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Maternal - There were no treatment-related effects on reproductive or pregnancy parameters in (untreated) female partners of treated males (Tab. A-7).

F₁ - There were no treatment-related changes in fetal weight (Tab. A-7) or external alterations in fetuses (Tab. A-8).

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TABLE A-7. MK-0462: ORAL FERTILITY STUDY IN MALE RATS. TT 893-729-0
SUMMARY OF LAPAROTOMY DATA FROM F0 FEMALES

TREATMENT GROUP:	CONTROL	5 MG/KG/DAY	35 MG/KG/DAY	250 MG/KG/DAY
FEMALES				
TOTAL FEMALES	24	24	24	24
PREGNANT	23	24	22	24
EXAMINED LIVE LITTER	23	24	22	24
DIED	0	0	0	0
SACRIFICED	0	0	0	0
ABORTED	0	0	0	0
NOT PREGNANT	1	0	0	0
LIVE	1	0	1	0
DIED	0	0	1	0
SACRIFICED	0	0	0	0
NOT BRED	0	0	1	0
CORPORA LUTEA				
CORPORA LUTEA	399	407	393	412
CORPORA LUTEA/PREGNANT FEMALE	17.3	17.0	17.9	17.2
% PREIMPLANTATION LOSS (LITTER MEAN)	6.9	7.6	8.6	6.5
IMPLANTS				
IMPLANTS	369	375	357	383
IMPLANTS/PREGNANT FEMALE	16.0	15.6	16.2	16.0 NS
RESORPTIONS AND DEAD FETUSES				
RESORPTIONS	26	15	19	12
% RESORPTIONS/IMPLANTS (LITTER MEAN)	7.5	3.7	5.4	3.1
DEAD FETUSES	1	0	0	0
% DEAD FETUSES/IMPLANTS (LITTER MEAN)	0.3	0.0	0.0	0.0
% (RESORP+DEAD FET)/IMP (LITTER MEAN)	7.8	3.7	5.4	3.1 NS
LIVE FETUSES				
LIVE FETUSES	342	360	338	371
FEMALES	164	202	161	199
MALES	178	158	177	172
SEX RATIO (LITTER MEAN)	0.47	0.56	0.48	0.54
LIVE FETUSES/PREGNANT FEMALE	14.9	15.0	15.4	15.5 NS
LIVE FETAL WEIGHT (GM, LITTER MEAN)				
FEMALES	5.23	5.29	5.22	5.28
MALES	5.56	5.65	5.48	5.58

% PREIMPLANTATION LOSS = ((NO. CORPORA LUTEA - NO. IMPLANTS) / NO. CORPORA LUTEA) X 100
SEX RATIO = (TOTAL NO. LIVE FEMALE FETUSES/TOTAL NO. LIVE FETUSES)
NS = TREND NOT STATISTICALLY SIGNIFICANT (P > 0.05) THROUGH INDICATED DOSE.

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TABLE A-8. MK-0462: ORAL FERTILITY STUDY IN MALE RATS. TT 893-729-0
SUMMARY OF EXTERNAL EXAMINATION OF FETUSES FROM F0 FEMALES

SUMMARY OF EXTERNAL EXAMINATION OF FETUSES

TREATMENT GROUP:	CONTROL	5 MG/KG/DAY	35 MG/KG/DAY	250 MG/KG/DAY
LIVE FETUSES/LITTERS EXAMINED	342/23	360/24	338/22	371/24
DEAD FETUSES/LITTERS EXAMINED	1/ 1	0	0	0
FETUSES WITH MALFORMATIONS (% LM)	1 (0.26)	1 (0.28)	0	0
LITTERS WITH MALFORMATIONS (%)	1 (4.3)	1 (4.2)	0	0
FETUSES WITH VARIATIONS (% LM)	0	0	0	0
LITTERS WITH VARIATIONS (%)	0	0	0	0
TYPE AND NUMBER OF FETAL ALTERATIONS (% LM)				
	CLASS			
MICROGNATHIA	(M)	1 (0.26)	0	0
MICROGLOSSIA	(M)	1 (0.26)	0	0
POLYDACTYLY	(M)	0	1 (0.28)	0

(LM) = LITTER MEAN (M) = MALFORMATION

C.5. Genetic Toxicology

- a. Microbial Mutagenesis Assays (TT #91-8036, TT #91-8017)
- b. *In Vitro* Alkaline Elution/Rat Hepatocyte Assay (TT #91-8357, TT #91-8371)
- c. V-79 Mammalian Cell Mutagenesis Assay (TT #93-8552, TT #93-8553, TT #93-8564)
- d. Assay for Chromosomal Aberrations *In Vitro* in Chinese Hamster Ovary Cells (TT #91-8696, TT #95-8605)
- e. Assay for Chromosomal Aberrations in Mouse Bone Marrow (TT #94-8688)

All studies were conducted by Merck Research Laboratories and complied with GLP.

C.5.a. Microbial Mutagenesis Assays

(Reports: TT #91-8017, TT #91-8036; Vol. 23)

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Summary:

RIZ was not mutagenic in either the presence or absence of metabolic activation when evaluated in two separate Ames tests (different lots) under appropriate test conditions. Positive controls produced the expected results.

Report #	Lot	Strains	Concs (solvent)	Results
91-8017	002X003	<i>S. typhimur.</i> : TA1535, TA 97a, TA98, TA100 <i>E. coli</i> : WP2, WP2 uvrA, WP2 uvrA pKM101	100, 300, 1000, 3000, 10000 µg/plate (DMSO)	negative
91-8036	004B003	<i>S. typhimurium</i> : as above <i>E. coli</i> : WP2 uvrA pKM101	50, 150, 500, 1500, 5000 µg/plate (DMSO)	negative

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C.5.b. *In Vitro* Alkaline Elution/Rat Hepatocyte Assay

(Reports: TT #91-8357, TT #91-8371; Vol. 23)

Summary:

This assay is not part of the ICH core battery or recommended in OECD guidelines, but the sponsor has included the study findings in their labeling statement.

RIZ was tested for its capacity to induce DNA strand breaks in cultured rat hepatocytes at concentrations up to 10 mM. Neither cytotoxicity nor an increases in the mean elution slope (an indicator of DNA strand breaks) were observed. The positive control aflatoxin B₁ caused a 12.6-fold increase in elution slope at a concentration of 1 µM. Thus, RIZ is considered negative in this assay.

C.5.c. V-79 Mammalian Cell Mutagenesis Assay

(Reports: TT #93-8552, TT #93-8553, TT #93-8564; Vol. 23)

Summary:

The mutagenic activity of RIZ (lots 004B003 and 004B007) was assessed by determining mutations at the *hprt* locus that confer resistance of V79 Chinese hamster lung cells to 6-thioguanine (TG). The first experiment, a cytotoxicity range-finder with RIZ concentrations of 3-8 mM in DMSO, determined survival ranges of 91% to 18% in the presence of S-9, and 96% to 41% in the absence of S-9. For the definitive experiments, the top RIZ concentration in the presence of S-9 remained 8 mM (3, 5, 7, 8 mM), but was increased to 9 mM in the absence of S-9 to achieve the recommended degree of cytotoxicity (20%). Under appropriate test conditions, RIZ did not increase the number of TG-resistant colonies above background. Positive controls produced the expected results. Thus, RIZ was not mutagenic under the conditions of this assay.

C.5.d. Assay for Chromosomal Aberrations *In Vitro* in Chinese Hamster Ovary Cells

(Reports: TT #91-8696, TT #95-8605; Vol. 24)

Summary:

RIZ (lot 004B003, in dH₂O) was evaluated at concentrations 1-10 mM with and without S-9 for its propensity to induce chromosomal aberrations in Chinese hamster ovary cells. In the first experiment, the high doses that caused less than a 50% reduction in cell count after a 20 hr exposure were 4 mM and 7 mM in the presence and absence of S-9, respectively. The frequency of chromosomal aberrations in cultures exposed to RIZ up to these concentrations () did not exceed background levels under appropriate test conditions. Similar results were obtained in a repeat study of slightly higher RIZ concentrations. Positive controls produced the expected increases in chromosomal aberrations. Thus, RIZ was not clastogenic under the conditions of this study.

C.5.e. Assay for Chromosomal Aberrations in Mouse Bone Marrow

(Report: TT #94-8688; Vol. 23)

Summary:

RIZ (lot 004B015; 0, 12.5, 41.7, 125 mg/kg as free base in 0.5% McCell) was given as a single gavage dose to female CD-1 mice (n = 8-12/group). Animals were sacrificed 24 or 48 hr after treatment with negative controls or RIZ, and 24 hrs after positive control treatment (Mitomycin, 1 or 3.5 mg/kg, i.p.). Animals were treated with colchicine 3 hrs prior to sacrifice to fix cells in metaphase. At sacrifice, femoral marrow was collected and smeared on slides for analysis of chromosome aberrations. No significant increases in the chromosome aberration frequency were detected in marrow cells from RIZ-treated animals. Mitomycin C produced the expected increase in chromosomal aberrations.

Under the study conditions, RIZ was devoid of a clastogenic effect. However, the study was not designed and conducted under conditions consistent with OECD guidelines. The most obvious design flaw was in dosage selection. The guideline states that "the highest dose is defined as the dose producing signs of toxicity such that higher dose levels, based on the same dosing regimen, would be expected to

produce lethality". The sponsor cites a 14-week oral toxicity study of 125, 250 and 500 mg/kg/day as their basis of dosage selection. In that study, the only toxicity noted at the LD and MD were slight decreases in body weight. Two of 20 animals at the HD died, with the earliest death occurring at week 2. Clearly, the HD used in the *in vivo* chromosomal aberration does not meet the requirements for a high dose set forth in the OECD guidelines. However, the sponsor provided toxicokinetic evidence demonstrating that mouse plasma exposures during week 5 at the 125 mg/kg level were 200 times the anticipated human exposure. Because of the high relative plasma exposure in mice, absence of any positive mutagenicity findings in any other assay, and the computerized MULTICASE QSAR assessment (see Appendix 1) that RIZ is not expected to be a trans-specific/trans-gender rodent carcinogen, an additional study with higher doses is not warranted.

It is also noted that the number of cells/animals that were scored was lower than OECD guidelines (50 rather than 100).

