhage, thrombosis and necrosis were seen at the injection sites (tail) of rats treated with mid and high doses. Additionally, 1 out of 10 low dose treated females had ulceration/necrosis at the injection site. Extramedullary hematopoiesis in spleen were seen in high dose treated rats (males: 3/10 and females: 5/10).

10. **Plasma Levels of the Drug (MDL 73,147) and its Metabolite (MDL 74,146) (Report # K-93-0217-D)**: Levels of MDL 73,147 and MDL 74,156 were determined by HPLC methods. At 15 minutes after drug administration levels of the drug (MDL 73,147) increased with increasing dosages, and levels in females were about 2 - 3 times greater than males. There was no indication of accumulation after 28 repeat daily dosing and 6 hr after drug

administration levels of the parent drug were below detection limit. The metabolite (MDL 74,156) levels at 15 min after drug administration also increased with increasing dosages and there were no gender differences and no accumulations after repeat dosing.

TABLE 1: Average fifteen-minute concentrations of MDL 73,147 (free base) and its metabolite MDL 74,156 in the plasma of male and female rats on days 1 and 28 of an intravenous one-month toxicity study of MDL 73,147EF.

Dose Group (mg/kg/d)	Sex	n	MDL 73,147 (ng/mL) Day 1	MDL 73,147 (ng/mL) Day 28	MDL 74,156 (ng/mL) Day 1	MDL 74,156 (ng/mL) Day 28
15	M	3	385 ± 150	343 ± 76	335 ± 168	450 ± 80
	F	3	835 ± 178	384 ± 155	839 ± 104	314 ± 168
	Both sexes	6	610 ± 287	364 ± 112	587 ± 303	382 ± 139
30	M	3	1297 ± 158	354 ± 172[a]	1607 ± 587	652 ± 404[a]
	F	3	2578 ± 936	804 ± 199	1401 ± 201	935 ± 120
	Both sexes	6	1937 ± 923	624 ± 297[b]	1504 ± 408	822 ± 268[b]
60	M	3	1687 ± 1443	788 ± 42[a]	2177 ± 1915	2436 ± 43[a]
	F	3	4916 ± 86	1957 ± 480	3542 ± 688	2320 ± 518
	Both sexes	6	3302 ± 1991	1489 ± 725[b]	2860 ± 1488	2366 ± 372[b]

a n=2
b n=5

Sponsor's Table 1, Vol. 1.56, Page. 212

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.

1-Month Oral Toxicity Study in Rats
(Report # C-91-0071-T)

Testing Laboratories: Toxicology Department
Marion Merrell Dow Inc.
Cincinnati, OH

Study Started: April 20, 1990

Study Completed: March 7, 1991

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

Animals: Male and Female Sprague Dawley rats (about 53 days old; wt.: .

Drug Batch No.: C-46711

Methods: In this study dose selection was based on 2-week oral dose ranging study (report # C-88-0018-T: 100, 200 and 300 mg/kg/day) in which salivation was seen in treated rats at 200 mg/kg and higher dose levels. Increased liver weights and ventral prostate weights were seen in all treated rats without any abnormal histopathological findings. Based on these findings and information of oral LD_{50} values (see above), sponsor selected 0 (vehicle: water), 200, 300, 400 and 500 mg/kg/day dose levels for the present study. There were 10 rats/sex/group and volume of administration of vehicle or drug was fixed at 3 ml/kg via gavage. During the last two weeks of the study period, rats were dosed after overnight fast. All animals were observed daily for clinical signs and mortality. Body weights were recorded on days 16, 23 and 30 of the study. Food intakes were recorded weekly. Ophthalmoscopic examinations were performed at pretest and on day 30 of the study. Blood samples were collected by cardiac puncture at the end of the study periods for hematological and serum chemistry tests. All surviving rats were sacrificed at the end of treatment period and subjected to complete necropsy. Only control and high dose group rats were examined histologically. Kidneys from 400 mg/kg/day group were also examined microscopically.

Results:

1. **Observed Effects**: No treatment related clinical signs were seen when rats were dosed in non-fasted state (i.e. during the first 2 weeks). However, clinical signs such as salivation, depression, ataxia and tremors were seen in treated rats when drug was administered after overnight fasting.

2. Mortality: During the study period, 7 rats (2 male from 200 mg/kg group, 1 female from 400 mg/kg group, and 2 male and 2 female from 500 mg/kg group) died due to dosing accident. During the last 2 weeks of the study, there were 12 treatment related deaths (males: 1 at 400 mg/kg, 3 at 500 mg/kg; females: 1 at 300 mg/kg, 4 at 400 mg/kg and 3 at 500 mg/kg) and the cause of death was not be established.

3. Body Weight/Food Consumption/Water Consumption: At the end of treatment period, body weight gains were reduced by 10.3%, 14.2% and 20% in males and 3.8%, 9.2% and 92% in females at 300, 400 and 500 mg/kg/day dose levels respectively, when compared to respective control values. Food intakes were reduced by 14% and 22% in 400 and 500 mg/kg treated males compared to control values.

