CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 21024

CHEMISTRY REVIEW(S)

DIVISION OF SPECIAL PATHOGEN AND IMMUNOLOGIC DRUG PRODUCTS—HFD-590

Review of Chemistry, Manufacturing and Controls Section

NDA #: 21-024 (originally NDA 50-752)
CHEMISTRY REVIEW #: 1 **REVIEW COMPLETED**: 06/12/98

SUBMISSION TYPE	DOCUMENT DATE	CDER DATE	ASSIGNED DATE
Original Submission	12/22/97	12/24/97	01/20/98
BC	02/03/98	02/05/98	?
BC	03/03/98	03/05/98	03/06/98
SU	03/04/98	03/06/98	03/10/98
BC	04/28/98	04/30/98	05/04/98
BC	04/30/98	05/01/98	05/07/98
BC	05/22/98	05/26/98	06/01/98
BC (faxed)	06/10/98	?	?

NAME/ADDRESS OF SPONSOR: Hoechst Marion Roussel, Inc.
10236 Marion Park Drive
P.O. Box 9627
Kansas City, MO 64134-0627

DRUG PRODUCT NAME:
Proprietary: Priftin®
Nonproprietary: Rifapentine

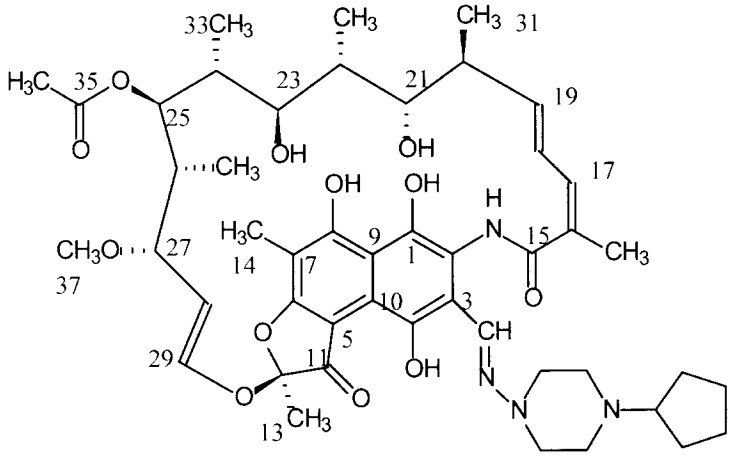
CHEM. TYPE/THER. CLASS: 1P/Priority
DRUG CLASS: Ansamycin
PHARMACOLOGICAL CATEGORY: antibacterial
INDICATION: Treatment of pulmonary tuberculosis
DOSAGE FORM/STRENGTH: tablets, 150 mg
ROUTE OF ADMINISTRATION: Oral

CHEMICAL NAME/STRUCTURAL FORMULA:

Rifamycin, 3-[[(4-cyclopentyl-1-piperazinyl)imino]methyl]-

3-[*N*-(4-Cyclopentyl-1-piperazinyl)-formimidoyl]rifamycin

5,6,9,17,19,21-Hexahydroxy-23-methoxy-2,4,12,16,18,20,22-heptamethyl-8-[*N*-(4-cyclopentyl-1-piperazinyl)-formimidoyl]-2,7-(epoxypentadeca[1,11,13]trienimino)-naphtho[2,1-*b*]furan-1,11-(2*H*)-dione 21-acetate



(rifamycin numbering system, not IUPAC)

CAS Registry: 61379-65-5
Molecular Formula: $C_{47}H_{64}N_4O_{12}$
Molecular Weight: 877.04

SUPPORTING DOCUMENTS:
None

RELATED DOCUMENTS:

REMARKS/COMMENTS:

LABELING —

ENVIRONMENTAL ASSESSMENT — A categorical exclusion was claimed.

CONCLUSIONS & RECOMMENDATIONS:
Recommend: Approval with Phase 4 Commitment

John Smith, /S/ Chemist

Concurrence:
HFD-590/NSchmuff

cc:

	HFD-590/CSO
HFD-590/Div. File	HFD-590/P/T
HFD-590/ NSchmuff	HFD-590/Micro
HFD-590/MO	HFD-590/JSmith

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ENVIRONMENTAL ASSESSMENT AND/OR FONSI

3.E. Environmental Assessment

The applicant claims a categorical exclusion for the action requested, as the action qualifies for categorical exclusion under 21 CFR Part 25, 25.31(b) Action on an NDA. The action increases the use of the active moiety, but the estimated concentration of the substance at the point of entry into the aquatic environment will be The action complies with the categorical exclusion criteria and to the applicant's knowledge, no extraordinary circumstances exist.

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 21024

PHARMACOLOGY REVIEW(S)

Submitted: December 24, 1997
Assigned: December 29, 1997
Completed: April 30, 1998
HFD-590

Sponsor: Hoechst Marion Roussel Inc.
Box 9627 F3-M3032
Kansas MO 64134-0627

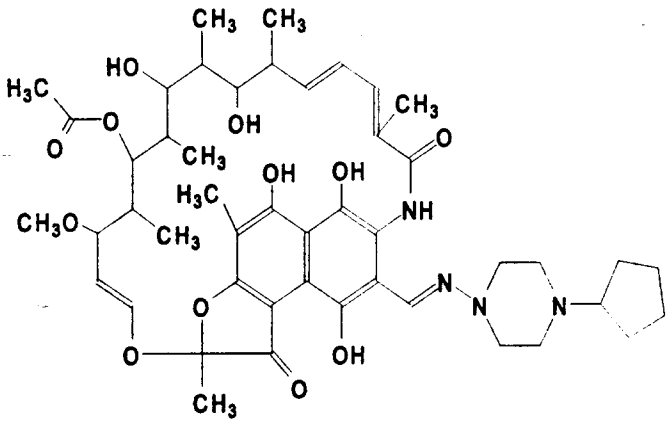
Drug: Priftin
Generic Names Rifapentine (INN, USAN, BAN)
Code Numbers MDL 473, L11473, DL473-IT, DL473-IT(A/F ciclopentil), rifamycin A/F cyclopentyl and M000473

Chemical Names Rifamycin, 3- [[(4-cyclopentyl-1-piperazinyl)imino]methyl]-;.[N- (4-Cyclopentyl-1-piperazinyl)formimidoyl]rifamycin 2,7-(Epoxypentadeca [1,11,13] trienimino) naphtho[2,1-b]furan-2,4,12,16,18,20,22-heptamethyl-8- [N- (4-cyclopentyl-1-piperazinyl)- formimidoyl]- 21-acetate

Molecular formula: $C_{47}H_{64}N_4O_{12}$
Molecular Weight 877.04
Physical and Chemical Characteristics Rifapentine is a red-orange, odorless, crystalline powder.

CAS Registry Number CAS-61379-65-5

Structure:



Indication: Treatment of pulmonary tuberculosis.

Formulation: Priftin is formulated in a tablet containing 150 mg of rifapentine, calcium stearate, disodium EDTA, FD & C Blue No. 2 aluminum lake, hydroxypropyl cellulose, hydroxypropyl methylcellulose, microcrystalline cellulose, polyethylene glycol, pregelatinized starch propylene glycol, sodium ascorbate, sodium lauryl sulfate, sodium starch glycolate synthetic red iron oxide, and titanium dioxide.

Introduction:

The rifamycins are a group of macrocyclic antibiotics derived from *Streptomyces mediterranei.* Rifapentine is a rifamycin derivative in which a cyclopentyl ring is substituted for a methyl group on the piperazinyl moiety, resulting in a more lipophilic drug. The rifamycins exert their antibiotic activity by forming a stable complex with the DNA-dependent RNA polymerase of susceptible bacteria, suppressing the initiation of chain formation in RNA synthesis. Rifapentine demonstrates a profile of microbiological activity similar to rifampin (also known as rifamycin B) but with a serum elimination half life about five times longer than rifampin. Since compliance with dosing is a major factor in treatment failures and the development of resistant strains of tuberculosis, the longer half life of rifapentine may theoretically improve compliance due to reduced frequency of dosing. Priftin is administered twice weekly in the initial phase of dosing and once weekly in the continuation phase of tuberculosis therapy. Priftin is administered with one or more of the following drugs: isoniazid, pyrazinamide and /or ethambutol.

This submission contains final reports of preclinical pharmacokinetics and toxicology studies submitted to support the NDA.

Preclinical Pharmacokinetics

1. of MDL 473 (rifapentine) in serum or buffer. Report V- 86-49.
2. Assay of rifapentine (MDL 473) in biological fluids. Project Report V-86-57.
3. for cyclopentyl rifampicin (MDL 473, rifapentine) in the presence of amikacin. Project Report IP-87-177.
4. Method for determination of DL 473-IT and its metabolite L14583 in plasma of humans, monkeys and rats. Project Report V-86-84.
5. DL 473 assay in serum: a comparison Report V-86-77.
6. Pharmacokinetic profile of rifapentine, a new long lasting rifamycin, in the rat, the mouse and the rabbit. Report V-89-17.
7. The pharmacokinetics of 14C-DL 473-IT in rats. Part II. Report V-86-73.
8. Pharmacokinetics of rifapentine (MDL-473), SPA-S-565 and rifampicin in mice after single 10 mg/kg intravenous or oral doses. Report V-87-39.
9. DL 473-IT (rifampicin AF/cyclopentyl) preliminary kinetic studies in mice and rats. Report V-88-22.
10. Pharmacokinetic study of rifapentine hydrochloride in male rats after a single oral administration. Comparison with rifapentine. Report V-88-71.
11. Physiological disposition of a series of rifamycins in rats: a comparative study. Report V-86-64.
12. Serum levels of DL 473-IT in rats treated orally for 3 months. Report V-88-23.
13. DL 473-IT: blood levels and toxicity during repeated oral administration to cynomolgus monkeys for four weeks. Report V-88-24.
14. Serum levels of DL 473-IT in cynomolgus monkeys treated orally for 3 months. Report V-86-86.
15. Protein binding of a series of [rifamycins] to bovine serum albumin as measured by "two-phase partition dialysis." Report V-86-72.
16. Studies of binding C3-substitute rifamycins to human and bovine serum albumin. Report V-86-65.

Pharmacokinetic Studies Review

Single dose studies

The following pharmacokinetic parameters were determined following single oral doses of rifapentine:

Species	Dose mg/kg	C_{max} µg/ml	Tmax (h)	$T_{1/2\beta}$ (h)	$AUC_{0-\infty}$ µg/ml	V/F (L/kg)	Cl (mL/kg/h)
Mouse*	10	14	2	18	377	-	-
Rat (male)[1]	3	4.5	8	18	137	0.6	22
Rat (male)[1]	10	7	8	21	317	1.0	32
Rat ,male[2]	10	8	4-8	17	1979	0.8	34
Rat (female)	10	9	4-8	15	317	0.7	32
Monkey[3]	10	10	(6)	(13)	-	-	-
Monkey[3]	20	17	(6)	(13)	-	-	-
Monkey[3]	40	34	(6)	1(3)	-	-	-
Monkey[3]	80	54	(6)	1(3)	-	-	-
Man[4]	8	10	9	12	394	-	-

V/F: Apparent volume of distribution.
*Strain Crl:CD-l(ICR)BR; determined by using *Sarcina lutea* ATCC 9341.
[1]Wistar rats; determined
[2]Wistar rats; determined
[3]Cynomolgus monkeys; determined
[4]Determined

Rifapentine appears to be rapidly and extensively absorbed after oral administration. In male CD-1 mice following oral administration of 10 mg/kg rifapentine, the mean plasma concentration at 15 minutes after dosing was 10.5 µg/mL, or about 77 % of the C_{max} (= 13.6 µg/mL) observed at 2 hours. The blood concentration-time curve tended to plateau at near C_{max} from 0.5 to 8 hours. Comparison of i.v. and oral AUC values indicated ~100 % oral bioavailability (F). Following single oral and i.v. doses of either 3 or 10 mg/kg given to Wistar rats (both sexes), comparison of AUC values demonstrated F = 84 % and 65 %, respectively. As in mice, high plasma concentrations were achieved rapidly and tended to plateau for up to 8 hours. Seven days after giving single oral doses of 10 mg/kg rifapentine to CF1 mice, mean serum concentrations were 0.36 µg/mL and the calculated terminal half-life was 31 hours (not shown in table). In male Crl:CD(SD)BR rats, rifapentine base demonstrated better oral bioavailability than the hydrochloride salt (with respective AUC values of 249 and 152 µg-hr/mL following 10 mg/kg doses.) Distribution studies in mice and rats following oral administration demonstrated that peak tissue concentrations of rifapentine

occured at ~ 8 hours after dosing. Highest concentrations of drug were observed in adrenals, liver, pancreas, submaxillary glands, kidneys, fat, heart, thymus, spleen, and lungs. In rats after oral administration of 10 mg/kg l4C-rifapentine, the following tissue drug concentrations were observed at 8 hours: adrenals, 53.83 µg/g; liver, 39.71 µg/g ; pancreas, 27.49 µg/g ; submaxillary glands, 18.61 µg/g ; kidneys, 18.55 µg/g ; fat, 13.98 µg/g ; heart, 13.70 µg/g ; thymus, 11.53 µg/g ; spleen,10.35 µg/g; and lungs, 9.49 µg/g . The ratios of tissue to blood concentration were greater than one for all time points (1, 8,24, and 72 hours.) In the rat, maximum tissue concentrations occurred in the liver before being observed in other tissues. In mongrel dogs, i.v. administration (15 mg/kg bolus followed by 1.88 mg/kg/hr infusion for 4 hours) resulted in bone concentrations of of tissue. Rifapentine does not appear to penetrate the blood-brain barrier to any significant extent. In rats, of l4C-rifapentine in plasma was protein-bound. The observed association constants indicate low-affinity, nonspecific binding to albumin. There appeared to be about one rifapentine binding site per protein molecule.