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C.6. Carcinogenicity

C.6.a. 100-Week Oral Carcinogenicity Study in Mice

(GLP; Report: TT#94-061-0,-1; Vols. 26-27)

Conducted by: MRL, West Point, PA

Study Dates: 9/8/94 - 8/1/96

Summary:

RIZ was administered by gavage at doses of 0, 2, 25 and 125 mg/kg/day to CD-1 mice (50/sex/dose group, 2 control groups). The initial study plan called for a 106 week study; the study was terminated at week 100 because the mortality incidence exceeded 50% in most groups. Because RIZ is relatively non-toxic to rodents, dosage selection was based on toxicokinetic information from subchronic toxicity and toxicokinetic studies. The mouse:human plasma exposure ratio [based on the sponsor's value of human exposure at the projected therapeutic dose of 10 mg] from those studies indicated that the exposure ratios achieved in HDM (440-fold) and HDF (314-fold) are well in excess of the 25-fold ratio recommended by ICH. The ratios based on the human exposure at the MRHD (30 mg/day; AUC = 160 ng.hr/ml) were approximately 200 in both sexes. A limited number of parameters were assessed (body weight, ophthalmology, palpable masses, gross/histopathology).

According to the sponsor's survival analysis, there were no differences in mortality among treatment groups; the Agency's analysis also found no evidence of a treatment-related mortality trend (see Appendix 2). The body weight gain in HDM and HDF were significantly reduced (10%) based on group mean values at study initiation and termination. However, there were no differences in terminal body weights, which is considered a more appropriate parameter for assessing toxicity in a chronic study. No other treatment-related effects were evident on any other parameter. The NOAEL for the study is greater than 125 mg/kg/day.

In the opinion of the reviewer, the study was acceptable as a valid assessment of the carcinogenic potential of RIZ in mice as the deficiencies of the study (lack of toxicokinetic data from the study animals, slightly premature termination of the study) are considered minor. Toxicokinetic data, especially at later time points, would have been useful for verifying that a 25-fold mouse:human exposure ratio was actually achieved. Based on the wide margin between expected clinical exposures and exposures in the mouse 5-week toxicokinetic study, it is unlikely that a 25-fold ratio was not achieved in the present study. Thus, under the condition of the study, there was no evidence of tumorigenic potential associated with long-term RIZ administration in mice at plasma exposures well in excess of expected human exposures.

Methods:

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Animals: Crl: CD-1 (ICR)BR mice, 6 wks old; males: , females:

N: 250/sex (50/sex/group; 2 controls);

Housing: Group 2-3 cage

Dosages: 2, 25, 125 mg/kg/day (calculated as the free base)

Dose selection was based on toxicokinetic considerations because RIZ is relatively non-toxic in rodents. Toxicokinetics were determined in a 5-week study of 25, 125, 250 and 500 mg/kg/day. The relevant comparisons of mouse and human pharmacokinetic data (corrected for plasma protein binding) are shown in the sponsor's TABLE 1:

TABLE 1

MK-0462 (L-705,126)					
CD-1 Mouse Pharmacokinetics and Mouse to Human AUC Ratios					
Study (Duration)	Dose (mg/kg/day)	Mouse AUC*		AUC* Ratio (Mouse/Human)**	
		Male	Female	Male	Female
TT #93-086-0 (5 weeks)	25	5.74	5.15	96	64
	125	26.38	25.15	440	314
	250	60.85	57.36	1014	717
	500	132.41	100.15	2207	1252

* AUC unit: $\mu\text{g}\cdot\text{hr}/\text{ml}$ (unbound drug = total drug AUC x 0.82 for mice or total drug AUC x 0.86 for humans)
 ** Comparison is based on human male and female plasma unbound drug AUC of 0.06 and 0.08 $\mu\text{g}\cdot\text{hr}/\text{ml}$, respectively, following the proposed oral therapeutic dose of 10 mg or 0.2 mg/kg.

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Toxicokinetics were not determined in the CA study, so any potential changes in exposure that occur with repeated administration of RIZ to mice are not known. Also, it is noted that different vehicles were used in the subchronic (0.5% methylcellulose) and 2-year (water) studies. However, the data from the 5-week study suggest that the mouse:human exposure ratios far exceed the 25-fold ratio recommended by the ICH. The reviewer considers it unlikely that repeated drug treatments or different vehicles would result in a lowering of plasma exposures to RIZ to an extent whereby a 25-fold exposure ratio would not be achieved.

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Route/Freq: one daily gavage administration
 Vehicle: deionized water (10 ml/kg)
 Lots: 004B015, 004B006, 004B007, 004B008, 004B010, 004B013, 004B014, 004B015, 004B024 ().

Parameters monitored:

- clinical signs - weekly
- palpation for masses - monthly beginning week 26
- body weights - 1-2X weekly
- ophthalmic exam* - predose, wks 53 and 93
- hematology - at pathologist's discretion for leukemia diagnosis
- histopathology - see Appendix table for tissues

* indirect ophthalmoscopy for routine exams; lens exam on control and HD at week 70

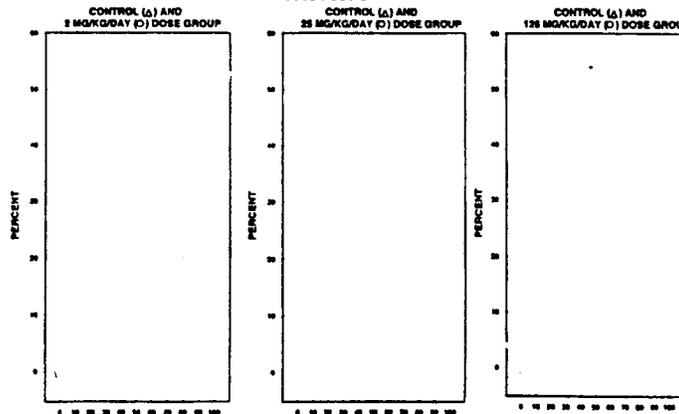
Statistics: The primary method of tumor incidence analysis was the Trend Test (Peto *et al.*, 1980); a secondary test of significant findings was conducted that adjusted for the multiplicity of tumor sites that were analyzed. For additional details, the Agency's statistical review should be consulted.

Results:

Mortality: The mortality among groups is shown in the table below, and also graphically in the sponsor's figures 1 and 2. According to the sponsor's statistical analysis, there was no significant increase in mortality among the drug-treated groups. The graphical presentation suggests a possible trend of increased mortality during weeks 0-50 in HDF, weeks 30-50 in MDF, and over the latter portions of the study in HDM. The Agency's statistical analysis should be consulted for confirmation of the sponsor's analyses. No increases in unusual causes of death or reasons for sacrifice were seen in the drug-treated groups.

		% Mortality	
Group		males	females
Con1		62	52
Con2		34	52
2	mg/kg	44	54
25	"	38	52
125	"	58	42

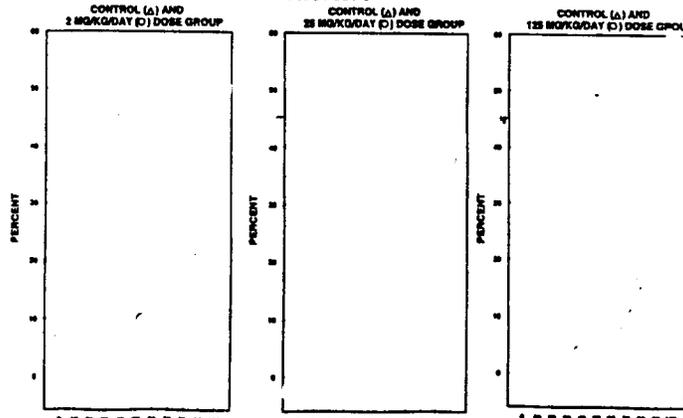
FIGURE 1: CUMULATIVE PERCENT MORTALITY - FEMALE
MK-0462 : 100 - WEEK ORAL CARCINOGENICITY STUDY IN MICE
TT#94-061-0



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FIGURE 2: CUMULATIVE PERCENT MORTALITY - MALE
MK-0462 : 100 - WEEK ORAL CARCINOGENICITY STUDY IN MICE
TT#94-061-0



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Clin Obs: No treatment-related signs were observed/reported by the sponsor.

Body Wt: The body weight time-course profiles suggest that HD group weights tended to be lower than other treatment groups beginning at weeks 30-40 (Sponsor Figures A-1, A-2). At termination, the body weight gain reduction in the HD groups was statistically significant. The difference in weight gain reported by the sponsor for MDF was not the same as shown below (and it is unclear what value the sponsor used as their pretest value). Although the body weight gain reductions suggest that the HD was close to the MTD, group mean differences in terminal body weight were negligible.

FEMALES

	pretest	WK 98	Δ	Δ bwg (%)	Δ term bw (%)
C1	22.5	34.6	12.1	-	-
C2	23.2	36.9	13.7	-	-
2 mg/kg	22.9	35.7	12.8	0	0
25 "	22.9	34.0	11.1	-14%	-5%
125 "	23.2	34.8	11.6	-10%	-3%

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MALES

	Pretest	WK 98	Δ	Δ bwg (%)	Δ term bw (%)
C1	28.7	43.9	15.2	-	-
C2	28.6	43.0	14.4	-	-
2 mg/kg	29.0	44.3	15.3	+3%	+2%
25 "	29.2	44.6	15.4	+4%	+3%
125 "	29.6	42.9	13.3	-10%	-1%

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Figure A-1. MK-0462: 100-Week Oral Carcinogenicity Study in Mice. TT #94-061-0
Average Body Weights for Female Mice

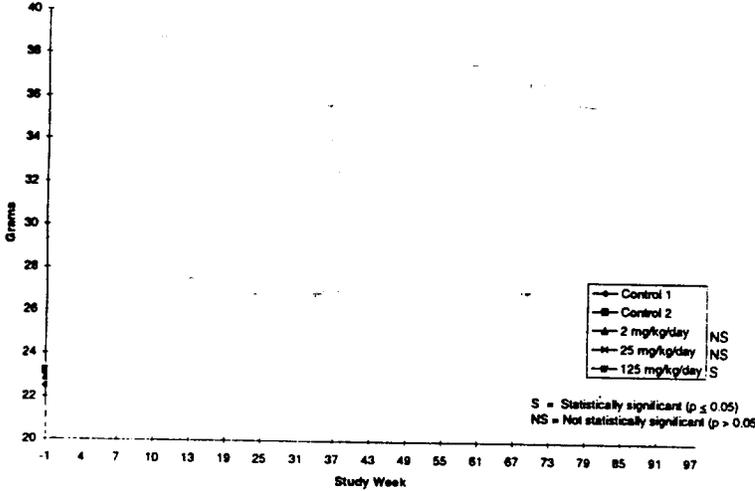
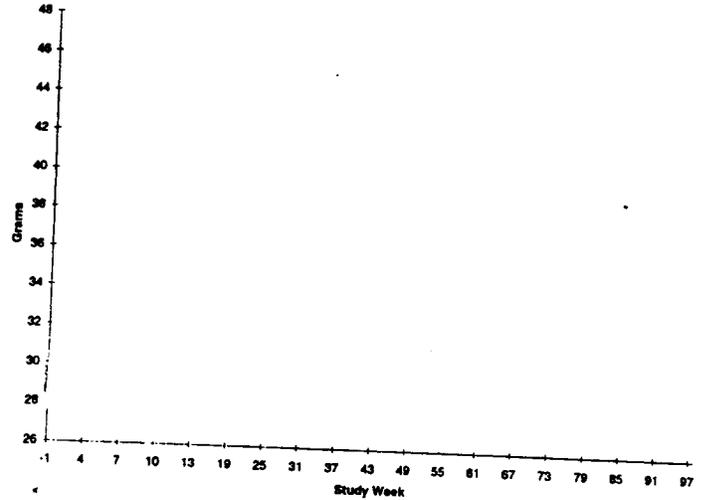


Figure A-2. MK-0462: 100-Week Oral Carcinogenicity Study in Mice. TT #94-061-0
Average Body Weights for Male Mice



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Ophthalmoscopy: No treatment-related effects

Palpable Masses: No treatment-related effects

Gross/Histopathology:

Non-neoplastic lesions: There was no evidence of treatment-related gross pathological or histopathological lesions.