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

5. Blood Chemistry/Urinalysis: Only in high dose (500 mg/kg/day) treated males increases in SGPT (43%), SGOT (46%) and cholesterol (70%) were seen. These increases were mainly due to the high values seen in rat # 048M. If this rat is excluded from consideration then no treatment related effects were seen.

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

7. Organ Weights: In females, relative wt. of the liver were increased by at 400 mg/kg/day dose level.

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

9. Histopathology: Histopathological examinations of renal pelvis and ureter were not done "properly". However, according to sponsor epithelial necrosis, suppurative inflammation, and/or hemorrhage were seen at the junction of the renal pelvis and ureter and/or in the ureter in 5/10 males treated with 500 mg/kg/day and 2/10 females treated with 400 mg/kg/day (but not seen in 500 mg/kg/day treated females).

In this study, 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.

3-Month Oral Toxicity Study in Rats
(Report # C-89-0017-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: May 11, 1988 to June 29, 1989.

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

Animals: Sprague-Dawley rats weighing and 41 days of age were used.

Methods: Four groups of animals each consisting of 27 males and 27 females were given MDL 73,1477EF dissolved in water by gavage at constant volume of 3 ml/kg and at dose levels of 0, 10, 30 and 100 mg/kg/day. Fifteen/sex/dose group were used for toxicity study. Three/sex/dose group were bled 2 and 24 hrs after the first and 30the doses for pharmacokinetic study. According to sponsor, dose selection was based on the 2 week dose range study (Report No. T-88-15). However, rationale in dose selection was not stated.

Results:

Clinical signs (daily): Normal.

Mortality: One female of the 100 mg/kg/day group died of a dosing accident.

Body weight and food consumption (weekly): No effect in body weight was noted. increases in food consumption were observed in the 100 mg/kg/day group.

Hematology (week 13): Normal.

Urinalysis (day 90): Presence of crystal in the urine was observed in two of ten males in the 100 mg/kg/day group.

Organ weight: Increases in liver (29%), brain (5%), heart (14%) and kidney (14%) weights were noted in the females of the 100 mg/kg/day group.

Gross and histopathological examinations: Normal.

Pharmacokinetics: No accumulation of parent drug or the metabolite (MDL 74156) was seen after 30 single daily doses of 10-100 mg/kg/day. Plasma levels of MDL 73147 and MDL 74156 were higher in female rats than in male rats.

In conclusion, MDL 73147EF given by gavage for three months produced increases in liver, brain, heart and kidney organ weights as well as the presence of crystal in the urine of the 100 mg/kg group. Thus, a no effect dose of 30 mg/kg/day was established. Although sponsor stated that dose selection was based on the 2 week, dose-range study, rationale in dose selection was not stated. The highest dose used in the [illegible] appears to be too low to [illegible]

1-year Oral Toxicity Study in Rats
(Report # I-93-0020-T)

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

Study Started: August 23, 1990

Study Completed: May 7, 1993

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

Animals: Male and female Crl:CD(BR) Sprague Dawley rats (days old; males: g and females: g).

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

Methods: Groups of rats (68/sex/group) were given orally (gavage) MDL 73,147EF at daily doses of 0 (vehicle: water), 30, 75 and 400 mg/kg/day for up to 1 year (in this study, rats were treated for 6 months or 1-year and then allowed to recover for 1-month). Rats were fasted for 6 hr prior to administration of vehicle or drug. The volume of administration was fixed at 3 ml/kg. All rats were observed for clinical signs and mortality twice daily. Body weights and food intakes were recorded weekly. Just before sacrifice blood samples were collected for hematological and serum chemistry tests. Additionally, blood samples were also collected at 2 and 24 hr after drug administration on days 1, 30 and 366 of the study to monitor levels of MDL 73,147EF and its active metabolite MDL 74,156 in plasma (3 rats/sex/dose/time point were used). At the end of 6-month treatment (about 20/sex/group), 6-month treatment plus 1-month recovery (4-5/sex/group), 1-year treatment (about 20/sex/group), and 1-year treatment plus 1-month recovery (4 - 5/sex/group) were sacrificed and subjected to complete necropsy. Only control and high dose group rats were examined microscopically. Additionally, kidneys and ureters from the remaining rats were also examined microscopically.

Results:

1. Observed Effects: At ≥75 mg/kg/day, excessive salivation, increased incidence of nasal discharge, red/brown urine (in males), convulsions, depression and tremors were seen. Sporadic convulsions were also seen in 3/68 low dose treated males.

2. Mortality: There were 78 deaths (males: 3 in low dose group, 10 in mid dose group and 32 in high dose group; females: 2 in control group, 2 in low dose group, 5 in mid dose group and 24 in high dose group). Twenty-three out of 78 deaths (1 male and 1 female at 75 mg/kg/day, 18 males at 400 mg/kg/day and 3 females at 400 mg/kg/day) were most likely treatment related and cause of deaths were suppurative upper urinary tract inflammation, urinary tract lesions or convulsions. Cause of remaining deaths were accidents/undermined.