Single-dose studies indicate that rifapentine is not extensively metabolized. In both rats and mice, unmetabolized rifapentine accounted for most of the 14C observed in blood. Plasma levels of 25-desacetyl rifapentine were consistently < 0.2 µg/ml and accounted for about one-third of the rifapentine dose at 48 hours. Rifapentine appears to be a potent inducer of hepatic mixed function oxidase in mice. Pretreatment of male Kunming mice with 40 mg/kg rifapentine (by gavage) every other day for 10 days resulted in a 31 % reduction in pentobarbital sleeping time and a 38 % reduction in plasma antipyrine concentrations (compared to 93 % and 28 % reductions, respectively, in phenobarbital pretreated mice.) NADPH-cytochrome c reductase and NADPH oxidase activities were increased 88 % and 82%, respectively. Relative liver weights and microsomal protein and cytochrome P450 content were also increased.

Rifapentine is primarily excreted unmetabolized in the bile. In rats, 92 % and 6 % of a 10 mg/kg oral dose were recovered in the feces and urine, respectively, within 12 days, with residual radioactivity in the carcasses accounting for 0.96 % of the dose. Elimination did not appear to be dependent on route of administration: following single 10 mg/kg doses of rifapentine given i.v. to rats, 89 % and 10 % were recovered in the feces and urine, respectively, within 12 days. Elimination of rifapentine appeared to follow first-order kinetics. Rats given single i.v. doses of 10 mg/kg demonstrated the following concentrations of drug and principal metabolite in the bile:

Time after dosing	Rifapentine (µg/mL)	25-Desacetylrifapentine (µg/mL)
0-4		
20-24		
44-48		

The ratio of drug to metabolite decreased from 33.2 within the first hour of dosing to 3.2 at 48 hours.

Although pharmacokinetics appeared to be consistent in most species examined, results of one study (Project Report V-89-17) suggested that the rabbit is unique. Following single i.v. doses of 10 mg/kg given to New Zealand white rabbits, the plasma half-life was 1.8 hours and the volume of distribution was 0.44 L/kg. The AUC determined as the parent drug (51 µg*h/mL) was roughly equivalent to the same value determined for the same dose in mice (when the value is adjusted for relative body surface areas.) However, when total AUC was determined as 14C and compared to the same value determined as parent drug, in the rabbit 73 % of total exposure was as rifapentine, whereas in the mouse total exposure as parent drug was 92 %. This indicates that in the rabbit systemic exposure to the parent drug is significantly less than it is for other species, possibly due to idiosyncratic metabolism.

Comparison of pharmacokinetic determinations indicates that rifapentine has a longer half-life and produces greater systemic exposure than rifampin. In mice following single 10 mg/kg oral doses of either rifapentine or rifampin, observed $AUC_{0-\infty}$ values were 376.6 and 127.6 µg*hr/mL, and mean residence times were 26.03 and 7.29 hours, respectively. At 96 hours after dosing, the observed plasma rifapentine concentration was 0.43 µg/mL, whereas the rifampin plasma level was below the detection limit.

Rifapentine does not appear to be excreted as quickly as rifampin. The sponsor suggested that differences in hepatic subcellular distribution of the two drugs could explain the differential rates of excretion. Specifically, the fraction of rifapentine found in hepatic cellular organelles (particulate fraction) relative to cytosolic concentration was greater than observed with rifampin.

Repeat-dose studies

Sprague-Dawley rats given 3 successive oral 40 mg/kg rifapentine doses every 7 days demonstrated a decline in serum drug concentrations from 19μg/mL obtained 2 hours after the first dose to 13 μg/mL obtained 2 hours after the last dose. In addition, plasma drug levels obtained 7 days after dosing tended to increase, from 0.5 μg/mL after the first dose, to 0.7 μg/mL after the last dose.

In a three month oral dosing study in Sprague-Dawley rats (Project Report V-88-23, which was part of a 3 month repeat-dose toxicology study, Project Report V-86-55), the following parameters were determined for rifapentine.

Dose mg/kg/day	Study day*	C_{max} (μg/mL) males	C_{max} (μg/mL) females	C_{min} (μg/mL) male	C_{min} (μg/mL) female
10	6	14	20	9	12
	92	37	36	24	24
40	6	45	60	38	39
	92	86	110	65	86
80	6	100	130	61	140
		250	360	180	200
20 (every 3 days)	10	17	19	3	3
	91	28	38	9	10
150 (every 3 days)	11	65	94	12	18
	91	65	100	27	32

*The first day represents steady-state, the last the end of the study.
C_{min} samples taken 3 hours after dosing.
C_{min} samples taken 24 hours after dosing (daily treatment), 72 hours after dosing (for the 20 mg/kg every 3 day group), or 120 hours after dosing (for the 150 mg/kg every 5 day group.)

There was clear evidence of accumulation in this study. In addition, accumulation was more extensive in females.

In a one year nonclinical toxicology study (Project Report IT-86-144), Sprague-Dawley rats given daily oral doses of 10, 20, or 40 mg/kg/day demonstrated no significant evidence of accumulation. The following blood concentrations of rifapentine were as follows:

C_{min} µg/mL males	C_{min} µg/mL females	C_{48} µg/mL males	C_{48} µg/mL males	C_{96} µg/mL males	C_{96} µg/mL females
23	17	-	-	-	-
21	23	-	-	-	-
26	24	21	13	6.5	5
48	50	-	-	-	-
32	28	-	-	-	-
23	49	30	31	20	26
82	96	-	-	-	-
40	41	-	-	-	-
37	58	48	42	24	34

*Values reported as µg/mL; samples were obtained 24 hours after the preceding daily doses. tSamples obtained 48 hours after the last dose. Samples obtained 96 hours after the last dose.

Although the results of the 3 month and 12 month rifapentine blood concentration studies appear to be incongruous, comparison of the C_{MIN} values obtained at 13 weeks (91 days) for the 10 and 40 mg/kg/day groups indicate otherwise. The following values were obtained:

Dose (mg/kg/day)	Study	[Priftin] µg/mL (Males)	[Priftin] µg/mL (females)
10	3 month	24	24
	1 year	23	17
40	3 month	65	86
	1 year	82	96

Except for the values observed in males in the 40 mg/kg/day group, the concentrations appear to be roughly comparable.

In rats after 13 weeks, C_{MIN} values either stabilized or, in the high-dose (40 mg/kg/day) group, began to decrease. This suggests that although bioaccumulation appears to be an important phenomenon in subchronic exposure, induced clearance (possibly due to increased metabolism) becomes important in chronic exposure.

Cynomolgus monkeys given oral doses of either 20 mg/kg every other day or 40 mg/kg every third day, both for a month, demonstrated rapid declines in trough serum concentrations during the course of the study. Blood concentrations of rifapentine at 24 hours after initial dosing were 16 and 25 µg/mL for the 20 and 40 mg/kg dose groups, respectively. C_{MIN} values obtained at the end of the study were 3 and 7 µg/mL for the respective 20 and 40 mg/kg groups.

Determination of rifapentine blood levels in a 3 month oral repeat-dose toxicology study in cynomolgus monkeys (Project Report V-86-86) demonstrated the following values:

Dose (mg/kg)*	Study day	C_{max} (µg/mL)	C_{min} (µg/mL)
10	1	10	-
	4	9	3.6
	92	5	1.4
20	1	17	-
	4	15	7.0
	92	8	3.7
80	1	54	-
	4	62	55
	92	42	26
40	1	34	-
	12	28	~1.7
	92	16	~0.5

C_{max} measured 6 hours after dosing.
C_{min} measured prior to dosing or before sacrifice.
* animals dosed every 3 days

There were no significant sex differences in blood drug levels. The sponsor suggested that the lack of dose-proportionality noted in the 80 mg/kg/day group was due to saturation of elimination mechanisms.

Comparison of the 3 month studies in rats and monkeys indicates that accumulation occurs more extensively in the rat. The only directly comparable dose group is the 40 mg/kg/day group in the rat study, which, based on relative body surface areas, is equivalent to the 20 mg/kg/day group in the monkey study. Mean C_{min} values obtained at the end of the studies were 75.5 µg/mL (rats) and 3.7µg/mL (monkeys).

In a one year repeat-dose toxicology study (Project Report IT-86122), cynomolgus monkeys were given oral rifapentine doses of 20, 40, and 80 mg/kg/day. The following blood levels were observed:

Dose	Study day	C_{max} (µg/mL)	C_{min} (µg/mL)
20	1	2.6	12
	87	1.5	3.6
	183	3.6	5.4
	364	4.4	5.2
40	1	23	32
	87	2.1	5.5
	183	2.2	9.0
	364	6.5	7.7
80	1	67	54
	87	12	11
	183	17	18
	364	22	21

Plasma concentrations of rifapentine were consistently higher in females in the 20 and 40 mg/kg/day groups. There was clear evidence of increased elimination in the mid- and high-dose groups, probably by induction of metabolism. No drug or metabolites were detected 96 hours after the final doses.

Comments: Several factors complicate interpretation of the nonclinical pharmacokinetic studies that were reported by the sponsor. Many of the studies are old In the pivotal repeat-dose studies, the microbiological assay (with *Sarcina lutea* as the indicator organism) was used. As reported by the sponsor, this assay is sensitive to both rifapentine and its presumed principal metabolite, 25-desacetyl rifapentine. However, since HPLC analysis was not used for most of the pharmacokinetic studies, the presence of unknown metabolites inactive in the microbiological assay cannot be ruled out. The sponsor has not addressed the issue of differential metabolism in any systematic fashion. Certainly, the rabbit appears to be unique in its relatively rapid elimination of parent drug and 25-desacetyl metabolite. Evidence in long-term studies indicates that accumulation could occur in the rat but not extensively in monkeys. Induced metabolism appears to occur with chronic exposure in both rats and monkeys. In fact, counterbalancing accumulation and metabolic induction with increased clearance would be consistent with observed pharmacokinetic parameters in rats and monkeys. Although there was speculation that enterohepatic recirculation (which is a characteristic of rifampin-type drugs) could in part account for accumulation, no studies were performed to specifically evaluate this possibility.

PHARMACODYNAMIC SAFETY STUDIES

Activities of DL 473-IT on cardiovascular and central nervous systems Study V-86-87

Oral administration of 50 or 100 mg/kg rifapentine for 3 consecutive days to spontaneously hypertensive rats did not affect systolic blood pressure. Oral doses of 10 or 50 mg/kg given to normotensive conscious mongrel male dogs produced only slight and transient increases in heart rate and no effects on blood pressure. Male beagle dogs given i.p. doses of 10 mg/kg rifapentine (0.54 times the human dose, based on body surface area comparisons) demonstrated no effects of treatment on blood pressure or heart rate inhibited by vagal stimulation or carotid occlusion. Vascular responses to concurrent adminstration of either norepinephrine, acetylcholine, isopropylnorepinephrine, histamine, or angiotensin were similarly unaffected by 10 mg/kg given i.p.

Mice given i.p. doses of 6 mg/kg demonstrated no significant effects. Mice given i.p. doses of 10, 30, 60, or 100 mg/kg (0.8 times the human dose) demonstrated slight decreases in spontaneous activity and muscle tone, slight motor incoordination, mild tremors, and slight mydriasis at the highest of these doses. Doses of 300 and 600 mg/kg i.p.(2.5 to 5 times the human dose) produced marked decreases in spontaneous activity and muscle tone, marked motor incoordination, mild mydriasis and exophthalmos, and 3/3 mice in the high-dose group died within 48 hours of treatment. Most of these effects were observed within 15 to 20 minutes of treatment, were maximal at 60 to 120 minutes, and (at doses of 100 mg/kg) persisted up to 48 hours after dosing.

Adminstration of rifapentine, 75 mg/kg i.p., (0.6 times the human dose) to male CF-1 mice had no protective effect on pentylenetetrazole-induced convulsive death. Rifapentine (25 mg/kg i.p., 0.4 times the human dose) had no effect on conditioned behavior in the rat.

Toxicology Studies Summary

Acute studies

1. Oral median lethal dose (LD_{50}) determination of DL-473-IT in mice .
2. Acute toxicity studies with DL 181-IT, DL 507-IT, DL 777-IT, DL 809-IT, DL 899-IT, DL 473-IT, and Rifudin in CD1 mice.
3. Acute toxicity tests with DL 473-IT (AF/ciclopentil).
4. DL473-IT-Batch VIII (-B/7/79). Oral acute toxicity study in mice.
5. Acute toxicity tests with batches VI and VII of DL 473IT differently prepared to improve the bioavailability.
6. Acute toxicity studies with DL 473-IT in mice. The influence of solubility of different batches on the toxicological response.
7. Bioavailability of DL 473-IT.
8. Oral median lethal dose (LD50) of DL-473-IT in rats.
9. DL 473-IT-Batch V and VIII. Oral acute toxicity studies in rats.
10. Acute toxicity in the guinea pig by the oral route.
11. Acute toxicity studies with DL 473-IT in beagle dogs.