Neoplastic lesions: By the sponsor's analysis, a significant ($P < 0.05$) positive trend was found for one tumor type:

	C1	C2	2	25	125	p
liver hemangioma (M)	0	0	0	3	1	0.029

A trend test adjusted for the multiplicity of tumor sites analyzed was not significant. The lack of dose-relationship supports the sponsor's contention that the tumors were not treatment-related. In addition, this tumor type was seen in females with group distribution more consistent with that of a spontaneously-arising tumor (2 Con, 3 LD, 2 MD, 1 HD).

C.6.b. 106-Week Oral Carcinogenicity Study in Rats

(GLP; Report: TT#94-060-0; Vols. 25-26)

Conducted by: MRL, West Point, PA Study Dates: 8/17/94 - 8/22/96

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Summary:

RIZ benzoate was administered by gavage at doses of 2, 25 and 125 mg/kg/day to Sprague-Dawley rats (50/sex/dose group, 2 control groups) for 106 weeks. Because RIZ is relatively non-toxic in rodents, dosage selection was based on toxicokinetic information from subchronic and chronic toxicology studies [an AUC value for the 125 mg/kg dose was interpolated based on the linear kinetics of RIZ over a range from]. The rat:human plasma exposure ratios [based on human exposures at the projected therapeutic dose of 10 mg] were 687-fold in HDM and 519-fold HDF, which far exceed the 25-fold ratio recommended by ICH. The ratios based on the human exposure at the MRHD (30 mg/day; AUC = 160 ng.hr/ml) were greater than 300. A limited number of parameters were assessed in the carcinogenicity study (body weight, ophthalmology, palpable masses, gross and histopathology).

According to the sponsor's analysis, the mortality rate in the HD groups was significantly greater than controls (48 and 52% in HDM&F, respectively vs. 36 and 38% in ConM&F). The Agency's statistical analysis found no evidence of a treatment-related mortality trend, and that survival rates were adequate for tumorigenicity assessment purposes. No specific drug-related causes of death were identified. Body weight gain was significantly reduced in MDM and HDM, but the mean reductions in terminal body weight or weight gain did not exceed 10% in any group. Drug-related clinical signs were minimal (salivation - HD, foot pad reddening - MD, HD).

No clearly treatment-related neoplastic or non-neoplastic lesions were observed. A positive ($P < 0.05$) trend test was observed for two tumor types, uterine stromal sarcoma and pancreatic islet cell adenoma. However, uterine tumor incidence was low (1 LD, 2 HD), and the background incidence of pancreatic tumors was high. Thus, a treatment relationship is unlikely. The NOAEL was 2 mg/kg in males, and 25 mg/kg in females (AE = clinical signs).

The absence of treatment-related lesions supports the sponsor's contention that the drug is nontoxic in rodents, and justifies the use of plasma exposure data as the basis for dose selection. The sponsor did not conduct a toxicokinetic study in the test animals or a satellite group. However, the rat:human exposure ratios that were determined in previous studies were sufficiently high to allow for the acceptance of this approach. Thus, under the conditions of the study, there was no evidence of tumorigenic potential associated with long-term RIZ administration in rats at plasma exposures well in excess of expected human exposures.

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Methods:

Animals: Sprague-Dawley rat, [CrI:CD(SD)BR]; 38 days old; M: F:

N: 250/sex (50/sex/group; 2 controls);

Dosages: 2, 25, 125 mg/kg/day (calculated as the free base)

Dose selection was based on toxicokinetic considerations because RIZ is relatively non-toxic in rodents. The toxicokinetic studies used for the basis of selection were a 21-week study of 10, 50, and 250 mg/kg/day, and a 4-week study of 500 and 1000 mg/kg/day. The relevant comparison of rat and human exposures are shown in the sponsor's TABLE 1:

TABLE 1
MK-0462 (L-705,126)
SD Rat Pharmacokinetics and Rat to Human AUC Ratios

Study (Duration)	Dose (mg/kg/day)	Rat AUC*		AUC* Ratio (Rat/Human)**	
		Male	Female	Male	Female
TT #93-011-0 (21 weeks)	(2)***	0.49	0.49	8	6
	10	2.47	2.55	41	32
	50	16.49	16.60	275	208
	(125)***	41.23	41.49	687	519
	250	94.86	86.29	1581	1079
TT #93-085-0 (4 weeks)	500	134	176	2233	2200
	1000	431	348	7183	4350

* AUC unit: $\mu\text{g}\cdot\text{hr}/\text{ml}$ (unbound drug = total drug AUC x 0.82 for rats or total drug AUC x 0.86 for humans)
** AUC ratio (rat/human) comparison is based on human male and female unbound drug AUC of 0.06 and 0.08 $\mu\text{g}\cdot\text{hr}/\text{ml}$, respectively, following the oral proposed therapeutic dose of 10 mg (0.2 mg/kg).
*** AUC values for the 2 and 125 mg/kg/day dose groups were approximated from the observed pattern of linear pharmacokinetics that was dose proportional over the dose range of 10 to 250 mg/kg/day (TT #93-111-0).

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The sponsor used the intended therapeutic human dose (10) mg for comparisons. The maximum recommended human dose is 30 mg, so the exposure ratios should be decreased by approximately 3-fold. Toxicokinetics were not determined in the CA study so a true exposure ratio cannot be derived for the study. However, the data presented above suggest that the rat:human exposure ratios far exceed the 25-fold ratio recommended by the ICH. Note also that the sponsor presents the ratios corrected for plasma protein binding, which was relatively similar in the two species.

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Route/Freq: one daily gavage administration
Vehicle: deionized water (5 ml/kg)
Lots: 004B014, 004B015, 004B021, 004B024
Housing: Individual

Parameters monitored:

- clinical signs - weekly
- palpation for masses - monthly beginning week 26
- body weights - 1-2X weekly
- ophthalmic exam* - predose, wk 53-54 and 102-103
- hematology - at pathologist's discretion for leukemia diagnosis
- histopathology - see Appendix table for tissues

* indirect ophthalmoscopy for routine exams; slit-lamp was used at the discretion of the examiner)

Statistics: The primary method of tumor incidence analysis was the Trend Test (Peto et al., 1980); a secondary test of significant findings was conducted after adjusting for the multiplicity of tumor sites that were analyzed. For additional details, the Agency's statistical review can be consulted.

Results:

sponsor's analysis of

Mortality: The mortality among groups is shown in the table below, and also graphically in the sponsor's figures. The increase in mortality was statistically significant at week 102 for HDF and week 99 for HDM [the sponsor considers the survival rate at the HD adequate for tumorigenicity assessment purposes]. There was no increase in unusual causes of death or reasons for sacrifice in these groups.

Sex		C ^a	L ^b	M ^c	H ^d
Female	R	33	21	20	26
	NT	100	50	50	50
Percent Dead		33%	42%	40%	52%
Trend P-value ^e				0.173	0.028
Male	R	33	19	18	24
	NT	100	50	50	50
Percent Dead		33%	38%	36%	48%
Trend P-value ^e				0.422	0.049

R = Number of animals dying prior to scheduled sacrifice.
NT = Number of animals tested in the study.