3. Body Weight/Food Consumption/Water Consumption: Throughout the study period, body weights were adversely affected at high dose level. At the end of treatment period, body weight gains were reduced by 18.6% and 23.9% in high dose treated males and females respectively, when compared to their respective control values (the final body weights in high dose treated males and females were about 19% and 24% lower than the respective control values). During the first six months of treatment, food intakes of high dose treated males were decreased by 8-19% (no effect on food intakes in treated females were evident) when compared to control values.

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

5. Blood Chemistry/Urinalysis: At the end of 6-month of treatment, increases in BUN (38%), serum phosphorus (20%) and alkaline phosphatase (90%) were seen in high dose treated males. At the end of study period (i.e. 1-year) only 1 male survived, therefore no meaningful comparison is possible. In females, at the end of treatment period, only serum alkaline phosphatase levels were increased by 178% in high dose treated groups. Additionally, in high dose treated females partial thromboplastin times (PTT) were significantly increased at both sampling points (6-month and 1-year) (control: 18 sec at 6-month and 17.3 sec at 1-year, high dose: 21.4 sec at 6-month and 26.3 sec at 1-year). Hematuria was seen in most of the high dose treated rats (both sexes).

6. Organ Weights: At he end of 6-month, adrenal weights were increased by 21% (relative wt.: 66%) and 16% (relative wt.: 26%) in high dose treated males and females respectively. At the end of 1-year of treatment period, adrenal weights were increased by 50% (relative wt.: 99%) in high dose treated females (only 1 high dose treated male survived 1-year treatment, therefore no comparison is possible). Additionally, increases in relative weights of liver (30%), kidneys (15%), brain (28%), heart (27%) and uterus (28%) were seen in high dose treated females at 1-year necropsy.

7. Gross Pathology: Bloody urine in the urinary bladder was seen in 5/56 high dose treated rats.

8. Histopathology: Increased incidences of renal proximal convoluted tubule degeneration (in both sexes) and lower urinary tract epithelium irritation (suppurative inflammation and/or reactive hyperplasia of the renal pelvic epithelium, mainly in males) were seen in treated rats.

Histopathological Findings*					
		Dose (mg/kg/day)			
Findings	Sex (M/F)	0	30	75	400
Renal PCT Degeneration	M	0/40	0/43	0/45	10/51
% Affected		0	0	0	19.6
	F	1/42	0/42	0/44	8/52
% Affected		2.4	0	0	15.4
Lute Irritation	M	3/40	5/43	6/45	24/51
% Affected		7.5	11.6	13.3	47.0
	F	1/42	2/42	1/44	4/52
% Affected		2.4	4.8	2.3	7.7

* = Data from unscheduled deaths, 6-month and 1-year necropsy are combined.
PCT = Proximal convoluted tubule.
LUTE = Lower urinary tract epithelium.

9. Plasma Levels of the Drug and Its Metabolite (MDL 74,156): MDL 73,147 levels increased with increasing dosages. Levels of active metabolite (MDL 74,156) also increased with increasing dosages. At 2 hr, MDL 73,147 levels were significantly higher in females than males. There is an evidence of accumulation of drug and its metabolite (MDL 74,156) during 1-year course of treatment.

Levels of 73,147 (ng/ml)							
Dose (mg/kg/day)	Sex (M/F)	Day 1		Day 30		Day 371-374	
		2 hr	24 hr	2 hr	24 hr	2 hr	24 hr
30	M	--	--	16 ± 5.5	--	14 ± 6.4	--
	F	29 ± 7.0	--	160 ± 77.7	--	169 ± 99	--
75	M	35 ± 11.5	--	24 ± 11.8	--	62.3 ± 50.9	--
	F	184 ± 199	--	191 ± 38.2	16 ± 9.8	1125 ± 130	--
400	M	110 ± 126	--	200 ± 128	60 ± 53	334[1]	--
	F	1029 ± 813	318 ± 127	855 ± 529	464 ± 161	5777 ± 2670	290 ± 129

Levels of 74,156 (metabolite: ng/ml)							
Dose (mg/kg/day)	Sex (M/F)	Day 1		Day 30		Day 371-374	
		2 hr	24 hr	2 hr	24 hr	2 hr	24 hr
30	M	292 ± 59.5	--	480 ± 96.2	--	1196 ± 149	13 ± 4.6
	F	327 ± 26.4	--	423 ± 201	--	1221 ± 318	--
75	M	1374 ± 187	--	902 ± 520	30 ± 19.8	4162 ± 1934	54 ± 9.5
	F	1020 ± 539	?	1236 ± 404	129 ± 142	3740 ± 993	25 ± 16
400	M	2480 ± 589	846 ± 237	5353 ± 1457	1563 ± 1355	12421[1]	--
	F	3529 ± 1440	1902 ± 789	4843 ± 1248	1741 ± 633	14748 ± 4396	3305 ± 226

[1] = Only on rat was used.
Limit of detection = 10 ng/ml.

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).