Repeat dose studies

Mouse studies

1. MDL 473: Three-week dietary tolerance study in mice.
2. MDL 473 Three month oral toxicity study in mice.

Rat Studies

1. MDL 473: Four week dietary tolerance study in rats.
2. 31-Day oral toxicity study and blood level evaluation in rats treated with DL 473-IT (AF/Cyclopentil).
3. Three-month oral toxicity study with DL 473-IT (Rifamycin AF/Cyclopentyl) in the rat.
4. Three-month oral toxicity study - rats (Study V-86-71).
5. Serum levels of DL 473-IT in rats treated orally for 3 months.
6. MDL 473: One year oral toxicity study in Sprague-Dawley rats.

Monkey Studies

1. DL 473-IT: Blood levels and toxicity during repeated oral administration to cynomolgus monkeys for four weeks.
2. 13-week oral toxicity study in cynomolgus monkeys with DL 473-IT (Rifamycin AF/Cyclopentyl) and relative blood levels.
3. Serum levels of DL 473-IT in cynomolgus monkeys treated orally for 3 months.
4. MDL 473: A twelve month oral toxicity study in cynomolgus monkeys.

Reproductive Toxicology Studies

1. Results of reproduction and postnatal studies with MDL 473-IT in the rat.
2. Fetal toxicity study in the rat treated with MDL 473 administered by the oral route.
3. Preliminary fetal toxicity study in the rat with MDL 473 administered by the oral route.
4. Fetal toxicity and perinatal studies in rabbit with DL 473-IT (Rifamycin AF/cyclopentyl) administered by oral route.

Mutagenicity Studies

1. Study of the mutagenicity of DL 473-IT with Salmonella typhimurium (Ames test).
2. Evaluation of rifapentine (MDL 473, DL 473-IT) for potential mutagenicity with the Ames test*.
3. Report on the mutagenicity experiment performed on the substance DL 473-IT of the Firm Lepetit S.p.A.-Milan.

Immunogenicity Studies

1. Immunogenicity of rifamycins.
2. Effect of DL 473-IT (AF/cyclopentil) on immunological responsiveness.
3. Effect of rifampin and its cyclopentyl derivative, MDL 473, on the expression of delayed type hypersensitivity in the BALB/c mouse.
4. Comparative effect of the naphthalenic ansamycins rifamycin SV, rifampin and cyclopentylrifampicin on murine neutrophil function.

Toxicity Studies Review

Acute Studies

1. Oral median lethal dose (LD_{50}) determination of DL-473-IT in mice . . Project Report I-82-0006-T . IT-88-510 and NBX-488, . April 1981. Drug lot # DX-2636.GLP study.

This study was designed to determine the LD_{50} of rifapentine when administered to mice by oral gavage. The first study performed was a pilot study,(groups of mice, 5/sex/dose were treated with rifapentine at doses between 1 and 10 g/kg, results not shown) from which doses were selected for the definitive LD_{50} study. In the definitive LD_{50} study, CRI:CD-1(ICR)BR mice (10/sex/dose) were given single oral doses of DL-473-IT (4.0, 8.0, 9.24, or 12.32 g/kg) by oral gavage and observed for 14 days. The following LD_{50} values were determined at 7 and 14 days after treatment (given as g/kg and 95W confidence limits):

	Males	Females
7 days	5.3	7.0
14 days	5.2	6.1

Adverse effects observed included severe depression, ataxia, head wobble, dyspnea, rough coat, and mortality in all dose groups. Red fecal pellets and light orange ears, tails, and urine were due to the test material color. Decreased body weight gains were observed in males at ≥ 8.0 mg/kg. Clinical signs and mortalities were generally observed beginning the day after treatment. All mortalities were observed within 9 days of treatment. Necropsy revealed bright red coloration of the lungs, gastrointestinal contents, and slight orange coloration of the viscera, effects probably due to the color of the drug. In two high-dose group, males that died on study, increased liver weights, prominent lobulation of the liver, and pale livers were observed on necropsy.

2. Acute toxicity studies with DL 181-IT, DL 507-IT, DL 777-IT, DL 809-IT, DL 899-IT, DL 473-IT, and Rifudin in CD1 mice. Study G-80-0018-T Study V-84-48)

Groups of CD1 and CF1 mice (number of mice not reported) were treated with single oral doses (1, 2, 3, 4, 6 g/kg) of a number of experimental compounds including DL-473-IT. These results were reported as part of a study comparing two strains of mice for determining LD_{50} values with different

experimental compounds. No statistically significant differences in lethal doses for DL 473-IT were observed between the strains of mice. The LD_{50} values in CD-1 and CF1 mice were 4310 and 2330 mg/kg , respectively.

3. Acute toxicity tests with DL 473-IT (AF/ciclopentil) Report G-76-0019-T . Study V-89-20, 1976)

Groups of CF1 mice and Sprague-Dawley rats; (between 4 and 8 animals /sex/dose) were treated with single oral or intraperitoneal doses of drug. Doses used were 2, 3, 4, 5 g/kg (mice, oral); 600, 700, 800, 900 mg/kg (mice, i.p.); 1, 2, 4 g/kg (rats, oral); 500, 600, 800 mg/kg (rats, i.p.); no controls

The following LD_{50} values were determined (given as mg/kg):

Route	Mice	Rats
Oral	3210	2350
Intraperitoneal	710	585

Mice given DL 473-IT by i.p. injection demonstrated reduced spontaneous activity, reduced palpebral fissures, tail rigidity, and increased reactivity. The day following treatment, the mice demonstrated mild sedation, tremors, ataxia, lacrimation, and slight encrustations around the eyes. Surviving animals returned to normal by day 5 or 6. Following oral administration, mice demonstrated mild sedation and slight encrustations around the eyes. Surviving animals were normal by day 4 or 5.

Rats given DL 473-IT by i.p. injection demonstrated sedation, ataxia, and hair bristling. The day following treatment, rats were sedated and demonstrated encrustations around the eyes, hair bristling, and ataxia. Surviving rats were normal by day 4 or 5. The only prominent effect observed after oral administration was sedation, which persisted for 6 to 7 days.

For mice, most deaths occurred between days 1 and 3, regardless of route of administration. In rats given drug i.p., deaths occurred within 16 hours of treatment. Following oral administration, deaths in rats occurred throughout the 7 day observation period. The most prominent effects observed at necropsy were liver and intestinal adhesions in mice given drug i.p. No other significant effects were seen.

4. DL473-IT-Batch VIII (-B/7/79). Oral acute toxicity study in mice. Study G-76-0019-T, Study V-86-69.

This study was conducted to determine the LD_{50} of a batch of rifapentine drug product prepared by dissolving "batch VII" in methanol and precipitating in water. Batch VII had demonstrated poor intestinal absorption in previous acute toxicity studies. Groups of mice (CF1 mice, 8/sex/dose) were treated with single oral doses of DL473-IT, (1, 2, 3, 4, 6 g/kg). The LD_{50} (2000 mg/kg) did not differ significantly from values obtained with previous drug batches. The LD_{50} of batch V (which has been used in a number of the animal studies performed to date) was 2330 mg/kg.

5. Acute toxicity tests with batches VI and VII of DL 473IT differently prepared to improve the bioavailability. Report G-79-0015-T (V-84-148)

This study was conducted to determine the differential acute toxicities of four batches of drug product prepared by different methods. Groups of mice (strain CF1, 8 animals/sex/dose) received single oral doses of DL 473IT at 2, 3, 4, 6, 8, 12, 16, 18 g/kg.

Table 1. LD50 values for different batches of rifapentine

Rifapentine Batch	LD50 (mg/kg)
Batch VI ground twice in	> 18,000
Batch VII dissolved and crystallized at T <50 C	10, 000
Batch VII dissolved and crystallized at T >50 C	>18,000
Batch VII dissolved and precipitated in water	2,940

Only batch VII, prepared by dissolving in methanol and precipitating in water, produced LD_{50} values comparable to previous studies. Apparently, the other batches in this study had poor oral bioavailabilities, resulting in much higher LD_{50} values.

6. Acute toxicity studies with DL 473-IT in mice. The influence of solubility of different batches on the toxicological response. Report G-79-0014 (V-84-147)

lot # A/4/78, B/11/78 (= VI), B/11/78 (= VII); April 1979. B/11/78

Groups of mice (CF1 mice, 4/sex/dose) received single oral or intravenous doses of DL 473-IT. Doses were 1, 1.5, 1.75, 2 g/kg (oral); 125, 150, 200 mg/kg (iv.). Vehicle controls for the intravenous group received distilled water with 14% 0.1 N NaOH, pH 11.9, while controls for the oral group received distilled water with 23.3% 0.1 N NaOH, pH 12.1.

The acute toxicity of DL 473-IT was demonstrated to be dependent on the solubility of variously prepared batches of drug product in alkaline solution.

7. Bioavailability of DL 473-IT - mice. Report G-79-0016-D

This study compared the LD_{50} values obtained in mice with various drug batches in order to determine the relationship between formulation and bioavailability. The solubility of DL 473-IT was inversely proportional to its crystallinity. The amorphous form of DL 473-IT, based on the relative magnitude and reproducibility of LD_{50} values, appeared to have the greatest oral bioavailability.

Drug Batch	LD50
Laboratory scale (0.18 kg)	3300
Pilot Plant scale (2.5 kg)	2330
Pilot plant scale (17.1 kg)	15800
Ground in mortar	3200
Ground in	>12,000
Dissolved in methanol and precipitated in water	2940

8. Oral median lethal dose determination of DL-473 in rats. Chemical Study IT-88-511/ I 82-0007-T. Drug lot # DX-2670. July 1981 GLP study.

Sprague-Dawley rats; (5/sex/dose,probe study ,10/sex/dose, LD_{50} study) received single oral doses of drug by gavage.; doses ranged from 2000 to 8000 mg/kg in the probe study and in the LD_{50} study doses were: 1259, 1995, 2512, 3162, 3981, 6310 mg/kg (males); 631, 1000, 1259, 1585, 1995, 3162, 5012, 6310 mg/kg (females).

Oral median lethal dose (LD_{50}) value for males was 2473 mg/kg and for females was 2086 mg/kg. Most mortalities occurred between 24 and 48 hours post-dose, with all occurring by day 6. Clinical signs included depression, rough coats, and red coloration of extremities and fecal pellets. Adverse effects were absent at day 12. No consistent treatment-related effects were observed when animals were necropsied 14 days after dosing.

9. DL 473-IT-Batch V and VIII. Oral acute toxicity studies in rats. Report G-79 0019-T (Study V-86-74).

Groups of Sprague Dawley rats (CD, 10 rats /sex/dose) were treated with single oral doses of drug at 1350, 2000, 3000, 4500 mg/kg. The acute toxicities of two batches of drug were compared. The oral LD_{50} of batch VIII (1700 mg/kg) was significantly less than batch V (3100 mg/kg).

10. Acute toxicity by the oral route in guinea pigs. Report G-77-0012-T (Study V-89-19)

Groups of guinea pigs (4/sex/dose) were administered single oral doses of drug (250, 500, 1000, 2000, 3000, 4000 mg/kg). Equivalent doses of rifampicin used as comparative controls.

At 14 days, the LD_{50} for AF/cyclopentyl was 378 mg/kg, compared to the LD_{50} for rifampicin of 590 mg/kg .

11. Acute toxicity studies - beagle dogs. Report G-79-0025-T (Study V-89-18)

Groups of beagle dogs (1 animal/sex/dose, except for the 1 g/kg oral dose in which only one male was used) were treated with rifapentine as single oral doses (1, 1.5, 2, 2.5, or 3 g/kg , or single i.p. doses (200, 400, 600, or 800 mg/kg , suspended in 0.5% Methocel HG 90).

No mortalities occurred in the oral treatment group. Signs observed between 2 and 5 days after dosing included sedation, ataxia, dyspnea, salivation, vomiting, and reddish coloration of the mucosa, skin, and feces. Dogs in the 3 g/kg group demonstrated prostration and convulsive seizures. Losses of appetite and body weight were observed in the higher dose groups, but these effects did not persist after about 4 days. Necropsy demonstrated no significant effects.

Both dogs given 800 mg/kg i.p. died on day 3. Signs observed in the 600 and 800 mg/kg groups included prostration, dyspnea, salivation, convulsive seizures, diffuse clonus, vomiting, anorexia, and body weight loss. Dogs in lower dose groups demonstrated sedation, ataxia, muscle tremors, evidence of abdominal pain, and dysorexia. Signs of toxicity were seen in the 600 mg/kg group for up to 43 days after treatment; signs in the lower dose groups persisted for 5 - 11 days. Necropsy demonstrated thickening of the peritoneum, visceral adhesions, ascites, increased hepatic volume, and reddish coloration of various tissues.

Multiple dose studies Mouse Studies

12. Three-week dietary tolerance study - mice

1. Project Report I-85-0021-T (Study IT-85-96)

Drug lot # DX-2636. May, 1981. GLP study.

Groups of mice, (CRL:CD-1 (ICR) BR mice, 3/sex/dose) were treated with rifapentine by dietary admixture for 3 weeks at 10, 20, 40, 80, 160, 320, 640, or 1280 mg/kg/day. Controls received diet mixture without drug.

Mortalities occurred on study in both the 640 (2/3 males) and 1280 (3/3 males and 2/3 females) mg/kg/day groups. Necropsy of these animals demonstrated hepatocellular hypertrophy and vacuolation, myocardial inflammation, decreased spleen cellularity, dilated lymphatics in the small intestines and mesenteric lymph nodes, lymphoid cell depletion, and lung hemorrhage.