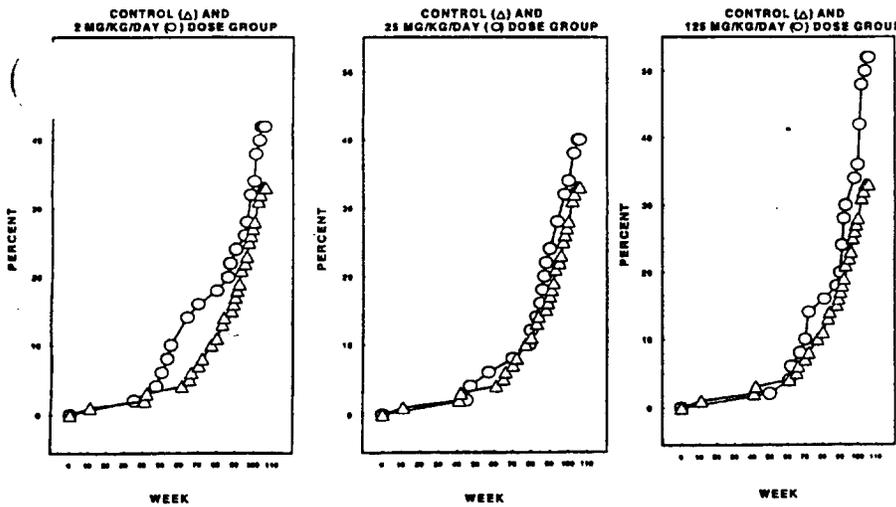


FIGURE 1 : CUMULATIVE PERCENT MORTALITY - FEMALE
MK - 0462: 106 - WEEK ORAL CARCINOGENICITY STUDY IN RATS
TT#94-060-0

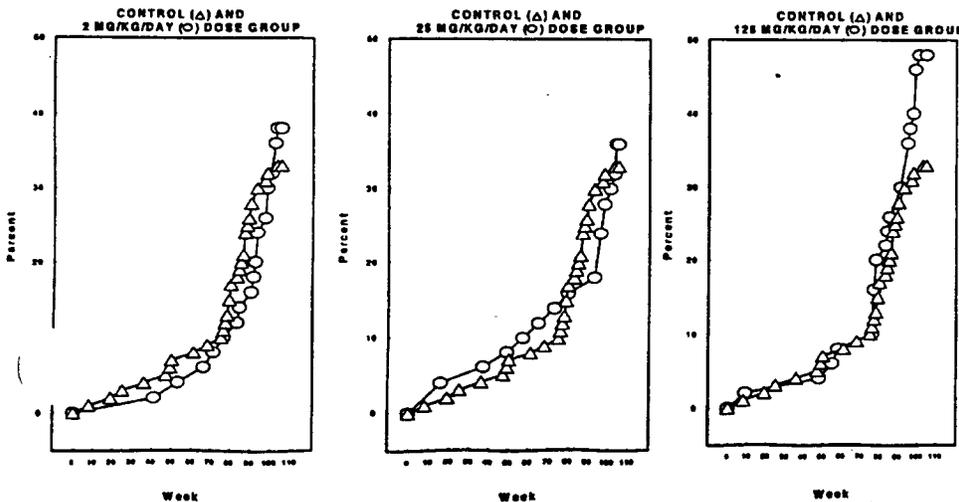


FIGURE 2 : CUMULATIVE PERCENT MORTALITY - MALE
MK - 0462 : 106 - WEEK ORAL CARCINOGENICITY STUDY
TT#94-060-0

Clin Obs: Post-dose salivation was recorded in HDM and HDF during weeks 1-4. Recording of this sign was then discontinued by the sponsor on the basis that it had been adequately documented in previous studies. Foot pad reddening was evident in MD and HD animals between at 30 min and 6 hrs after dosing.

Body Wt: The body weight-time course profiles suggest that weights in the HD groups tended to be lower than the other treatment groups beginning at weeks 30-40 (Sponsor Figures A-1, A-2). However, as shown in the Table below, the magnitude of the changes in either terminal body weights or body weight gains did not exceed 10%, and should not have negatively impacted the study.

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FEMALES

	pretest	WK 104	Δ	Δ bwg (%)	Δ term bw (%)
C1	141	329	188	-	-
C2	148	319	171	-	-
2 mg/kg	143	319	176	-2%	-2%
25 "	143	324	181	0	0
125 "	142	309	167	-7%	-6%

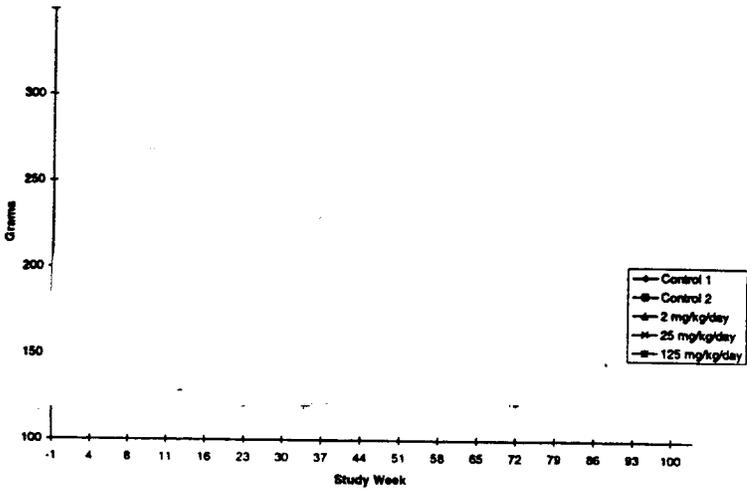
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MALES

	Pretest	WK 105	Δ	Δ bwg (%)	Δ term bw (%)
C1	178	611	433	-	-
C2	173	612	439	-	-
2 mg/kg	178	617	439	0	0
25 "	178	601	423	-3%	-2%
125 "	180	572	392	-9%	-6%

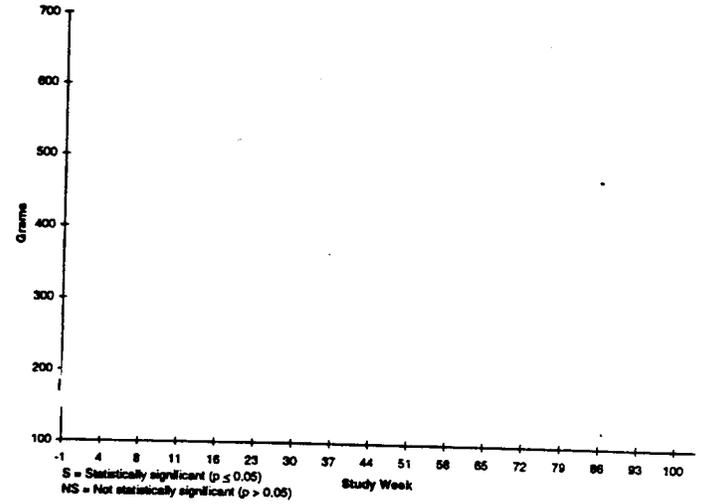
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Figure A-1. MK-0462: 106-Week Oral Carcinogenicity Study in Rats. TT #94-050-0
Average Body Weights for Female Rats



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Figure A-2. MK-0462: 106-Week Oral Carcinogenicity Study in Rats. TT #94-060-0
Average Body Weights for Male Rats



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Ophthalmoscopy: No treatment-related effects

Gross/Histopathology:

Non-neoplastic lesions: There was no evidence of dose-related increases in the incidence of any gross pathological or histopathological lesions.

Neoplastic lesions: By the sponsor's analysis, a significant ($P < 0.05$) increased trend was found with only two tumor types:

	C1	C2	2	25	125	p
Uterus - Stromal Sarcoma	0	0	1	0	2	0.048
Pancreas - Islet Adenoma (M)	5	5	8	5	10	0.045

The pancreatic tumor is not significant when considered as a common tumor (significance level $p < 0.01$). The uterine tumor was not significant by a Trend test adjusted for the multiplicity of tumor sites analyzed. The use of this analysis should be considered by the Agency statistician, but the low incidence of the tumors and absence from the MD group suggest that the likelihood of an incidental occurrence.

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D. PHARMACOKINETIC/ADME STUDIES

The following table summarizes the parameters of the pharmacokinetic studies conducted with RIZ. Individual reports of each study were not provided in most cases. Generally, individual study results were combined and summarized in a single report.

Type of Study	[Reference No.]	Species/Sex	Route	Duration of Treatment	Dose	Conformity to GLP Y/N
Mass balance, <i>in vivo</i> metabolism	[G-1; G-12]	Rat/Male	i.v., p.o.	Acute	3 mg/kg	N
Biliary excretion	[G-2]	Rat/Male	i.v., p.o.	Acute	3 mg/kg	N
Mass balance, <i>in vivo</i> metabolism, pharmacokinetics, absorption kinetics	[G-1; G-12]	Dog/Male	i.v., p.o.	Acute	1 mg/kg	N
Biliary excretion	[G-2]	Dog/Male	i.v.	Acute	1 mg/kg	N
Pharmacokinetics	[G-1; G-3]	Rat/Male	i.v.	Acute	3 mg/kg	N
Absorption kinetics	[G-1; G-3]	Rat/Male	p.o.	Acute	3, 10 mg/kg	N
Pharmacokinetics, absorption kinetics	[G-3]	Dog/Male, Female	i.v., p.o.	Acute	1 mg/kg (i.v.) 2 mg/kg (p.o.)	N
Pharmacokinetics, absorption kinetics	[G-3]	Monkey/Male	i.v., p.o.	Acute	1 mg/kg (i.v.) 2 mg/kg (p.o.)	N
Extrahepatic clearance	[G-1]	Rat/Male	i.v., i.a.	Acute	3 mg/kg	N
Dose dependence, <i>in vivo</i> metabolism	[G-1; G-12]	Dog/Male	p.o.	Acute	1, 2, 5 mg/kg	N
Tissue distribution	[G-8]	Rat/Male	i.v., p.o.	Acute	3 mg/kg	N
<i>In vitro</i> plasma protein binding	[G-1; G-9; G-26]	Rat, Mouse, Rabbit, Dog, Human plasma	N/A	N/A	0.05-5 µg/ml	N
<i>In vitro</i> erythrocyte partitioning	[G-1; G-10]	Rat, Dog, Human whole blood	N/A	N/A	1 µg/ml	N
<i>In vivo</i> metabolism	[G-13]	Human/Male	p.o.	Acute	60 mg	N
<i>In vivo</i> metabolism	[G-14]	Rat/Male, Female	p.o.	Acute	3 mg/kg	N
<i>In vivo</i> metabolism	[G-14]	Mouse/Male, Female	p.o.	Acute	3 mg/kg	N
<i>In vivo</i> metabolism, mass balance, pharmacokinetics, absorption kinetics	[G-15]	Human/Male	i.v., p.o.	Acute	3 mg (i.v.) 10 mg (p.o.)	N
<i>In vivo</i> metabolism	[G-16]	Rat/Male, Female	p.o.	Chronic (53 weeks)	10, 50 mg/kg/day	N
<i>In vivo</i> metabolism	[G-16]	Mouse/Male, Female	p.o.	Chronic (5 weeks)	25, 125 mg/kg/day	N
<i>In vivo</i> metabolism	[G-27]	Rabbit/Female	p.o.	5 days	50 mg/kg/day	N
<i>In vivo</i> metabolism	[G-17; G-18; G-19]	Human/Male, Female	p.o.	Acute	10 mg	N
<i>In vitro</i> 5HT receptor binding activity	[G-20]	Human brain cortical homogenates	N/A	N/A	N/A	N
<i>In vitro</i> metabolism	[G-1]	Rat liver, lung, kidney microsomes	N/A	N/A	N/A	N
<i>In vitro</i> metabolism	[G-1]	Rat, Dog, Human liver microsomes	N/A	N/A	N/A	N
<i>In vitro</i> metabolism	[G-21]	Human liver slices	N/A	N/A	N/A	N
Metabolizing enzyme identification <i>in vitro</i>	[G-22]	Human liver microsomes and S9 fraction	N/A	N/A	N/A	N
Moclobemide interaction <i>in vitro</i>	[G-23]	Human liver S9 fraction	N/A	N/A	N/A	N
β-Blocker interaction <i>in vitro</i>	[G-24]	Human liver S9 fraction	N/A	N/A	N/A	N
<i>In vitro</i> cytochrome P-450 inhibition	[G-25]	Human liver microsomes	N/A	N/A	N/A	N

i.v. = intravenous p.o. = oral N/A = not applicable

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Reference List of PK/ADME Reports