Clinical signs observed on study included reddened ears and extremities due to the color of the test material (all groups), rough hair coats (≥ 320 mg/kg/day groups); and severely decreased activity (≥ 640 mg/kg/day groups). Decreased body weight gains were observed in males (≥ 320 mg/kg/day groups) and females in the 1280 mg/kg/day group. Food consumption was decreased in the 640 and 1280 mg/kg/day groups (although this effect was statistically significant only in male groups at sporadic time points.)

Necropsy of animals surviving the study demonstrated dose-related increases in frequency and/or severity of hepatocellular hypertrophy (≥ 20 mg/kg/day groups), coloration of various tissues (≥ 40 mg/kg/day groups), "accentuated lobular liver patterns" in males (≥ 40 mg/kg/day groups) and females (≥ 80 mg/kg/day groups), decreased body fat and/or "loss of body condition" (≥ 80 mg/kg/day groups), hepatocellular vacuolation (≥ 80 mg/kg/day groups), increased relative liver weights (males, ≥ 80 mg/kg/day groups; females, ≥ 160 mg/kg/day groups), increased absolute liver weights (≥ 160 mg/kg/day groups), and dilation of small intestinal lacteals and mesenteric lymph node lymphatics (males, ≥ 160 mg/kg/day groups; females, ≥ 320 mg/kg/day groups).

Serum concentrations of MDL 473, determined after 21 days of exposure, were increased in a dose-related manner in the 10 to 80 mg/kg/day groups. In dose groups ≥ 160 mg/kg/day, serum drug concentrations increased in an inconsistent manner.

The NOAEL in this study was 10 mg/kg/day, which converts to a human dose of about 0.8 mg/kg/day (based on relative body surface areas). Although the inconsistent serum drug levels seen in high dose groups could in part be explained by decreases in food consumption, other factors, such as saturation of absorption or enzyme induction, could also partially contribute to the observed effect.

15. MDL 743: Three month oral toxicity study in mice. Report K-96 0704-T 1996. Study # 96.0509 Hoechst AG, HMR Preclinical Development Germany. Drug lot # 77736 December 1995. GLP study.

This study was Groups of NMRI mice (15/sex/dose with 30/sex/dose for blood drug level determinations) received daily oral (gavage) doses of rifapentine (10, 20, 40, 80, or 160 mg/kg/day) for 92 consecutive days, dose volume: 10 ml/kg; vehicle controls (0.5% aqueous methylcellulose).

One male animal in the control group was found dead on study day 12: the cause of dead was not determined. No other deaths occurred on study. There were no significant effects of treatment on body weights, body weight gains, or food consumption. All animals in the 80 and 160 mg/kg/day group demonstrated orange discoloration of the hairless areas, beginning on study days 31 and 10, respectively. This discoloration was presumed by the sponsor to be due to the test article. No other clinical signs were observed on study. No ocular effects were demonstrated.

Clinical pathology determinations demonstrated no significant effects of treatment on hematology or coagulation parameters. Dose-dependent increases in serum bilirubin were observed (males ≥ 20 mg/kg/day groups; females ≥ 80 mg/kg/day groups). Serum alkaline phosphatase levels were significantly increased in high-dose group males, with mean value ~ 1.7-fold greater than concurrent vehicle controls at the end of the study. Observed decreases in serum creatinine (80 and 160 mg/kg/day groups) did not correlate with other effects and this change does not appear to be significant.

Necropsies, performed the day after administration of the final doses, demonstrated orange discoloration of the pancreas and liver in the higher dose groups (≥ 40 mg/kg/day). In addition, accentuated lobular patterning of the liver was observed in the 80 and 160 mg/kg/day groups (especially in males). Statistically significant increases in group mean relative liver weights were seen in the 80 and 160 mg/kg/day groups. Relative spleen weights were also increased (≥ 40 mg/kg/day), although no histologic effects were observed. Group mean relative spleen and kidney weights were also observed in females in the low-dose group and males in the 20 mg/kg/day group, respectively. Histopathology demonstrated centrilobular hepatocyte fatty changes in males (≥ 20 mg/kg/day) and in females (≥ 80 mg/kg/day). Based on this change, the sponsor determined the maximum tolerated dose to be 40 mg/kg/day and the NOAEL to be 20 mg/kg/day.

Potential effects of drug on drug metabolizing enzyme activities were determined by obtaining liver tissue from mice sacrificed during study weeks four and 12 (pharmacokinetics satellite group animals). Slight to marked dose-dependent increases in virtually all enzyme activities were observed. The most significant increases were observed in glucuronyltransferase I (GT 1), pentylresorufin *O*-dependentylase (PROD), ethylresorufin *O*-deethylase (EROD), ketamine *N*-demethylase (KAND), and glutathione *S*-transferase (GST), although the overall increase in enzyme activity did not appear to be consistent with rifapentine being a strong metabolism-inducing agent.

Blood drug level concentration determinations demonstrated the following pharmacokinetics parameters:

Dose (mg/kg)	Day	C_{MAX} (µg/ml)		$AUC_{(0624)}$ (µg.hr/ml)	
		Males	Females	Males	Females
10	1	10	13	218	227
	30	39	24	607	412
	87	38	28	728	439
20	1	19	21	339	379
	30	55	36	949	559
	87	61	42	1150	727
40	1	32	38	638	782
	30	99	99	1980	1520
	87	128	90	2320	1300
80	1	51	61	1020	1180
	30	180	136	3500	2790
	87	154	143	3420	2550
160	1	82	121	1690	2100
	30	218	181	3880	3880
	87	189	169	3000	3450

There was clear evidence of accumulation at all doses: C_{MAX} and *AUC* values were 2- to 4-fold greater on days 30 and 87 compared to day 1 for all doses. Although systemic exposure increases were not apparent between study days 30 and 87 in the 80 and 160 mg/kg/day groups, such increases appeared to continue over the same interval in the lower dose groups.

According to the sponsor, the most likely maximum dosage of rifapentine for clinical use would be 600 mg twice a week for two months, followed by 600 mg once a week for four months. In a 60 kg human, these doses would be 10 mg/kg twice a week (or - 2.9 mg/kg/day over the treatment period) or 10 mg/kg/week (or 1.4 mg/kg/day over the treatment period). Following single 600 mg doses of rifapentine given to humans, the average observed C_{MAX} was 24 µg/ml and the *AUC* was 367 :µg*hr/mL. In mice, the respective values observed following three months exposure to 40 mg/kg/day (equivalent, based on comparison of body surface areas, to a human dose of - 3.3 mg/kg/day) were 90 - 128 :µg/ml and 1300 - 2320 :µg*hr/ml, respectively. Thus, at 40 mg/kg/day (reported by the sponsor to be the MTD), systemic exposure in mice was 3.5- to 6.3-fold greater than observed in humans at the anticipated therapeutic dose.

The sponsor is conducting a two year carcinogenicity bioassay in NMRI mice. The sponsor has proposed a standard study (two control groups, 50/sex/dose with an extra 15/sex/dose for Toxicokinetics determinations, daily oral gavage dosing). The proposed doses are: 4, 12, and 36 mg/kg/day. The high-dose was chosen based on the observation of centrilobular hepatocellular fatty changes in male mice given daily oral doses of 40 mg/kg/day in a three month oral toxicity study. This effect was claimed by the sponsor to delimit the MTD. Fatty liver was also seen in female mice, although at a higher dose. This effect was not associated with decreases in body weight gains or survival. However, systemic exposure at 40 mg/kg/day was significantly higher than observed with therapeutic doses in humans. The mid-dose was chosen based on the observation that systemic exposure achieved at this dose was equivalent to human exposure at therapeutic doses. The low dose was chosen in order to establish a NOAEL if tumors are observed at higher doses.

The study is acceptable. Although the adverse effect used to delimit the MTD was not associated with changes in parameters normally used, this nevertheless appears to be reasonable. Evidence from nonclinical studies previously conducted by the sponsor suggests that rodents may be uniquely susceptible to rifapentine, possibly due to decreased capacity to clear the drug after chronic exposure. The fatty changes observed in male mice in the three month range-finding study in fact should be taken to presage more serious toxicity in longer-term studies.

Multiple dose rat studies

13. Four week dietary tolerance study - rats Report I-85-0020-R (Study IT-85-59)

lot #

DX-2670. July 1981. GLP study.

Groups of Sprague-Dawley rats (3/sex/dose) were treated with rifapentine by dietary admixture for 4 weeks; at 2.5, 5, 10, 20, 40, 80, 160, 320 mg/kg/day. Controls received diet mixture without drug.

Mortalities occurred in 3/3 males in the 320 mg/kg/day group and in 1/3 females in both the 160 and 320 mg/kg/day groups. Necropsy of animals that died on study demonstrated yellowish discoloration of the skin and viscera, hepatocellular vacuolation, hemorrhages in the brain, heart, testes, bone marrow, and adrenals, decreased spermatogenesis and/or testicular degeneration, decreased spleen and bone marrow cellularity, lymphoid cell depletion, decreased thyroid colloid, and skeletal muscle mineralization and inflammation.

Clinical signs observed on study included decreased spontaneous activity, rough hair coats, ataxia, and marked decreases in body weight gains and food consumption (both sexes, ≥ 80 mg/kg/day groups). Necropsy demonstrated yellowish skin discoloration, probably due to the drug color, in females (≥ 10 mg/kg/day groups) and males (≥ 20 mg/kg/day groups), hepatocellular vacuolation (males, ≥ 40 mg/kg/day

groups; females, ≥ 80 mg/kg/day groups), yellowish livers and increased relative liver weights (both sexes, ≥ 80 mg/kg/day groups), other liver changes (friable and pale livers with accentuated lobular patterns and increased size; males, ≥ 80 mg/kg/day; females, ≥ 160 mg/kg/day), dilated small intestinal lacteals (both sexes, ≥ 80 mg/kg/day groups), dilated lymphatics in mesenteric lymph nodes (males only, ≥ 80 mg/kg/day groups), prominent adipose tissue in bone marrow (females, ≥ 80 mg/kg/day groups; males, ≥ 160 mg/kg/day groups), and testicular degeneration and depletion of spermatozoa in the epididymides in one male in the 160 mg/kg/day group.

Serum drug levels were dose-proportional, ranging from a mean of 1.21 µg/ml in the 2.5 mg/kg/day group to a mean of 194.20 µg/ml in the 320 mg/kg/day group.

Table 3. Serum levels of rifapentine in rats after 28 days of treatment

Dose	Rifapentine levels (µg/ml) Males	Rifapentine levels (µg/ml) Females
0	0	0
2.5	1.3	1.2
5.0	5.5	4.2
10	14	10
20	29	28
40	42	63
80	139	145
160	177	175
320	---	194

Comments: According to the sponsor, the NOAEL in this study was 20 mg/kg/day for males and 40 mg/kg/day for females (equivalent, based on relative body surface areas, to human doses of about 3 mg/kg/day for males and about 6 mg/kg/day for females.) Thus, the converted human equivalent doses at which no adverse effects were seen are about the same for both the rat and mouse dietary admix studies. Unlike the mouse dietary admix study, serum drug levels were roughly dose-proportional throughout the dosing range.

14. 31-Day oral toxicity study and blood level evaluation in- rats treated with DL 473-IT(AF/cyclopentil) Report G-77-0011-T (Study V-86-201)

Groups of Sprague-Dawley rats; 10/sex/dose; were dosed with rifapentine by oral gavage either daily (20, 80 mg/kg/day)or once every 5 days (7 total doses total at 150, 300 mg/kg every 5 days), both for 31-32 days. Control animals received vehicle (5% methocel)

No mortalities occurred on study. Clinical signs observed on study included orange coloration of skin, backs of the eyes, and urine, a slight, inconsistent dose-related decrease in body weight gains, and moderately increased water consumption in females. Clinical pathology demonstrated a statistically significant, dose-related increase in serum total bilirubin concentrations. Bilirubin concentrations observed in the 80 mg/kg/day group were 5-fold greater in males and 18-fold greater in females compared to respective control values. Aspartate transaminase (AST) serum concentrations were increased inconsistently (with statistical significance only in the 20 mg/kg/day and 150 mg/kg/5 day groups.)

Necropsy demonstrated dose-dependent "brick-red" coloring of the tissues, sporadic and moderate gastric dilation and repletion (all except the 150 mg/kg/5 day group), reduced absolute kidney, liver, and ovary weights, dose-related hepatic and renal steatosis, and zonal rarefaction of the testicular seminiferous epithelium in 7/10 males in the 80 mg/kg/day group. Also observed were signs of subacute and chronic

interstitial nephritis, although this finding was inconsistent and considered by the sponsor to be of uncertain relationship to treatment. Signs of chronic pancreatitis were seen in one female in the 300 mg/kg/5 day group and renal cortical cysts were seen in 2 females in the 80 mg/kg/day group.

Blood drug level determinations demonstrated dose-dependent concentrations at 24 hours after dosing, persistently high blood levels for up to 5 days (in the 150 and 300 mg/kg/5 day groups), and evidence of bioaccumulation in animals receiving daily treatment. Evidence of accumulation was not seen in animals dosed every 5 days.

Table Blood levels of rifapentine in rats once-daily therapy.

	20 mg/kg (Male)	20 mg/kg (Female)	80 mg/kg Male	80 mg/kg Female
24h after 1st dose	11	13	55	62
24h after 7th dose	15	22	61	100
24h after 14th dose	16	22	61	89
24h after 31st dose	37	40	94	195

Table Blood levels of rifapentine (µg/ml) 5 days after treatment once every 5 days.