- G-1. Absorption, Distribution, Metabolism, and Excretion of L-705,126 in Rats and Dogs.
- G-2. Biliary Excretion of [¹⁴C]MK-0462 in Rats and Dogs.
- G-3. L-705,126, A 5-HT_{1D} Receptor Agonist for the Treatment of Migraine: Nonclinical Data.
- G-4.* MK-0462: Fifty-Three-Week Oral Toxicity Study in Rats with a 27-Week Interim Necropsy (TT #93-111-0)
- G-5.* MK-0462: Fourteen-Week Oral Range-Finding Study in Rats (TT #93-085-0).
- G-6.* MK-0462: Five-Week Oral Toxicokinetic Study in Mice (TT #93-086-0).
- G-7. L-705,126: Fourteen-Week Oral Toxicity Study in Dogs (TT #92-104-0).
- G-8. Tissue Distribution of [¹⁴C]MK-0462 in Rats Following Intravenous or Oral Administration at 3 mg/kg.
- G-9. Binding of [¹⁴C]MK-0462 to Mouse Plasma Proteins.
- G-10. Blood-to-Plasma Partitioning of [¹⁴C]MK-0462 in Human Blood: Time and Temperature Dependence.
- G-11. MK-0462: Oral Toxicokinetic Study in Pregnant Rats with Secretion in Milk (TT #95-701-0).
- G-12. *In Vivo* Metabolism of [¹⁴C]MK-0462 in Rats and Dogs: Confirmation of Mono-N-Desmethyl-MK-0462 (L-706,248) in Urine.
- G-13. Isolation and Identification of Urinary Metabolites of MK-0462 in Humans.
- G-14. Metabolite Profiles of [¹⁴C]MK-0462 in Mouse Plasma and Urine and Rat Plasma following a 3 mg/kg P.O. Dose of [¹⁴C]MK-0462.
- G-15. An Open Study to Investigate the Disposition of a Single Oral and a Single Intravenous Dose of [¹⁴C]MK-0462 in Healthy Male Volunteers.
- G-16. Metabolite Profiles of MK-0462 from Safety Assessment Studies in Rats and Mice.
- G-17. Metabolite Profiles of MK-0462 in Human Plasma and Urine Following Single or Multiple 10 mg Oral Doses.
- G-18. A Double-Blind, Randomized, Placebo-Controlled, Two-Period Crossover Study to Investigate the Effects of Moclobemide on the Pharmacokinetics of MK-0462 in Young Healthy Subjects.
- G-19. Effects of Moclobemide on Metabolism of MK-0462 in Humans.
- G-20. MK-0462 (L-705,126), A 5-HT_{1D} Receptor Agonist for the Treatment of Migraine: Additional Nonclinical Data.
- G-21. Metabolism of [¹⁴C]MK-0462 by Human Liver Slices.
- G-22. *In Vitro* Metabolism of MK-0462 by Human Liver Subcellular Fractions: Identification of MAO-A as a Primary Enzyme Responsible for Metabolism.
- G-23. Comparative Metabolism of MK-0462 and its N-Mono- and Di-Desmethyl Metabolites, L-706,248 and L-733,283, by Human Liver S9.
- G-24. Effect of β -Blockers on the Metabolism of [¹⁴C]MK-0462 and [¹⁴C]Sumatriptan by Human Liver S9 Fractions.
- G-25. Evaluation of MK-0462 as an Inhibitor of Human Liver Microsomal Cytochrome P-450 Activities.
- G-26. Binding of [¹⁴C]MK-0462 to Rabbit Plasma Proteins.
- G-27. Metabolite Profiles of MK-0462 in Rabbits.

Reports located in Vol. 30.

D.1. Single Dose Pharmacokinetics/Absorption/Elimination

D.1.a. Animal Studies (Refs. G-1, G-2)

The single-dose pharmacokinetics and elimination of [¹⁴C]-RIZ were evaluated in rats (i.v.: 3 mg/kg; p.o.: 3 and 10 mg/kg) and dogs (i.v.: 1 mg/kg; p.o.: 1, 2 and 5 mg/kg) (Ref. G-1). Results from the rat intravenous studies indicated a short half-life, a plasma clearance rate similar to hepatic flow, and a moderately high volume of distribution. The amount of radiolabel absorbed after oral administration was 78%, and RIZ bioavailability was moderately high. Absorption was usually rapid, but in some animals a more prolonged, sustained absorptive phase was observed. Generally constant levels were apparent for 2 hrs. The PK profile in dogs was similar to that seen in rats (short terminal half-life, moderate Vd, good bioavailability, high absorption).

Single-Dose [¹⁴C]-Rizatriptan Pharmacokinetics in Rats and Dogs

Species	Dose, route	t _{max} (hr)	C _{max} (ng/ml)	AUC _(0-inf) (ng.h/ml)	Vd _{ss} (l/kg)	Cl (ml/min.kg)	t _{1/2} (min)	F
Rat (n = 3-4)	3, i.v.		~1000	709	4.3	70.5	65	
	3, p.o.	71	324	454			102	64
	10, "	26	1803	2256			103	95
Dog (n = 3-4)	1, i.v.			367	3.2	45.9	72	
	1, p.o.	26	152	173			67	47
	2, "	30	300	408			70	56
	5, "	19	1008	1284			76	70

Excretion studies in rats demonstrated that [¹⁴C]-RIZ was eliminated in both urine and feces, mostly within 24 hr; the urinary fraction was slightly greater after i.v. treatment. Renal excretion was the primary route in dogs after either i.v. or p.o. administration. The urinary:fecal excretion results were confirmed by a biliary excretion study in bile duct-cannulated rats and dogs (Ref. G-2), except that a similar percent of dose was excreted in rat bile after either route (14-18%).

Excretion of [¹⁴C]-Rizatriptan in Rats and Dogs

Species	Dose	Interval	Urine	Feces	Total
Rat (n = 4)	3, i.v.	0-24 hr	51	20	71
		0-144 "	58	23	81
	3, p.o.	0-24 "	40	37	77
		0-144 "	45	42	87
Dog (n = 4)	1, i.v.	0-24 "	70	9	79
		0-96 "	75	12	87
	1, p.o.	0-24 "	70	9	79
		0-96 "	75	13	88

D.1.b. Human Studies (Ref. G-15)

The single dose pharmacokinetics of [¹⁴C]-RIZ was studied in 6 healthy males after administration of 3 mg/kg, i.v. (30 min infusion) or 10 mg/kg, orally. The similar urinary recoveries after the i.v. and p.o. routes suggest that the radiolabel is well-absorbed, but the bioavailability of RIZ was only 47%, possibly because of significant first pass metabolism. The renal clearance of RIZ exceeded the GFR indicating active tubular secretion, but was only 25% of the plasma clearance implicating non-renal routes as the primary means of RIZ elimination. Most of the radiolabel was excreted in urine within 24 hr (Table below; shown is 0-120 hr interval).

Single-Dose [¹⁴C]-Rizatriptan Pharmacokinetics in Human Males

Dose, route	Analyte	t _{max} (hr)	C _{max} (ng/ml)	AUC _(0-inf) (ng.h/ml)	Vd _{ss} (l/kg)	Cl _p (ml/min)	Cl _r (ml/min)	t _{1/2} (hr)	F
10, p.o.	RIZ	1.4	19.8	59.8			396	2.2	47
	[¹⁴ C]	1.8	59.0	333.8				5.6	
3, i.v.	RIZ			38.1	154	1325	349	2.4	
	[¹⁴ C]			125.2				5.8	

n = 6

Excretion of Radioactivity by Humans

	% Dose Excreted (0-120 hr)		
	urine	feces	total
3, i.v.	89.5	4.4	93.9
10, p.o.	82.4	11.5	93.9

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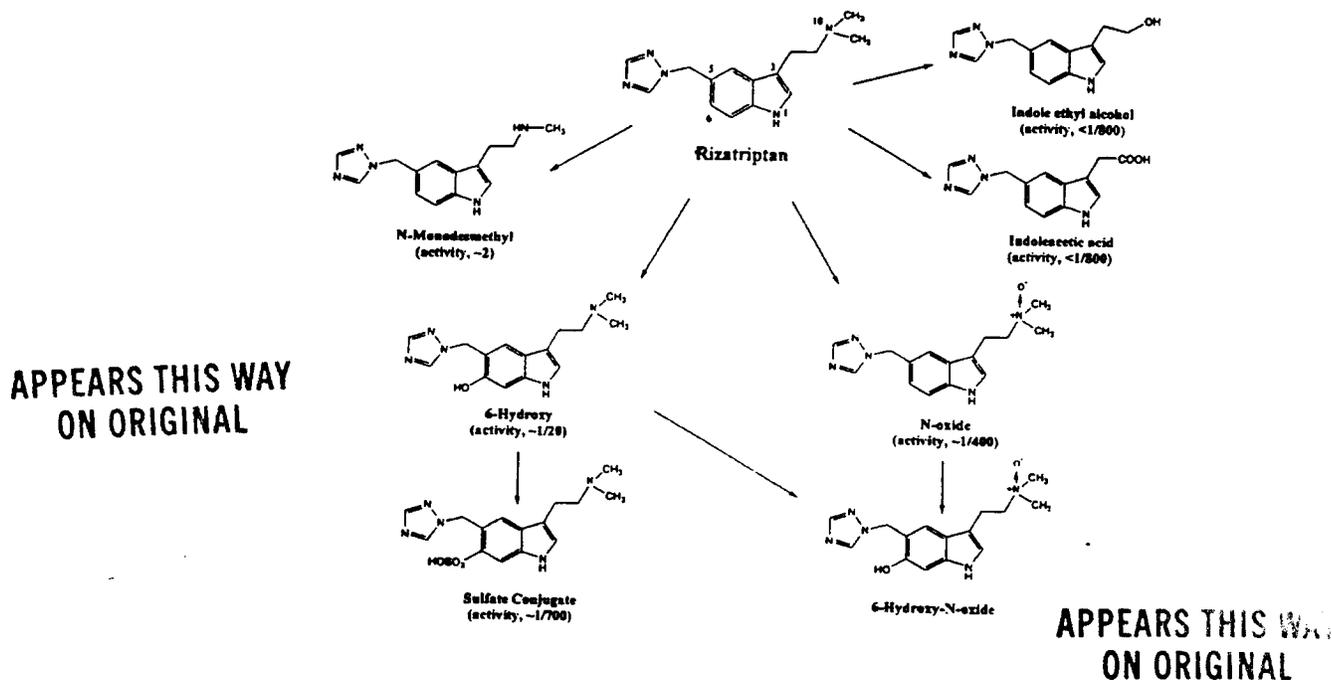
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D.2. Metabolism

In vivo studies of RIZ metabolism in rats, dogs, mice, rabbits and humans identified several qualitatively similar pathways among species. The major metabolic routes for RIZ were aromatic hydroxylation at 6-position of the indole ring (6-hydroxy-RIZ), N-oxidation of the tertiary amine (RIZ-N¹⁰-oxide), oxidative deamination to the indoleacetic acid (RIZ-IAA), and sulfate conjugation of 6-hydroxy-RIZ (see sponsor Figure G-1). A subsequent analysis (Ref. G-12) confirmed that a small amount of N-demethylated metabolite is also present in rat, dog and human urine.