Time point	150 mg/kg Male	150 mg/kg Female	300 mg/kg Male	300 mg/kg Female
5 days after 1st dose	4.5	8.9	9.4	24
5 days after 2nd dose	4.2	8.9	12	28
5 days after 3rd dose	3.1	6.2	17	20
5 days after 4th dose	4.3	4.5	11	17
5 days after 5th dose	5.4	11	12	22
5 days after 5th dose	4.1	5.6	13	14

Comment: The finding in this study of dose-related decreases in liver weights is inconsistent with results obtained in dietary admixture studies.

15.Three-month oral toxicity study - rats (Study V-86-71)

Groups of CD (Sprague-Dawley) rats; (25/sex/dose) were treated with rifapentine by oral gavage for 13 weeks. Doses were 10, 40, 80 mg/kg/day, or 20 mg/kg every 3 days, or 150 mg/kg every 5 days. Control animals received vehicle (0.5% methocel[7])

Significant mortality rates were observed in the 80 mg/kg/day groups: 20/25 females and 5/25 males, with most deaths occurring in study weeks 2, 12, and 13. Rats in this dose group demonstrated significant loss of appetite, poor condition, decreased water consumption, and decreased body weight gains prior to death. Ophthalmic examinations at weeks 6 and 12 demonstrated orange discoloration of the fundus, an effect not observed in other dose groups. Signs of toxicity included increased serum liver enzymes (AST and alkaline phosphatase) and urea (BUN), and decreased total serum proteins. Necropsy of rats in the 80 mg/kg/day group that survived treatment demonstrated moderate to marked hepatic steatosis, hypertrophy and slightly cloudy swelling of the adrenal fasciculata epithelium, modest hyperplasia and hypertrophy of the splenic subcapsular histiocytes, dilation and filling of the stomach and hypotrophy of the gastric glands, dilation of the lacteal vessels and/or apparently empty cysts in the mesenteric lymph nodes, diffuse myocarditis, thymic atrophy, moderate amounts of erythrocytes in mesenteric lymph node sinuses, pancreatic acinar cell degeneration, and renal steatosis. Necropsy of rats that died on study demonstrated hepatic steatosis and steatonecrosis, renal steatosis, thymic atrophy, uterine or prostate and seminal vesicle atrophy, myocarditis, and congestion of the adrenal cortical sinusoids.

Dose-related effects seen in all treatment groups included bilirubinemia, hepatic steatosis, cytoplasmic vacuolization and/or signs of degeneration in pancreatic acinar cells, decreased body weight gains (males only), and decreased leukocytes (females only). Severity of hepatic steatosis observed after 13 weeks of treatment was greater than observed after 6 weeks. Serum ALP levels were increased in the 40 mg/kg/day and 150 mg/kg/5 day groups, but alterations in other serum parameters associated with hepatic damage were seen only in the 80 mg/kg/day group. Splenic subcapsular histiocytic hyperplasia and hypertrophy, an effect seen in the 80 mg/kg/day group, was also present in lesser severity in the 20 mg/kg/3 day and 150 mg/kg/5 day groups. Other effects, such as dilation and repletion of the stomach, dilation of the ileal and/or duodenal lacteals, myocarditis, renal steatosis, and increased BUN, seen in the 80 mg/kg/day group, were also occasionally seen in lower dose groups, but with both lower incidence and severity. Reductions in body weight gains, seen in males in all dose groups, also occurred in females in all but the 10 mg/kg/day group, but the effect was much less severe. Orange-red discoloration of skin, tissues, and urine, was observed in all but the 10 mg/kg/day and 20 mg/kg/3 day groups. Although there were decreases in absolute liver, kidney, and heart weights, these appeared to be related to decreased body weights. The sponsor did not report changes in relative organ weights. Most adverse effects were not seen in animals after a one month recovery period. Effects that persisted after the one month recovery period included decreased leukocytes in females in the 150 mg/kg/5 day group, decreased body weights relative to untreated controls in some of the higher dose group males, and discoloration of tissues in some of the higher dose groups.

Although the sponsor states that the NOAEL in this study was 10 mg/kg/day for 13 weeks, in fact a true NOAEL was not established. Males in both the 10 mg/kg/day and the 20 mg/kg/3 day groups demonstrated decreased body weight gains (- 7% less than controls), and slight to moderate hepatic steatosis and some pancreatic effects were seen in both sexes in the 10 mg/kg/day group.

Serum drug levels were determined as part of the 3 month oral toxicity study in rats. Rats were killed 3 hours after dosing and serum drug concentrations determined by microbiological assay (using *Sarcina lutea* as the indicator organism). Females had higher serum drug levels, longer serum half-lives, and lower elimination rate constants than males, for most dose groups. Serum concentrations appeared to increase in proportion to dose over the 10 to 80 mg/kg range. Saturation of absorption was seen in the 150 mg/kg group. Evidence of bioaccumulation was seen at the end of the 3 month treatment period.

Three month oral toxicity study. Study # 96.0766/11-5-96. Laboratory: Hoechst Aktiengesellschaft Frankfurt, Germany. Drug lot # 77736. GLP study.

Groups of Wistar rats; (10/sex/dose for the main study and with 10/sex/dose for toxicokinetic study) received daily doses of rifapentine (10, 20, 40, 80 mg/kg/day) by oral gavage for 91 consecutive days. Vehicle control animals received 0.5% aqueous hydroxyethylcellulose.

One male in the high-dose group died on study day 61: the cause of death was not established, but appeared to be due to drug toxicity. No other deaths occurred on study. Statistically significant dose-related decreases in body weight gains were observed in all male treatment groups. Body weight gains were also decreased in females, but the effect was statistically significant only in the 40 and 80 mg/kg/day groups. Mean body weights for males at necropsy were 86%, 89%, 96%, and 96% of concurrent controls in the 80, 40, 20, and 10 mg/kg/day groups, respectively, and in the respective female groups mean body weights were 87%, 92%, 98%, and 95% of concurrent controls. Marginal changes in food consumption were observed but did not appear to be related to body weight gain changes. Orange discoloration of the hairless areas was seen in animals in the 40 and 80 mg/kg/day groups beginning on study days 14 and 4, respectively. No ocular effects were demonstrated. Slight hematologic effects were observed, including slight decreases in thrombocytes in the high-dose group (both sexes) and slight (but dose-related and statistically significant) increases in reticulocytes and decreases in hemoglobin in females (all treatment groups). Clinical chemistry effects included dose-dependent increases in serum bilirubin (all treatment groups), alkaline phosphatase and urea (high-dose group, both sexes), and alanine transferase and aspartate transferase (high-dose group females). Necropsy demonstrated orange discoloration of the pancreas and

liver ($ 20 mg/kg/day) and yellow or orange discoloration of the eyes and testes ($ 40 mg/kg/day). Dose-dependent increases in absolute and relative thyroid weights were observed in all treated females and the effect was correlated with histologic demonstration of thyroid follicular colloid storage. Slightly increased relative thyroid weights were also seen in high-dose group males, also associated with colloid storage. Relative liver weights were increased in the high-dose group, with dose-dependent fatty changes seen in all dose groups. Other organ weight changes appeared to be related to decreased relative body weights. Other histopathologic effects observed in high-dose group animals included villus stroma lymphangiectasia of the duodenum and jejunum and lymphocytic infiltration of the Harderian gland.

Assay of liver samples from rats sacrificed at 12 weeks demonstrated slightly increased activities of glucuronyltransferase I and II (10 mg/kg/day groups), ethylresorufin *O*-deethylase (40 mg/kg/day groups), and pentylresorufin *O*-dependentylase (80 mg/kg/day group). Rifapentine does not appear to be a potent drug-metabolizing enzyme inducer in rats based on these analyses.

The following pharmacokinetic parameters were demonstrated:

Dose (mg/kg/day)	Day	C_{MAX} (µg/ml) (Males)	C_{MAX} (µg/ml) Females	$AUC_{(0-24)}$ (µg*h/ml) (Males)	$AUC_{(0-24)}$ (µg*h/ml) (Females)
10	1	6	8	127	149
	29	18	18	342	355
	86	25	28	530	487
20	1	12	14	245	276
	29	34	36	693	657
	86	46	46	1030	928
40	1	21	24	399	445
	29	46	48	982	1060
	86	58	67	1140	1470
80	1	33	34	619	708
	29	56	91	1010	1460
	86	69	94	1520	1880

Based on comparison of relative body surface areas, the doses used in this study, 10, 20, 40, and 80 mg/kg/day, are equivalent to human doses of approximately 1.6, 3.2, 6.3, and 12.7 mg/kg/day. According to the sponsor, the therapeutic dose will be 600 mg given twice a week for two months, followed by once a week dosing for four months. Calculated on a daily basis, the therapeutic doses are 2.8 mg/kg/day for two months, followed by 1.4 mg/kg/day for four months. The *AUC* observed in humans following a single 600 mg dose was 367 µg*h/ml roughly equivalent to the systemic exposure observed in rats in the 10 mg/kg/day group on study day 29.

17. One year oral toxicity study - rats. Report # I 86 0010 (Study IT-86-144)

drug lot # D-2980;October 1984. GLP study.

Groups of Sprague-Dawley rats (30/sex/dose) were treated with rifapentine via daily oral gavage doses for 1 year. Doses were 10, 20, or 40 mg/kg/day; and vehicle control animals received 0.5% methocel.

Five deaths occurred on study: 4 controls due to either injury or undetermined causes, and one male in the 40 mg/kg/day group (which died on study day 261.) The sponsor did not determine the cause of death in this high-dose animal, but stated the death did not appear to be treatment-related. Signs consistent with sialodacryoadenitis were seen in 7 rats, but this finding was not dose-related and probably not associated with treatment.

Dose-related decreases in body weight gains were seen in all treatment groups. Statistically significant decreases were seen in males in the 40, 20, and 10 mg/kg/day groups, beginning, respectively, on study weeks 12, 22, and 38. Body weight decreases in females in the 40 and 20 mg/kg/day groups were statistically significant beginning, respectively, on study weeks 4 and 8. At the end of 12 months treatment, the respective decreases in body weight gains (compared to controls) in male treatment groups 40, 20, and 10 mg/kg/day were 12%, 10%, and 6%. For females in the 40 and 20 mg/kg/day treatment groups, the respective decreases at 12 months were 19% and 9%. No statistically significant effect of treatment on body weight gain was seen in females in the 10 mg/kg/day group. Body weight gains tended to normalize in rats kept for an 8 week recovery period. There was no consistent effect of treatment on either food or water consumption.

There were statistically significant dose-related effects on hematologic parameters: decreases in mean corpuscular hemoglobin concentration (MCHC) were seen in both sexes at doses ≥ 20 mg/kg/day, decreases in mean corpuscular hemoglobin (MCH) were seen in both sexes in the 40 mg/kg/day group, and decreases in hemoglobin concentration (Hgb) were seen in males in the 40 mg/kg/day group. These effects tended to reverse in the recovery group animals.

Bilirubinemia was consistently observed in all treatment groups: this effect was statistically significant in females in all treatment groups, but in males only in the 10 mg/kg/day group. The effect was reversed in recovery group animals. Serum cholesterol levels were decreased in both males (≥ 20 mg/kg/day groups) and females (40 mg/kg/day group). The average decreases in serum cholesterol levels in both sexes in the 40 mg/kg/day group (60%) was statistically significant, but the effect in the 20 mg/kg/day group was significant only in males (38%). After the 8 week recovery period, the effect was still significant in males in the 40 mg/kg/day group (with an observed decrease of 24%.) Serum ALP elevations (~ 1.5 × control values) were seen in males in the high dose group.

Relative organ weights were increased in all treatment groups, but it is not clear that this was not the result of decreased body weight gains. Statistically significant increases noted included: adrenals (females in the 10 and 20 mg/kg/day groups; males in the 40 mg/kg/day group), brains (females in the 20 and 40 mg/kg/day groups; males in all treatment groups), livers and hearts (females in the 20 and 40 mg/kg/day groups; males in the 40 mg/kg/day group), spleens (both sexes, 20 and 40 mg/kg/day groups), and pituitaries and uteri (females in the 40 mg/kg/day group). Increases in relative kidney weights demonstrated no apparent dose-relationship. Some of the increases in relative organ weights persisted in the recovery groups.

The most consistent effect observed at necrospy was yellowish discoloration of the skin (≥ 20 mg/kg/day groups). This effect tended to reverse on recovery, but was still evident in 2/6 females in the 40 mg/kg/day group. Other gross pathologic effects seen included focal or multifocal pale hepatic foci (males, ≥ 10 mg/kg/day groups) and sporadic enlargement of the spleens and/or adrenals. Dose-related intraocular yellowish discoloration was seen in recovery-group animals but was not observed in animals sacrificed immediately after cessation of dosing. No ocular abnormalities were noted accompanying the discoloration. One subcutaneous tumor was observed in a male in the 20 mg/kg/day group.

Histopathology demonstrated centrilobular hepatic steatosis, slight to severe, in males only (3/10 in the 20 mg/kg/day group and 6/10 in the 40 mg/kg/day group.) This effect was not observed in females and was not seen in recovery group animals. Reactive lymphoid hyperplasia, associated with spleen enlargement, was occasionally observed but probably was not treatment-related. Hepatocellular carcinoma was seen in the one male in the 20 mg/kg/day group which also had a subcutaneous tumor (fibrosarcoma) in the head. Other neoplastic lesions observed included a pituitary adenoma (male, 40 mg/kg/day group), an adenoma of the adrenal cortex (female, 10 mg/kg/day group) which was associated with cystic cortical degeneration, and a hyperplastic nodule (adenoma) of the liver (male, 40 mg/kg/day group).

Serum drug concentrations were dose-proportional after 13 weeks of treatment (mean values of 20.1, 48.9, and 89.1 µg/ml in the 10, 20, and 40 mg/kg/day groups, respectively.) The serum drug levels remained essentially unchanged in the 10 mg/kg/day group at 26 and 52 weeks, but concentrations tended to decrease at the same time points in the higher dose groups. Although there was no evidence of accumulation, blood levels obtained 96 hours after the final doses (at 52 weeks) demonstrated concentrations that were 24%, 70%, and 59% of the concentrations obtained at the time of final dosing in the 10, 20, and 40 mg/kg/day groups, respectively.

Relative organ weights calculated using body weights are less instructive than would be organ weights compared to brain weight since administration of this drug was associated with decreased body weights. Effects of treatment on serum bilirubin and cholesterol concentrations are consistent with rifampin-type drugs. Although the sponsor states that hematologic effects were not drug-related, bone marrow smears were not examined in order to assess that possibility.

Monkey Studies

18. Four week oral toxicity study - monkeys Report G-76-0018. (Study V-88-24)

Groups of Cynomolgus monkeys (3/sex/dose) were treated with rifapentine by oral gavage either every 2 days (20 mg/kg) or every 3 days (40 mg/kg), for 4 weeks. Vehicle control animals received (0.5% gum tragacanth).

No animals died on study. There was no effect of treatment on body weight gains, clinical signs, or clinical biochemistry parameters. Signs of anemia, including decreases in Hgb, hematocrit (Hct), and total erythrocytes) were seen at 4 weeks. Sporadic increases in urine protein, hemoglobin, and erythrocytes were observed in animals in the 20 mg/kg group. No consistent effects of treatment on organ weights or gross pathology were observed, with the exception of discoloration of adipose tissue. Histopathology demonstrated effects on the liver, lungs, and kidney consistent with infections, (such as trypanosomiasis), typical of jungle-trapped monkeys. Sporadic changes seen in treated animals included focal myocarditis, thickening of the splenic capsule, macrophages with brown pigment, and increased absolute and relative liver weights. At 24 hours after initial doses, mean serum blood levels were groups, respectively. These values decreased during the treatment period to respective values of 3 and 7 µg/ml throughout the remainder of the study.

Comment: Although the sponsor states that signs of anemia were due to blood sampling, this effect was not observed in control animals which were also sampled and it is thus probable that anemia was an effect of treatment. Evaluation of this study is complicated by the fact that most, if not all, of the monkeys had one or more infections.

19. Thirteen-week oral toxicity study - monkeys Report G-79-0021-T (Study V-86-85)

Groups of cynomolgus monkeys (4/sex/dose) were treated with rifapentine by oral gavage either daily or every 3 days for 13 weeks (10, 20 or 80 mg/kg/day or 40 mg/kg every 3 days). Vehicle control animals received (0.5% methocel).

There were no deaths on study. No effects of treatment on weight gains, food or water consumption, behavior, or clinical signs were observed. Reddish discoloration of the feces and urine was commonly observed in the 80 mg/kg/day group, and also occasionally in other dose groups. No consistent effects of treatment on clinical chemistry or urinalysis parameters were seen. Signs of anemia, including decreases in Hct, Hgb, and erythrocytes, were observed in two females in the 80 mg/kg/day group.

Necropsy demonstrated dose-related increases in absolute and relative liver weights (slight in the 40 mg/kg/3 day group; moderate to marked in the other treatment groups), occasionally accompanied by accentuated hepatic lobular patterns; moderate increases in absolute and relative spleen weights (20 and 80 mg/kg/day groups); and slight increases in absolute and relative adrenal weights (females in the 10 and 20 mg/kg/day groups). These effects were not observed in recovery group animals (held for 1 month after cessation of treatment; 1/sex/dose.) Liver weight increases were statistically significant in animals given daily doses and adrenal weight increases were significant only in females in the 20 mg/kg/day group. Histopathology demonstrated hepatic steatosis associated with increased liver weights. Lipidosis of the adrenal zona fasciculata was occasionally observed, especially in the 80 mg/kg/day group. Signs of hepatic steatosis persisted in the 80 mg/kg/day recovery group animals.

Serum levels were as follows:

Dose regimen	Serum level µg/ml 24 h after dose #1	Serum level µg/ml 24 h after dose #4	Serum level µg/ml 24 h after dose #91
10 mg/kg/day	9.8	9.1	4.7
20 mg/kg/day	17	15	8.1
80 mg/kg/day	54	62	42
40 mg/kg every 3 days	34	28	16

Drug concentrations were measured by microbiological assay (using *Sarcina lutea* as the indicator organism.) The values obtained were given previously in the review of nonclinical pharmacokinetics. Serum drug concentrations decreased slightly over the course of the study in all but the 80 mg/kg/day group. Animals in the 80 mg/kg/day group maintained relatively constant blood levels throughout the study. No evidence of drug bioaccumulation was observed.

21. One year oral toxicity study - monkeys (Study IT-86-122) October 1984.

Drug lot # D-2980.October 1984. GLP study.

Groups of cynomolgus monkeys (4/sex/dose except in the high-dose group where there were 6/sex/dose) were treated with rifapentine by daily oral gavage doses for 12 months (20, 40 or 80 mg/kg/day). Vehicle control animals received 0.5% methylcellulose. Two animals /sex from the high dose group were placed in a 3 week recovery group to determine the reversibility of any toxicities encountered.

There were no deaths on study. The only consistent clinical sign observed in treated animals was red discoloration of the feces. Emesis was sporadically observed in treated monkeys, occurring in isolated instances in all dose groups. One female in the high-dose group demonstrated clinical signs consistent with acute hepatic damage, including inappetence, bilirubinuria, and markedly elevated alanine aminotransferase (ALT; 17-fold increase) and AST (3-fold increase), on study days 60-66. This animal was not given drug on study days 63-67 and 70-87, and later demonstrated emesis after dosing on isolated study days. Decreases in food consumption were seen occasionally, but no consistent pattern developed. No other significant clinical effects were observed.

The only clinical biochemistry parameter that was consistently affected was serum cholesterol, which was This effect was seen in all dose groups but was not observed after the recovery period in recovery-group animals. Other clinical biochemistry and hematology effects observed were sporadic, not dose-related, and/or were consistent with parasitic infections.

Gross pathology demonstrated statistically significant increases in relative liver weights in males in the 40 and 80 mg/kg/day groups and females in the 40 mg/kg/day group. Mean absolute liver weights were non-significantly increased in all treatment groups. Relative kidney weights were significantly increased in males in all dose groups. Females in the 80 mg/kg/day group demonstrated significantly increased relative ovary weights. Recovery group animals demonstrated no significant increases in organ weights. Inflammatory lesions of the lungs and intestines were considered by the sponsor to be related to parasitic infections.

Histopathology demonstrated inflammatory infiltrates and granulomatous lesions in many tissues, reported by the sponsor to be associated with parasites. Hepatic cytoplasmic granularity was observed in males in the 40 and 80 mg/kg/day groups and in females in the 20 and 40 mg/kg/day groups. No other treatment-related effects were observed. Examination of the female monkey in the 80 mg/kg/day group that developed signs of acute hepatitis on study demonstrated no hepatic effects. An adenoma of the pituitary pars intermedia was seen in this monkey, but was not considered by the sponsor to be related to treatment.

Serum rifapentine concentrations 24 hours after the first dose were 2.6, 23.3, and 67.1 μg/ml for males and 12.4, 31.7, and 54.1 μg/ml for females in the 20, 40, and 80 mg/kg/day groups, respectively. Serum drug concentrations decreased to 10-20% of the initial dose serum concentrations by study day 87 (for the 40 and 80 mg/kg/day groups.) Slight increases in serum drug concentrations were observed in the high-dose group on study days 183 and 364. Serum drug levels were stable throughout the study in the 20 mg/kg/day group. Serum drug concentrations decreased 63-77% at 48 hours after the final doses and no drug or microbiologically active metabolites were detected at 96 hours after cessation of treatment. There was no association observed between serum drug levels and adverse effects in the female monkey in the 80 mg/kg/day group which demonstrated signs of acute hepatitis.

Comments: This is the pivotal study in evaluating the safety of rifapentine. Although signs of parasitic infections complicate the interpretation of the study, for the most part 80 mg/kg/day (equivalent to a human dose of about 27 mg/kg/day) should be considered the NOAEL. It is difficult to determine if the adverse effect (acute hepatitis) observed in the female in the 80 mg/kg/day group was due to rifapentine. No histopathological effects were seen on necropsy which could be associated with drug toxicity. Returning the monkey to treatment after the acute hepatic syndrome appeared to resolve did not result in reappearance of clinical signs. With the exception of increases in liver, kidney, and ovary weights, no consistent treatment-related adverse effects were observed. Signs of hepatic enzyme induction, including increased liver weight and cytoplasmic granularity, accompanied by decreased serum drug concentrations with chronic drug exposure, are consistent with the known effects of rifampin and related congeners. The kidney and ovary weight increases were not associated with adverse biochemical or histopathological effects.

22. Results of Reproduction and postnatal studies with MDL 473 in rats. Report G-87-0061-T (Study V-89-53).

September 1978. Drug lot # A/4/78. GLP study.

Groups of Sprague-Dawley rats (20 males/dose and 24 females/dose for the fertility study and 20 females/dose for the perinatal-postnatal study) received daily oral gavage doses of rifapentine at 5, 10 or 20 mg/kg/day. Vehicle control rats received 0.5% methocel.

Effects of treatment on male fertility were determined by administering rifapentine for 84 days prior to mating. After the treatment period, each treated male was allowed two weeks to mate with two untreated females. Females were killed on gestation day 21 followed by examination of fetuses. There were no deaths in the treated males. Significant reductions in body weights were observed in males in the 20 mg/kg/day group beginning in the sixth week of treatment and in the 10 mg/kg/day group beginning in

the ninth week. Non-significant decreases in food consumption were also seen in these dose groups. There were no statistically significant effects of treatment on testicular weights or mating performance. No adverse effects of treatment on litter size, sex ratios, implantations, or post-implantation loses were observed. Mean fetal weights were slightly increased in all treated groups. No significant incidence of fetal abnormalities was observed.

Effects of treatment on female fertility were determined by administering rifapentine beginning 15 days prior to mating with untreated males and continuing throughout breeding, gestation, and lactation periods. On gestation day 21, 12 females per dose group were killed and fetuses were examined. The remaining 12 females per dose group remained on study until weaning of the pups at 21 days of age. Various developmental effects were assessed in the pups (F_1 generation). Two males and two females from each F_1 generation were mated with non-litter mates at approximately 100 days of age. The females were allowed to deliver and wean their young and reproductive parameters determined as with the F_0 females. All developmental effects determined for F_1 pups were also determined for F_2 pups. There were no deaths in the treated females. Body weight gains were significantly reduced in the 20 mg/kg/day group beginning in the second week of treatment and continuing throughout gestation and during the first week of lactation. Although the sponsor reported that there was a statistically significant decrease in food consumption in the first week of treatment in the 10 and 20 mg/kg/day groups, examination of the data indicates that this may have been an artifact due to excessive consumption by the control and low-dose groups. There was no effect of treatment on pregnancy rate. Resorption rate was significantly reduced in the 20 mg/kg/day group. Non-significant increases in fetal weights occurred in all dose groups. Rifapentine had no adverse effect on fetal survival or duration of gestation. Two females in the high dose group lost litters during lactation. No other significant adverse effects were observed during the lactation period. Pups in the 20 mg/kg/day group demonstrated statistically significant decreases in body weights on day 4 of lactation, but this effect was not seen on other lactation days. There were no effects of treatment on developmental parameters assessed in the F_1 pups. One male F_1 pup from a 20 mg/kg/day mother died at 146 days of age. Necropsy demonstrated a large (3 × 4 × 3 cm) dark red, multilobulated, circumscribed mass in the retrorenal area. The sponsor stated that this was a spontaneous event unrelated to treatment: no histopathologic examination was reported. No other adverse effects were reported in the F_1 generation. Following mating of F_1 generation pups, slight decreases in litter size, increases in resorption rate, and increases in pre-implantation losses were observed in the offspring of F_0 mothers in the 20 mg/kg/day group. The mean number of corpora lutea was higher in all pups from rifapentine-treated mothers. One abnormal fetus (bilateral, posterior pes varus) was observed in an F_1 mother descended from a 20 mg/kg/day group F_0 generation mother. No adverse effects on any assessed parameters were observed in F_2 generation pups.

Effects of treatment on perinatal-postnatal parameters were determined by treating females beginning on gestation day 15 and continuing through postpartum day 21. There were no maternal deaths or observed adverse effects during the treatment period. Pregnancy rates were decreased in all dose groups compared to controls. Gestational survival was significantly decreased in the 20 mg/kg/day group. Pup weights in the 20 mg/kg/day group were significantly decreased on post-partum days 1 and 4.