Figure G-1

Pathways of Metabolism of Rizatriptan^a [Refs. G-1; G-12; G-13; G-14; G-15; G-16; G-17; G-19; G-20; G-27]



D.2.a. *In vivo* Metabolism in Animals (Refs. G-1, G-12, G-14, G-16, G-27)

Metabolic profiles were determined in rats after administration of 3 mg/kg, i.v. or p.o., and in dogs following 1 mg/kg, i.v. or p.o. Similar metabolites appeared in rat urine after either route. Four major and 3-4 minor metabolite peaks were observed by ; the parent compound was the largest fraction by either route. In dog urine, five metabolite peaks were identified. The parent compound was largest fraction after intravenous administration, but comparable amounts of three metabolites were the major fractions after oral administration.

Comparison of Rat and Dog Urinary Profiles following [¹⁴C]-Rizatriptan

Fraction	Rat (0-8 hr urine)		Dog (0-24 hr urine)	
	3 mg/kg, iv	3 mg/kg, po	1 mg/kg, iv	1 mg/kg, po
rizatriptan	52.2	37.2	21.3	7.4
6-sulfate	3.1	4.4	5.6	5.4
UK-1	1.2	2.2		
UK-2	1.4	3.8		
6-hydroxy	1.0	0.9	6.9	7.4
UK-3	0.6	1.7		
6-hydroxy-N ¹⁰ -oxide	-	3.5	14.2	15.4
UK-4	4.5	5.3		
N ¹⁰ -oxide	19.3	16.7	19.5	14.9
indoleacetic acid	8.7	10.7	14.5	14.6

(n = 1)

The metabolite profiles of [¹⁴C]-RIZ were determined in mouse and rat plasma (30 or 60 min post-dose) and mouse urine (0-24 hr) following an oral dose of 3 mg/kg (Ref. G-16). Three drug-related compounds (RIZ, RIZ-N¹⁰-oxide, RIZ-IAA) were detected. The IAA metabolite was the major plasma fraction in both species, and the parent compound was the major mouse urinary fraction. No significant sex differences were evident (shown are the means of M+F):

APPEARS THIS WAY ON ORIGINAL			Percent Radiolabel Recovered		
			RIZ	RIZ-N ¹⁰ - oxide	RIZ-IAA
Mouse	Plasma	30 min	16	6	70
		60 min	8	3	86
	Urine	0-24 hr	54	9	22
Rat	Plasma	60 min	30	17	45

The metabolite profile of RIZ in rabbits was determined using a pooled plasma sample from 3 rabbits treated with 50 mg/kg/day for 5 days (ref. G-27). Four analytes were detected:

RIZ > N-monodesmethyl-RIZ > RIZ-IAA > 6-hydroxy-RIZ
 (73%) (18%) (10%) (2%)

D.2.b. *In vivo* Metabolism in Humans (Refs. G-13, G-15, G-17 - 20)

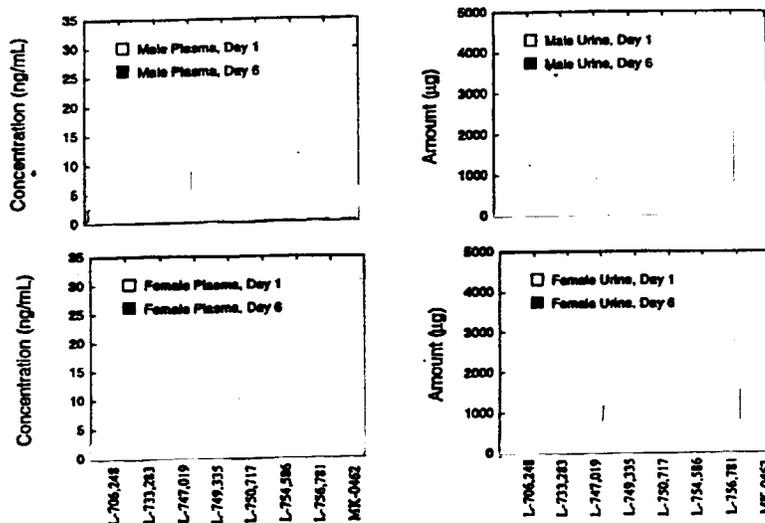
Five metabolites were identified in a pooled human urine sample after a 60 mg oral dose of RIZ to six healthy males: the indole-3-acetic acid, the N-10 oxide, 6-hydroxy-RIZ, the sulfate conjugate of 6-hydroxy-RIZ, and N-desmethyl-RIZ (Ref. G-13).

The plasma and urinary metabolic profile of [¹⁴C]-RIZ was determined in the PK study described in D.1. (6 healthy males; 3 mg/kg, i.v. or 10 mg/kg, p.o.; Ref. G-15). The major radioactive peaks in urine and plasma were the indoleacetic acid (IAA) metabolite and the parent compound (see Table below). Two other metabolites, possibly the 6-sulfate and the N-desmethyl metabolites, were detected in small amounts, but not conclusively identified ($\leq 5.7\%$)

Quantitation of RIZ and Metabolites in Human Samples

		% of Dose		
		RIZ	RIZ-N ¹⁰ -oxide	RIZ-IAA
urine (0-24 hr)	3, i.v.	29.3	3.7	34.6
	10, p.o.	14.0	2.3	51.0
plasma (1-4 hr)	3, i.v.	24.4	9.0	35.6
	10, p.o.	26.6	5.8	40.2

The effect of repeated dosing on RIZ metabolism was assessed in 3 males and 3 females after treatment with either a single 10 mg dose or four days of treatment with 10 mg, t.i.d. (Ref. G-17) Qualitatively similar profiles of plasma and urinary drug-related species metabolites were observed after either regimen. RIZ was the major component, followed by the IAA metabolite. This data is slightly inconsistent with the previous cited study in which RIZ-IAA accounted for approximately two-thirds of the drug-related activity in human urine. The sponsor contends that the profiles were quantitatively similar after single or repeated dosing, but a trend for higher levels of all species is evident in subjects after the repeated dose regimen. No gender differences were apparent.



L-706,248 - N-monodesmethyl
 L-747,019 - N-oxide
 L-749,335 - indole acetic acid
 L-754,586 - 6-hydroxyindole
 L-756,781 - sulfate conjugate
 of 6-hydroxyindole

D.2.c. *In vitro* Metabolism Studies (Refs. G-1, G-21, G-22, G-23, G-25)

In contrast to the *in vivo* studies, *in vitro* metabolism of RIZ in rat liver, lung and kidney microsomes, and dog and human liver microsomes was limited; only the N-oxide and/or IAA metabolites were detected in significant quantities (Ref. G-1):

Species	Prep	Metabolites				Total
		UK-1	6-hydroxy	N ¹⁰ -oxide	IAA	
Rat	Liver, control	0.5	-	1.4	-	1.9
	" , PB	2.4	-	1.9	1.1	5.4
	" , 3-MC	0.9	-	1.2	1.1	3.2
	Lung	-	-	8.8	0.8	9.6
	Kidney	-	-	18.3	0.6	18.9
Dog	Liver	-	1.7	12.7	0.9	15.3
Human (n=3)	Liver	-	-	-	7.6	7.6

An *in vitro* study with human liver slices confirmed the results demonstrating that the IAA was the only identifiable human *in vitro* metabolite of RIZ (G-21).

In vitro studies were conducted to determine the enzymes responsible for RIZ metabolism in humans (G-22). In a study with liver S9 fractions (G-22), the oxidative deamination of RIZ to RIZ-IAA was determined to be catalyzed by MAO-A based on the ability of clorgyline to inhibit the reaction (MAO-B inhibitors were effective only at high concentrations). The reaction was independent of NADPH, and blocked by SKF525A and ketoconazole only at high concentrations, indicating a small or negligible role for the P₄₅₀ system in this pathway. The indole ethyl alcohol metabolite of RIZ was also identified as a RIZ oxidative deamination product; its formation was favored (relative to the IAA) in the presence of NADPH.

A subsequent study on the deamination of RIZ and its N-mono- and di-desmethyl metabolites determined that the process favored the less substituted amines (G-23). Thus, the inability to detect the N-monodesmethyl metabolite in *in vitro* studies may have been due to its rapid deamination.

The inhibitory activity of RIZ versus several P450 isozymes (1A2, 2C9, 2C19, 2D6, 2E1, 3A4/5) was also evaluated *in vitro*. The results are summarized in the following table. The only effect of RIZ was WEAK competitive inhibition of 2D6 (K_i = 1400 nM) (Ref. G-25).

Isozyme	Assay	[Substrate] (μM)	[RIZ] (μM)	Result
1A2	phenacetin-O-deethylase	25-1000	0-10	~ 30% inhibition at 10 μM
2C9	diclofenac 4-hydroxylase	10-100	"	no effect
2C19	mephenytoin-4-hydroxylase	40-400	"	no effect
2D6	bufuralol-1-hydroxylase	5-50	"	$K_i = 1400 \text{ nM}$
2E1	chlorzoxazone-6-hydroxylase	20-500	"	no effect
3A4/5	testosterone-6 β -hydroxylase	25-250	"	no effect

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D.2.d. Metabolic Interactions (Refs. G-18, G-24)

Because of the importance of MAO-A in the biotransformation of RIZ, clinical studies addressed the potential interaction of RIZ with MAO inhibitors (Ref. G-18). These studies were included in the nonclinical section, but should be reviewed in more detail by the Biopharmaceutics reviewer. To summarize briefly, the MAO-A inhibitor moclobemide (150 mg, t.i.d.) did not alter the qualitative profile of RIZ metabolism, but markedly decreased the formation of the IAA metabolite and increased the exposure to RIZ (2.2-fold) and the N-monodesmethyl metabolite (5-fold). These studies suggest that an important drug interaction may exist between RIZ and inhibitors of MAO, particularly of the A isozyme.