Comments: The only significant toxicities observed in this study were decreases in paternal and maternal body weight gains and sporadic decreases in pup weights. Paternal weight gain decreases in the 10 and 20 mg/kg/day groups (equivalent to human doses of about 1.4 and 2.9 mg/kg/day, respectively, based on relative body surface areas) ranged and , respectively, of controls (in the male fertility study). The percentage decrease increased over time, but the statistical significance of this trend was not assessed by the sponsor. Maternal weight gain decreases in the 20 mg/kg/day group ranged from of controls. The percentage decrease tended to reverse over time, with the least effect (14% of controls) observed on gestation day 21, with the exception of a large effect (38% decrease compared to controls) observed on lactation day 7. The statistical significance of the trend was not assessed. Effects on paternal and maternal weight gains did not result in observed effects on mating parameters. Pup weights were only sporadically decreased and this effect was only seen in the progeny of

mothers in the 20 mg/kg/day group. Pregnancy rates were decreased in all dose groups compared to controls in the perinatal-postnatal study. Since pregnancy had been established 2 weeks prior to initiation of treatment, the observed effect on pregnancy rates suggests an adverse effect of rifapentine on gestational survival. Coupled with the small, but statistically significant, decrease in pup weights seen on post-partum days 1 and 4 in the 20 mg/kg/day group, a possible adverse *in utero* effect is suggested.

23. Fetal toxicity study in the rat treated with MDL 473 administered by the oral route. Report G-83-0044-T (Study V-86-47).

Groups of Sprague-Dawley rats (35 pregnant females/dose) were treated with rifapentine at 5, 20, 40 mg/kg/day daily by oral gavage. Animals were dosed from day 5 through day 15 of pregnancy and effects on maternal and fetal parameters determined. Vehicle control animals received 0.5% methocel. On gestation day 21, 25 animals were killed, and the remaining 10/dose were allowed to deliver litters and rear their offspring until weaning on post-partum day 21.

No maternal deaths occurred during the study. Maternal body weight gains and food consumption were significantly decreased in the 40 mg/kg/day group during the treatment period, resulting in maternal weights that were 16% less than concurrent controls on gestation day 15. After cessation of treatment, no significant differences in weight gains or food consumption were observed for the remainder of the gestation period. Weight gains were greater in the 5 and 40 mg/kg/day groups compared to controls in the lactation period, whereas weight gains in the 20 mg/kg/day group were slightly decreased. The only significant effect seen on necropsy at the end of gestation was orange-red coloration of various tissues in the high-dose group, an effect not seen in animals necropsied at the end of the lactation period.

Two dams, one in the control group and one in the 40 mg/kg/day group, lost pregnancies before delivery, both due to fetal and placental resorptions. There were no statistically significant effects of treatment on gestational parameters. Non-significant increases in resorption rate and post-implantation loss were observed in the 40 mg/kg/day group. Mean fetal weights were significantly decreased in the 40 mg/kg/day group.

Fetal anomalies were significantly increased ($p < 0.001$) in the 40 mg/kg/day group. Major malformations, including palatoschisis, cheilognathouranoschisis, right aortic arch, atresia of the aorta, left ventricular hypoplasia, and right ventricular hypertrophy, were observed in 10 fetuses (4.2%) from 5 litters (of 23 dams.) One control group fetus demonstrated major malformations (arhinia and ectrodactyly.) Significant incidence of minor anomalies was also seen in the 40 mg/kg/day group, including malformations of the tail, right subclavian artery, kidneys, testes, and bones. Although not specifically mentioned by the sponsor, there was an apparently dose-related incidence of fetal hydronephrosis. Skeletal anomalies included delayed ossification centra (skull, hyoid, sternebrae and falanges) and 14th ribs, the latter effect being observed in all fetuses in the high-dose group. Non-significant increases in 14th ribs were seen in all treatment groups. No malformations were observed in pups following delivery. Litter size and pup survival during lactation were non-significantly decreased in the 40 mg/kg/day group. No adverse effect on pup weight gains was observed.

Comments: The NOAEL in this study, 20 mg/kg/day group, is equivalent to a human dose of about 2.9 mg/kg/day, based on relative body surface areas. Significant adverse effects were observed in the 20 mg/kg/day group in the female fertility study reported previously (Study V-89-53), but the treatment period was considerably longer (about 56 days versus 11 days in this study.) Although some signs of adverse maternal effects were observed, the teratologic effects should be considered to be due to rifapentine. It should be noted that rifampin has been demonstrated to have teratologic effects in rodents.

24. Preliminary fetal toxicity study rats Report G-82-0052 (Study V-86-58) drug lot # A/4/78

Groups of Sprague-Dawley rats; (5 pregnant females/dose) were treated daily with rifapentine by oral gavage doses from day 5 through day 15 of pregnancy at 10, 20, 40 or 60 mg/kg/day. Vehicle control animals received 0.5% methocel.

There were no deaths during the study. Pregnant rats in the 60 mg/kg/day group demonstrated piloerection, hypomotility, and anorexia associated with significant decreases in body weights. Decreased body weight gains were also observed in the 20 and 40 mg/kg/day groups. After cessation of treatment, body weight gains normalized in all groups. Other clinical signs included reddish discoloration of extremities (≥ 40 mg/kg/day groups) and excreta (60 mg/kg/day group), and vaginal bleeding (one animal in the 60 mg/kg/day group). Necrospy demonstrated yellow-red discoloration of the viscera and serous and mucous membranes (≥ 40 mg/kg/day groups).

Only one rat (in the 40 mg/kg/day group) failed to become pregnant. Gestational parameters were normal in all but the high-dose group. In the 60 mg/kg/day group, 3/5 impregnated females demonstrated signs of resorption on necropsy. Litter sizes and general health indicators for pups from all but the high-dose group were essentially comparable to controls. Effects observed in the 60 mg/kg/day group included decreases in numbers of implantation sites, litter sizes, and fetal weights. No teratogenic effects were seen in any dose groups.

25. Fetal toxicity and perinatal studies in rabbit with DL 473-IT.(Rifamycin AF/cyclopentyl) administered by the oral route. Report G-80-0034. (Study V-86-88).

drug lot # A/4/78

December 1978. Non-GLP study.

Groups of New Zealand white rabbits, (4 pregnant rabbits/dose for the preliminary fetal toxicity study, 15 pregnant rabbits/dose for the main fetal toxicity study, 10 pregnant rabbits/dose for the perinatal toxicity study) were treated with rifapentine daily by oral gavage doses from day 6 through day 18 of pregnancy. Doses used were 10, 20, 40 or 60 mg/kg/day (preliminary fetal toxicity study), 10, 20 or 40 mg/kg/day (main fetal toxicity study)10 or 40 mg/kg/day (perinatal toxicity study). Vehicle control animals received (0.5% methocel®).

In the preliminary fetal toxicity study, one female in the 10 mg/kg/day group was found dead on gestation day 24: necropsy demonstrated 11 near-term fetuses and evidence of massive hemorrhage. One control group doe was sacrificed moribund on gestation day 23: no specific cause of death was established. Decreases in food consumption and body weight gains were observed in the 40 and 60 mg/kg/day groups. Body weight gains tended to normalize in these dose groups after the first week of treatment. Dose-related decreases in mean fetal body weights were accompanied by increases in litter sizes, thus complicating interpretation of this effect. Two major fetal malformations were observed: severe testicular atrophy in the 40 mg/kg/day group and arthrogryposis of the left foreleg in the 60 mg/kg/day group. No other significant adverse effects were observed.

In the main fetal toxicity study there were no deaths. One doe in the 40 mg/kg/day group aborted five fetuses on gestation day 28: necropsy performed after the animal was sacrificed the following day demonstrated evidence of pneumonia, probably resulting from a dosing error. Dose-related decreases in food consumption were observed in all dose groups, especially in the first week of treatment. Other than a slight decrease in body weight in the 40 mg/kg/day group observed in the first week of treatment, no significant effects of treatment on body weights or weight gains were seen. Necropsy on animals at the end of the study (gestation day 29) demonstrated evidence of mucoid enteritis in three does (one each in the control, 10 mg/kg/day, and 40 mg/kg/day groups). Only 1/14 and 1/11 fetuses were alive in the 10

mg/kg/day and 40 mg/kg/day group does, respectively, demonstrating enteritis on necropsy. No increase in pre-implantation losses was observed related to treatment. In addition to fetal losses associated with mucoid enteritis, 5/10 fetuses were dead in one 10 mg/kg/day group doe (with the surviving fetuses showing poor viability) and there was evidence of 10 resorptions in one 20 mg/kg/day group doe. No significant decreases in fetal weights were seen. Major malformations were seen in four fetuses: one with ovarian agenesis (10 mg/kg/day group), one with pes varus (20 mg/kg/day group), and two fetuses in the 40 mg/kg/day group with arhinia, microphthalmia, and irregularities of the ossified facial tissues. No significantly increased incidences of minor anomalies were observed in treated versus control group fetuses.

In the perinatal toxicity study, one animal in the 10 mg/kg/day group died on post-partum day 16. Necropsy demonstrated signs of mucoid enteritis. Non-significant decreases in food consumption and body weights were observed in treatment group animals. There were significant decreases in live-born pups in the 40 mg/kg/day group compared to concurrent controls. Post-partum survival and body weights were higher in treatment group pups compared to controls. No increased incidence of fetal malformations was observed.

Comments: Although the sponsor claims that this study demonstrated rifapentine to have no teratogenic potential, in fact dose-related malformations were observed that were somewhat consistent with effects observed in a previously discussed rat study (Study V-86-47). Specifically, malformations of the face were observed in the high-dose group (40 mg/kg/day) in both rats and rabbits. Although the total number and pattern of adverse effects were not entirely consistent, teratogenic effects were observed in both species. The actual systemic exposure of the rabbit to rifapentine was probably much lower than was achieved in the rat. Compared to the rat (and to humans) rifapentine plasma half-life is much shorter in the rabbit and both volume of distribution and *AUC* are also much lower. The general health of the animals used in this study appeared to be less than optimal based on the fairly high incidence of mucoid enteritis observed.

26. Study of the mutagenicity of DL 473-IT with *Salmonella typhimurium* Report G-79-0022-T (Study V-86-199).

The mutagenicity of rifapentine was examined using tester *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538. Mutant tester strains were isolated which were resistant to rifamycins in order to conduct this study. No mutagenic activity was observed using either the rifamycin-sensitive strains (at rifapentine concentrations of ≤ 0.5 μg/plate) or the rifamycin-resistant strains (at rifapentine concentrations of ≤ 500 μg/plate), with or without metabolic activation.

27. Evaluation of rifapentine (MDL 473, DL 473-IT) for potential mutagenicity with the Ames test Report G-85-0075-T(Study V-85-41) Drug lot # PRD-C/10/80.

The mutagenicity of rifapentine was examined using tester *Salmonella typhimurium* strains (TA98, TA100, TA1535, TA1537, TA1538) to confirm the findings of the previously performed mutagenicity assays. No mutagenic activity was observed using either the rifamycin-sensitive strains (at rifapentine concentrations of ≤ 1 μg/plate and ≤ 5 μg/plate without or with metabolic activation, respectively) or the rifamycin-resistant strains (at rifapentine concentrations of ≤ 1500 μg/plate and ≤ 3000 μg/plate without or with metabolic activation, respectively). Toxicity to the tester strains was observed at the highest doses used in these assays.

28. Report on the mutagenicity experiment performed on the substance DL 473-IT

The *in vitro* mutagenic potential of rifapentine was determined in a point mutation test using *Aspergillus nidulans*. The potential for *in vitro* and host-mediated (*in vivo* in male Swiss albino/CD mice) gene conversion was determined using *Saccharomyces cerevisiae*. No mutagenic activity was observed either *in vitro* (with or without metabolic activation, at concentrations up to 4000 µg/plate using *Aspergillus nidulans*, or at concentrations up to 10 mg/ml using *Saccharomyces cerevisiae*) or *in vivo* at 436 or 873 mg/kg (single doses) or at 72 or 291 mg/kg/day (5 daily doses.)

Evaluation of MDL 473 in an *in vitro* chromosome aberration assay utilizing rat lymphocytes. Study DR 0170-1695-002.

This study was designed to evaluate the clastogenic potential of rifapentine to cultured rat lymphocytes. Blood samples were collected by cardiac puncture from rats and whole blood cultures were set up in RPMI 1640 medium. Approximately 48 hours after the initiation of the cultures, cells were exposed to rifapentine at doses of 0 (negative control), 0.8, 2.7, 8.0, 26.7, 80.0, 266.7 and 800 µg/ml for the initial assay (Assay 1). In the absence of liver S-9, cells were exposed to drug for 24 hours and in the presence or absence of rat liver S9 activation system cultures were exposed to drug for 4 hours and harvested 20h after treatment termination. Cultures treated with mitomycin C (0.05 and 0.075 µg/ml) or cyclophosphamide (4 and 6 µg/ml) served as positive controls for the non-activation and activation assays, respectively. Based on mitotic indices, cells exposed to rifapentine doses of 0, 2.7, 8.0 and 26.7 µg/ml in the absence of rat S-9 and 0, 8.0, 26.7 and 80.0 µg/ml in the presence of S-9 were assessed for the incidence of chromosomal aberrations. A confirmatory experiment (Assay 2) was also conducted utilizing an additional harvest time (24 h after the initial harvest). In the absence of S9, the incidence of chromosomal abnormalities was determined at 0, 8, 20 and 27 µg/ml at the first harvest time and at 0 and 27 µg/ml at the second harvest time. In the presence of S9, chromosomal abnormalities were assessed at 0, 50, 80 and 140 µg/ml at the first harvest time and at 0 and 140 µg/ml at the second harvest time.

There was no significant increase in the incidence of abnormal cells in either asssy, compared to controls. The positive control cultures had significantly higher indices of abnormal cells. Rifapentine was considered to be negative in the *in vitro* chromosome aberration assay in rat lymphocytes.

Evaluation of MDL 473 in the mouse bone marrow micronucleus test. Study # DR-0170-1695-003.

This study was designed to determine the potential of rifapentine to induce micronuclei in the bone marrow polychromatic erythrocytes of CD-1 mice. Rifapentine was administered to CD-1 mice as a single dose by oral gavage at 0, 750, 1500 and 3000 mg/kg and animals were sacrificed at 24, 48 or 72 h after treatment. Cyclophosphamide was administered at 120 mg/kg to animals which served as positive controls. Positive control animals were sacrificed at 24h after treatment. At the end of the specified time periods animals were sacrificed and bone marrow samples obtained from both femurs. Cells were suspended in fetal bovine serum albumen, prepared on microscope slides and stained and examined. The numbers of polychromatic erythrocytes (PCE's), normochromatic erythrocytes (NCE's) and micronucleated polychromatic erythrocytes (MN-PCE's) were recorded. The MN-PCE frequency was calculated after examination of 1000 polychromatic erythrocytes.

The MN-PCE frequencies in the mice treated with rifapentine were not significantly different from those of the mice treated with vehicle. Positive control animals showed a statistically significant increase in MN-PCE's . Rifapentine is considered negative in the mouse bone marrow micronucleus test.

Evaluation of MDL 473 in the Chines Hamster Ovary cell/hypoxanthine-guanine-phosphoribosyl transferase (CHO/HGPRT) forward mutation assay. Study DR 0170-1695-001.

48674. June 1995. GLP study.

This study was designed to determine the potential of rifapentine to induce gene mutations at the HGPRT locus of the Chinese Hamster Ovary cells in culture. The CHO-K_1-BH_4 cell line was used in this study and these cell were exposed to rifapentine in the presence and absence of S9 liver homogenates prepared from Aroclor-1254 treated Sprague Dawley rats.

Dose levels of test materials ranged from 0.78 to 800 μg/ml. The solvent used to dissolve the rifapentine was used as the negative control treatment. Ethylmethanesulfate at 621 μg/ml was used as the positive control for the cultures without S9 while 20-methylcholanthrene (4 μg/ml) served as the positive control in the presence of S9.

The mutation frequencies observed in the test material treated cultures were not significantly different from the corresponding negative control values. The positive control cultures induced statistically significant increases in mutation frequency. In conclusion, rifapentine was not mutagenic in the CHO/HGPRT gene mutation assay under the above conditions.

29. Immunogenicity of rifamycins Report G-78-0009-R (Study V-86-53)

The potential for rifapentine to induce specific antibody formation was determined by injecting rabbits (strain and sex not given) with the drug emulsified in complete Freund's adjuvant. Booster immunizations were given once weekly for five weeks. Positive controls were injected with a 3-formyl rifamycin SV-bovine serum albumin conjugate (rifamycin-BSA). Specific antibody production was determined by equilibrium dialysis using γ-globulin fractions purified from sera samples obtained from immunized rabbits and [$^{14}C_{38}$]rifampicin. Rifapentine did not induce specific anti-rifampicin antibody production under conditions in which rifamycin-BSA induced high titers of specific antibodies. Rifamycin derivatives in which C_{38} is substituted with a thiazolic ring induced specific anti-rifampicin antibody formation. Under the conditions of this assay, rifapentine does not appear to react *in vivo* to form immunogenic drug-protein complexes.

30. Effect of DL 473 -IT (AF/cyclopentyl) on immunological responsiveness. Report G-77-0009 (Study V-86-68)

Potential immunosuppressive effects of rifapentine were determined in female CF/1 mice following either single- or multiple dose i.p., iv., or oral administration of either 12.5, 25, or 50 mg/kg/dose. Rifapentine demonstrated significant dose-related suppression of IgM antibody response to sheep red blood cells (SRBC) when administered i.p. during the four days of antigen injection. This effect was not seen when rifapentine was given i.p. either six days prior to immunization or as single doses on the day of immunization. IgG antibody response to SRBC was suppressed when rifapentine was administered i.p. for 12 consecutive days beginning on the day of initial immunization. No suppressive effect on IgG production was observed when drug was administered beginning on day four of immunization. Rifapentine did not affect IgG antibody production when administered after a primary immune response had been established.

Rifapentine inhibited macrophage function when administered i.p. concurrently with immunization using SRBC. Injection of macrophages primed *in vitro* with SRBC restored antibody responses in mice treated with rifapentine, supporting the concept that rifapentine-inhibition of macrophage function is the basis of immunosuppressive activity.

Rifapentine, administered during the period of skin sensitization to picryl chloride, significantly reduced cell-mediated immune response (determined as ear swelling after hapten challenge) compared to untreated controls. This effect was observed when the drug was administered either orally or i.p. Peritoneal cells, obtained from picryl chloride-sensitized and rifapentine-treated mice and incubated to remove adherent cells, were demonstrated to transfer induced sensitization to unprimed mice, which was taken by the sponsor to indicate that the suppressive effect of rifapentine on delayed-type hypersensitivity (DTH) was also due to its effects on macrophage function.

Comment: The immunosuppressive effects demonstrated in this study are similar to effects known to be associated with rifampin. The clinical relevance of these effects has not been determined.

31. Effect of rifampin and its cyclopentyl derivative, MDL 473 on the expression of delayed type hypersensitivity in the BALB/c mouse. Report I-87-0053-R (Study IP-87-75).

The ability of rifapentine to inhibit DTH response (determined as ear swelling) to a contact sensitizer, oxazolone, was determined in female BALB/c mice. Rifapentine was administered i.p. at 1, 10, 50, or 100 mg/kg either every other day (for a total of 5 doses) or every fourth day (for a total of 3 doses), beginning 3 days before sensitization and continuing until hapten challenge 5 days after sensitization. Control groups received i.p. injections of vehicle (0.9% saline + 0.05% methocel®) and positive controls received i.p. injections of 200 mg/kg hydrocortisone-21-acetate every 2 days for a total of 3 doses. Rifampin (1, 10, 50, or 100 mg/kg/day) was administered every day for a total of 9 doses as a comparative control. Neither rifapentine nor rifampin had suppressive effects on DTH reactions to oxazolone, whereas hydrocortisone essentially eliminated hypersensitivity reactions.

Comments: There are conflicting reports in the literature on the DTH-suppressive effects of rifampin-type drugs. It should be noted that the apparently discordant results obtained in studies V-86-68 and IP-87-75 could be explained by examining the details of the respective studies. Based on a comparison of the measured ear-swelling induced by picryl chloride versus oxazolone, the latter appears to produce about 40% greater response. The measured effects of rifapentine on picryl chloride-induced DTH, though statistically significant compared to vehicle control, in fact were not numerically striking. Also, in study V-86-68, rifapentine was administered daily, whereas in study IP-87-75 the compound was administered either every other day or every fourth day. Thus the actual dose delivered to macrophages (assuming this is the critical target of a proposed effect on DTH) was probably much greater in the study which demonstrated an effect of rifapentine on hypersensitivity reaction (using a probably weaker sensitizer).

32. Comparative effect of the napthalenic ansamycins rifamycin SV, rifampin and cyclopentylrifampicin on the murine neutrophilic function. Report I-88-0077 (Study IP-88-111).

The *in vitro* effects of rifapentine on murine neutrophil function were evaluated and compared to the effects of rifamycin SV and rifampin. Neutrophils were obtained by inducing peritoneal exudate with sodium caseinate injected i.p. into male BALB/c mice. The neutrophils were then harvested, cultured with various drug concentrations, and functional parameters determined. None of the drugs tested had effects on phorbol 12-myristate 13-acetate (PMA)-stimulated production of superoxide anion (O_2^-) or myeloperoxidase secretion, at drug cell culture concentrations of $\leq$ 80 µg/ml. Both rifapentine and rifamycin SV (at concentrations of 80 µg/ml) inhibited *N*-formyl-methionine-leucine-phenylalanine (FMLP)-stimulated neutrophil secretion of myeloperoxidase. Paradoxically, rifapentine (but not the other drugs tested) enhanced FMLP-stimulated myeloperoxidase secretion at a drug concentration of 1 µg/ml. Rifapentine (at concentrations of $\leq$ 80 µg/ml) had no inhibitory effect on nondirected or FMLP-directed neutrophil migration, although inhibition of these parameters was observed with rifamycin SV and rifampin at concentrations > 0.31 µg/ml and > 5.0 µg/ml, respectively.

Comment: The only significant effects of rifapentine on neutrophil functional parameters observed in this study were seen either at drug cell culture concentrations that are greater than blood concentrations usually seen with oral administration (inhibition of myeloperoxidase secretion at 80 μg/ml) or a slight, but significant enhancement of this parameter at a more biologically relevant concentration.

Summary and Conclusion

The following observations can be made from nonclinical toxicology studies (all of the dose comparisons are made based on relative body surface areas, and the human dose equivalent is given as human eq.)

There appear to be significant species differences in dose-related toxicities. These differences become apparent only in repeat-dose studies and appear to be related to both dose and duration of exposure. In the 3 week dietary toxicity study in mice, mortalities were observed in the ≥ 640 mg/kg/day groups, (human eq. = 53 mg/kg/day.) Clinical signs, such as decreased body weight gains, were observed at ≥ 320 mg/kg/day (human eq. = 27 mg/kg/day.) Increased relative liver weights were seen at ≥ 80 mg/kg/day (human eq. = 7 mg/kg/day) and the NOAEL was 40 mg/kg/day (human eq. = 3 mg/kg/day.) In the 4 week dietary toxicity study in rats, a similar profile is seen: significant mortalities occurred in the 320 mg/kg/day group (human eq. = 46 mg/kg/day); clinical signs, such as decreased body weight gains and liver, intestinal and bone marrow effects, were seen at ≥ 80 mg/kg/day (human eq. = 11 mg/kg/day), and the NOAEL was 20 mg/kg/day (human eq. = 3 mg/kg/day.) Thus, in rodents over about the same period of time, roughly the same pattern of dose-related toxicities occurred.

However, it is in comparing the 4 week study in rats to the 3 month study that significant differences emerge. In the 3 month oral toxicity study in rats, significant mortalities occurred at ≥ 80 mg/kg/day (human eq. = 11 mg/kg/day) which is about 1/4 the dose in which mortalities occurred in the 4 week study. Adverse effects, including clinical signs, decreased body weight gains, increased liver enzymes, hepatic and renal steatosis, myocarditis, thymic atrophy, and changes in various organs, were all seen at ≥ 10 mg/kg/day (human eq. = 1.4 mg/kg/day.) The severity of hepatic effects was greater at 13 weeks as compared to effects observed at 6 weeks. No true NOAEL was determined, and the dose at which adverse effects occurred was about 1/2 the NOAEL observed in the 4 week study. In the one year toxicity study in rats, one death occurred in the 40 mg/kg/day group (human eq. = 3 mg/kg/day), which was the NOAEL in the 4 week study. In addition, hematologic effects were observed that had not been previously reported. This evidence of cumulative toxicity was accompanied by evidence of significant accumulation observed in nonclinical pharmacokinetics studies in rats.

A similar pattern of cumulative toxicity was not seen in monkey studies. Some hepatic effects (increased relative liver weights, slight steatosis) and increased relative spleen and adrenal weights were seen at 80 mg/kg/day (human eq. = 27 mg/kg/day) in the 13 week toxicity study. In the 1 year study, a female in the 80 mg/kg/day group developed signs of acute hepatitis after about 2 months of dosing, but after a brief hiatus the monkey was returned to treatment without significant adverse effects. No evidence of drug-related pathology was observed at necropsy. Other than increased relative liver and kidney weights and increased hepatic cytoplasmic granularity (a phenomenon consistent with liver drug-metabolizing enzyme induction), no significant adverse effects were observed at any dose tested. The NOAEL in this study was 80 mg/kg/day (human eq. = 27 mg/kg/day.) There was no evidence of significant accumulation in nonclinical pharmacokinetics studies in monkeys.

Thus, after one year of exposure, human equivalent doses that produced no significant toxicities in monkeys were not tolerated in rats. There is clear evidence that this differential pattern of toxicity is related to differences in pharmacokinetics.

Ongoing Carcinogenicity Study

The sponsor is currently conducting carcinogenicity studies in rats and mice to determine the potential carcinogenic effects of rifapentine

Conclusion

The sponsor has provided adequate information to facilitate the approval of this compound. Rifapentine produces a toxicity profile , which is not unlike the drugs in this class. Its pharmacokinetic profile, with its longer half-life, facilitates less frequent and this may improve compliance with antituberculosis medication.

/S/

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Pharmacology/Toxicology Reviewer, DAVDP

Concurrences:
HFD-590/Ralbrecht
HFD-590/KHasting /S/ 6/12/98
Disk:
HFD-590/KHastings
cc:
HFD-590 Original IND
HFD-590/PM/BAtkins
HFD-590 Division File
HFD-590/Micro/LGosey
HFD-340
HFD-590/MO/JKorvick
HFD-590/Bio/KKumi
HFD-590/Pharm/OMcMaster
HFD-590/Chem/Jsmith

Notes

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