The potential influence of β -blockers on the oxidative deamination of RIZ was evaluated *in vitro* (Ref. G-24). Propranolol appeared to have significant inhibitory effects, whereas the remaining compounds tested had only slight (metoprolol) or negligible effects (nadolol, atenolol, timolol)

D.2.e. Pharmacodynamics of Metabolites (Ref. G-20).

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The radioligand binding activity of several RIZ metabolites at human 5-HT receptor subtypes was determined (G-20). There were no unique 5-HT binding activities of any of the metabolites. Only the minor N-desmethyl metabolite was similar to RIZ in its 5-HT_{1D} and 1A affinities. The major metabolite (RIZ-IAA; L-749,335) was devoid of 5-HT receptor affinity.

D.3. Distribution

D.3.a. Tissue distribution in Rats (Ref. G-8)

The tissue distribution of 3 mg/kg ¹⁴C-tolcapone was rapid after intravenous administration to SD rats. Maximum concentrations were reached generally within 5 minutes. Highest levels were in the liver (13.1% of dose) and kidney (3.5%) at 5 min, and small intestine (4.9%) at 1 hr. A similar pattern was observed after oral administration; highest levels were detected at 1 hr (the first time point). By 24 hrs, most of the label had dissipated (85% of i.v. dose recovered, 96% of p.o. dose recovered). There were no unusual tissue accumulations of label, but the tissue:plasma ratios suggested the label was cleared more rapidly from plasma than from tissue.

D.3.b. Plasma Protein Binding (Refs. G-1, G-9, G-16)

Test range = 50-5000 ng/ml

Species	% Bound
rat	18
mouse ^a	19
rabbit ^a	27
dog	12
human	14

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^a data from studies G-9 and G-26

D.3.c. Partitioning into erythrocytes (Ref. G-10):

Rizatriptan partitioned into rat, dog and human erythrocytes (blood:plasma ratios = 1.2 - 1.35) suggesting a possible slower clearance from blood than plasma.

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E. SPECIAL TOXICITY

The section presents some special tox studies of RIZ (inductive effects, hemolytic potential, corneal opacity and permeability, dermal irritation) and qualifying toxicology studies of the "potential" degradant L-783,540, a quaternary ammonium salt of RIZ.

E.1 Special Studies of RIZ

E.1.a. Exploratory P450 Induction Study in Rats of L-705,126, a 5HT_{1D} Receptor Agonist (TT #91-065-0,-3)

RIZ (100 mg/kg/day for 4 days) caused slight increases of cytochrome P₄₅₀-mediated 7-ethoxy-4-trifluoromethylcoumarin O-deethylase (EFCOD) activity in male (↑ 43%) and female (↑ 23%) rats. Liver weights were not increased. Phenobarbital (50 mg/kg/day), a positive control, caused a 7.8- and 5.3-fold induction in males and females, respectively.

The slightly increased EFCOD activity by RIZ is not considered a biologically significant inductive effect.

A rationale for limiting the study to a single substrate was not provided.

E.1.b. L-705,126 Hemolytic Assay: Washed Red Blood Cells and Whole Blood (TT #91-490-8; reported as a memo)

The hemolytic potential of RIZ was assessed in murine, canine and human washed red blood cells. No hemolysis or precipitation was observed.

E.1.c. L-705,126: Effect of L-705,126 in the Bovine Corneal Opacity and Permeability (BCOP) Assay (TT #93-4292)

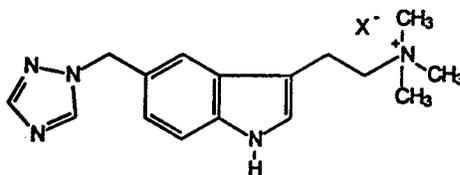
RIZ was evaluated in the bovine corneal opacity and permeability assay, an established *in vitro* test of ocular irritancy. A 20% suspension of RIZ was classified as a moderate ocular irritant.

E.1.d. MK-0462: Exploratory Acute Dermal Irritation Study in Rabbits (TT #93-2624)

The dermal irritancy of a 500 mg application of RIZ to the shaved skin of rabbits was evaluated after 24 hrs. The treatment caused neither systemic toxicity nor local irritation (erythema, eschar, edema).

E.2. Studies of the Degradant Mixture

L-783,540 is a quaternary alkyl trimethylammonium salt that is a degradation product in the RIZ RAPIDISC formulation.



Maximum levels that have been detected at common storage temperatures (25 and 30°C) are after 52 weeks. Higher levels are detected at higher temperatures or with longer storage; the highest levels reported thus far are after storage for 6 months at 40°C. The sponsor conducted the following toxicology qualification studies

- MK-0462/L-783,540: Microbial Mutagenesis Assay (WF #96-8007)
- MK-0462/L-783,540: *In Vitro* Alkaline Elution/Rat Hepatocyte Assay (TT #96-8414, TT #96-8416, TT #96-8420)
- MK-0462/L-783,540: Assay for Chromosomal Aberrations *In Vitro*, in Chinese Hamster Ovary Cells (WF #96-8613)
- MK-0462/L-783,540: Fourteen-Week Oral Toxicity Study in Rats (TT #96-034-0)
- MK-0462/L-783,540: Fourteen-Week Oral Toxicity Study in Dogs (TT #96-035-0)
- MK-0462/L-783,540: Oral Developmental Toxicity Study in Rabbits (TT #97-705-0)
- MK-0462/L-783,540: Oral Developmental Toxicity Study in Rats (TT #97-706-0)

E.2.a. MK-0462/L-783,540: Microbial Mutagenesis Assay (GLP; WF #96-8007; Vol. 31)

Summary:

The combination of RIZ (Lot 004B024) and the degradant was not mutagenic in either the presence or absence of metabolic activation in a standard Ames test under appropriate test conditions. The upper test concentration was limited by solubility.

Strains	Concs RIZ/deg (solvent)	Results
<i>S. typhimur.</i> : TA1535, TA 97a, TA98, TA100 <i>E. coli</i> : WP2, WP2 uvrA, WP2 uvrA pKM101	142.1/2.9, 426.3/8.7, 1421/29, 4263/87, 7105/145 µg/plate (DMSO)	negative
<i>S. typhimurium</i> : as above <i>E. coli</i> : WP2 uvrA pKM101	50, 150, 500, 1500, 5000 µg/plate (DMSO)	negative

Positive controls produced the expected results.

E.2.b. MK-0462/L-783,540: *In Vitro* Alkaline Elution/Rat Hepatocyte Assay
(GLP; TT #96-8414, TT #96-8416, TT #96-8420; Vol. 31).

Summary:

This assay is not part of the ICH core battery or recommended in OECD guidelines, so the study received a cursory review. RIZ + the degradant did not induce cytotoxicity or DNA strand breaks in rat hepatocyte primary cultures at concentrations up to 3.84/0.079 mg/ml. The upper concentration was limited by precipitate formation.

E.2.c. MK-0462/L-783,540: Assay for Chromosomal Aberrations *In Vitro* in Chinese Hamster Ovary Cells (GLP; WF #96-8613, Vol. 31).

Summary:

RIZ (lot 004B015, in dH₂O) plus the degradant was evaluated at concentrations of _____, with and without S-9, for its propensity to induce chromosomal aberrations in Chinese hamster ovary cells. The high doses that produced an approximate 50% reduction in cell count after a 20 hr exposure were 2.3/0.05 and 3.07/0.06 mg/ml in the presence and absence of S-9, respectively. The frequency of chromosomal aberrations in cultures exposed to RIZ + degradant up to these concentrations did not exceed background levels. Positive controls produced the expected increases in chromosomal aberrations. Thus, RIZ/degradant combination was not clastogenic under the conditions of this study.

E.2.d. MK-0462/L-783,540: Fourteen-Week Oral Toxicity Study in Rats

(GLP; Report: TT #96-034-0; Vols. 31-32)

Conducted by: MRL, West Point, PA

Study Dates: 5/7/96 - 8/5/96

Summary:

The toxicity of 98%:2% mixture of RIZ and the degradant product, L-783,540, was assessed in rats at doses of 2, 25 and 125 mg/kg/day by gavage for 14 weeks. The same clinical signs (hyperemia of the foot pads, salivation) and hepatic weight increases (15% in HDF) were the only notable findings, as observed in previous studies. Thus, no novel toxicities in rats are introduced by the presence of the degradant in the RIZ preparation.

Methods:

Animals: Crl:CD(SD)BR rats; 35 days old;

males: 122-166g, females: 100-132g

N: 15/sex/group

Dosages: 2, 25, 125 mg/kg/day (calculated as the free base) of a _____ of RIZ (lot 004B024) and the degradant L-783,540 (lot 002T001)

A basis for dosage level selection was not provided. It is noted that the HD was established in previous studies of RIZ alone as a No-Effect dose.

Route/Freq: one daily gavage administration
Vehicle: water (5 ml/kg)
Feeding: rationed; M: 22g, F: 16 g

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Parameters monitored:	clinical signs	-	daily
	body wt	-	1-2X weekly
	food cons	-	2X weekly
	ophthalmic exam	-	wks 6 & 12 (Con & HD); predose
	hematology*	-	wks 4, 8 & 12
	clinical chemistry*	-	"
	urinalysis	-	wks 8 & 12
	histopathology*	-	complete on Con & HD

* the same parameters as in the 14-week RIZ only study were assessed.
(see Appendix Table)

Results:

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Mortality: none

Clinical: Salivation at 125 mg/kg beginning wk 5; may be due to poor palatability. Hyperemia of foot pads at ≥ 25 mg/kg in M, and at 125 mg/kg in F.

Body Wt: ↓ bwg - 7% in MDM; 9% in HDM;

Ophth: The sponsor states that there were no treatment-related effects, but no data were provided.

Hematol: No treatment-related effects

Clin Chem: No treatment-related effects

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Urinalysis: No treatment-related effects

Organ Wts: Relative liver wts were slightly increased in MDF (8%) and HDF (15%).

Gross Path: No treatment-related effects

Histopath: No treatment-related effects

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Clinical: No data tables of clinical findings were submitted. The text states that mydriasis occurred in all dosage groups, similar to that reported in the study of RIZ alone.

Body Wt. FC: No treatment-related effects.

Ophthalmol: The sponsor states that there were no treatment-related effects, but no data were provided.

EKG: According to the text (no data submitted), HR was increased by a average in 7/8 HD animals at all time points.

Hematol: No treatment-related effects

Clin Chem: No treatment-related effects

Urinalysis: No treatment-related effects

Organ Wts: No treatment-related effects

Gross Path: No treatment-related effects

Histopath: No treatment-related effects

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E.2.f. MK-0462/L-783,540: Oral Developmental Toxicity Study in Rabbits

(GLP; Report: TT #97-705-0; Vol. 34)

Conducted by: MRL, West Point, PA

Study Dates: 1/28/97 - 2/19/97

Summary:

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The developmental toxicity of RIZ in combination with the degradant L-783,540 was assessed in rabbits by orally administering doses of 0, 5, 10 and 50 mg/kg/day on days 6-18 of gestation (n = 18 mated females per dose). Does were sacrificed on day 28.

There were no deaths or abortion. Average maternal body weights were decreased by 92%, and food consumption to a lesser extent in HD dams during the treatment period. Live fetal weights were slightly but significantly reduced in HD pups (9% in F; 5% in M). No other clearly treatment-related effects on pregnancy parameters or embryofetal development/morphology were observed. An increased incidence of missing lung lobes (caudate) was noted in HD fetuses, and may represent a developmental delay secondary to maternotoxicity.

The NOAEL in this study for maternal and developmental toxicity is 10 mg/kg.

Methods:

Animals: New Zealand white rabbits (premated); 25 wks; 3020-4398 g;

N: 18/group

Dosages: 0, 5, 10, 50 mg/kg/day of a 98:2 mixture of RIZ and L-783,540 (RIZ Lot: 004B024; calcd. as base).

Doses were the same as those used in the rabbit developmental toxicity study of RIZ alone.

Regimen: once daily on GD 6-18; all animals sacrificed on GD 28

Route: oral (gavage) in water (4 ml/kg)

Parameters: *Maternal* - clin signs, body wt, food cons, preg/non-preg, corpora lutea, implants, resorptions, live/dead fetuses, necropsy (thoracic, abdominal)
Fetuses - body wt, external exam, visceral exam, skeletal exam (alizarin red)

Results:

Maternal - There were no deaths or abortions. Mydriasis or slow pupillary reflex was the only clinical sign observed in HD animals. The average body weight gain in the HD group was markedly decreased (92%), particularly between days 12-18. The weight loss was paralleled by a decrease in food intake (sponsor Figures 1 & 2).

FIGURE 1. MK-0482/L-783,540: ORAL DEVELOPMENTAL TOXICITY STUDY IN RABBITS. TT 997-706-0
AVERAGE MATERNAL BODY WEIGHTS (GRAMS)

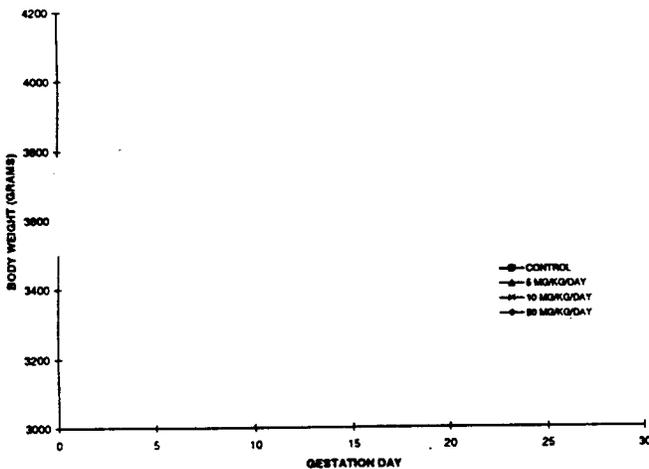
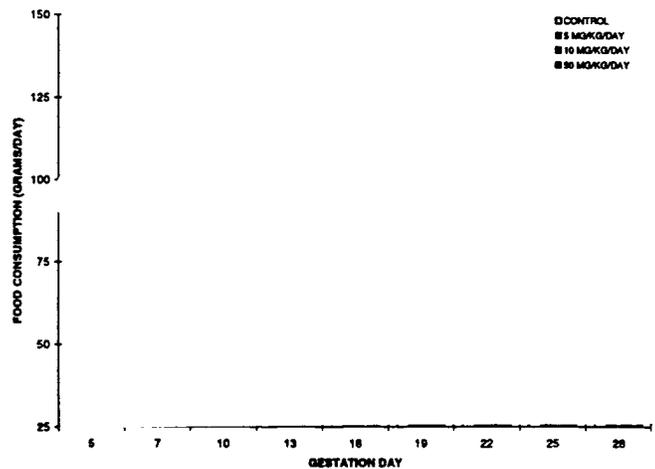


FIGURE 2. MK-0482/L-783,540: ORAL DEVELOPMENTAL TOXICITY STUDY IN RABBITS. TT 997-706-0
AVERAGE MATERNAL FOOD CONSUMPTION (GRAMS/DAY)



There were no treatment effects on pregnancy parameters (sponsor Table 4).

Fetal -

There were no treatment effects on fetal survival, external morphology, or skeletal examinations (sponsor Tabs. 4, 5, 7). Live fetal weights were significantly reduced at the HD (F: 9%, M: 5%; Tab.4).

The incidence of missing caudate lobe of lung was higher in HD fetuses (12%) than in other treatment groups. The sponsor contends that this visceral variation was not likely treatment-related because its incidence was "close to...the upper limit of historical control groups... and because there were no other indications of treatment-related effects on the lung". The sponsor's cited upper limit for historical control incidence of this variation is 7%, which appears considerably lower than the HD group incidence. Also, while no other lung variations were observed in this study, variation in lung lobation was noted only in drug-treated animals (albeit at a low incidence) in the rabbit teratology study of RIZ alone. Nonetheless, the finding is considered unexpected since placenta transfer of the degradant is probably limited. Therefore, the variation more likely represents a non-specific developmental delay secondary to maternotoxicity.

Incomplete ossification of the pelvic bone and the talus/calcaneus tended to increase with dose (sponsor Tab. 8). The incidences in the HD group (2.7 & 4.3%, respectively) were below historical controls (6 & 11%), and likely unrelated to treatment.

TABLE 4. MK-0462/L-783,540: ORAL DEVELOPMENTAL TOXICITY STUDY IN RABBITS. TT #97-705-0
SUMMARY OF LAPAROTOMY DATA

TREATMENT GROUP:	CONTROL	5 MG/KG/DAY	10 MG/KG/DAY	50 MG/KG/DAY
FEMALES				
TOTAL FEMALES	18	18	18	18
PREGNANT	17	18	17	17
EXAMINED LIVE LITTER	17	18	17	17
RESORBED OR DEAD LITTER	0	0	0	0
DIED	0	0	0	0
SACRIFICED	0	0	0	0
NOT PREGNANT	1	0	1	1
LIVE	1	0	1	1
DIED	0	0	0	0
SACRIFICED	0	0	0	0
NOT BRED	0	0	0	0
CORPORA LUTEA				
CORPORA LUTEA	177	192	172	170
CORPORA LUTEA/PREGNANT FEMALE	10.4 ± 1.8	10.7 ± 1.4	10.1 ± 2.1	10.0 ± 1.6
% PERI-IMPLANTATION LOSS (LITTER MEAN)	7.4 ± 11.5	16.6 ± 21.7	7.4 ± 8.6	4.5 ± 6.9
IMPLANTS				
IMPLANTS	164	159	159	162
IMPLANTS/PREGNANT FEMALE	9.6 ± 2.1	8.8 ± 2.5	9.4 ± 2.0	9.5 ± 1.6
RESORPTIONS AND DEAD FETUSES				
RESORPTIONS	5	12	7	14
% RESORPTIONS/IMPLANTS (LITTER MEAN)	2.7 ± 5.5	7.0 ± 10.6	4.4 ± 6.4	8.1 ± 12.6
DEAD FETUSES	1	0	0	1
% DEAD FETUSES/IMPLANTS (LITTER MEAN)	0.5 ± 2.2	0.0 ± 0.0	0.0 ± 0.0	0.8 ± 3.5
% POSTIMPLANTATION LOSS (LITTER MEAN)	3.3 ± 7.2	7.0 ± 10.6	4.4 ± 6.4	8.9 ± 13.5
LIVE FETUSES				
LIVE FETUSES	158	147	152	147
FEMALES	80	86	68	71
MALES	78	61	84	76
SEX RATIO (LITTER MEAN)	0.50	0.60	0.45	0.48
LIVE FETUSES/PREGNANT FEMALE	9.3 ± 2.0	8.2 ± 2.4	8.9 ± 1.9	8.6 ± 1.8
LIVE FETAL WEIGHT (GM, LITTER MEAN)				
FEMALES ^a	36.4 ± 4.3	37.2 ± 4.2	36.2 ± 5.5 ^{NS}	33.3 ± 4.6 ^S
MALES ^b	36.9 ± 4.1	38.8 ± 4.3	37.0 ± 4.7 ^{NS}	35.1 ± 3.4 ^S

% PERI-IMPLANTATION LOSS = ((NO. CORPORA LUTEA - NO. IMPLANTS) / NO. CORPORA LUTEA) X 100

% POSTIMPLANTATION LOSS = ((NO. RESORPTIONS + NO. DEAD FETUSES) / NO. IMPLANTS) X 100

SEX RATIO = (TOTAL NO. LIVE FEMALE FETUSES / TOTAL NO. LIVE FETUSES)

S = TREND STATISTICALLY SIGNIFICANT (P ≤ 0.05) THROUGH INDICATED DOSE.

NS = TREND NOT STATISTICALLY SIGNIFICANT (P > 0.05) THROUGH INDICATED DOSE.

a = STATISTICAL ANALYSIS WAS PERFORMED WITH AN ADJUSTMENT FOR NO. OF LIVE FETUSES PER LITTER.

b = STATISTICAL ANALYSIS WAS PERFORMED WITH AN ADJUSTMENT FOR NO. OF LIVE FETUSES PER LITTER AND TIME TO SACRIFICE.

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TABLE 5. MK-0462/L-783,540: ORAL DEVELOPMENTAL TOXICITY STUDY IN RABBITS. TT #97-705-0
SUMMARY OF EXTERNAL EXAMINATION OF FETUSES

TREATMENT GROUP:	CONTROL	5 MG/KG/DAY	10 MG/KG/DAY	50 MG/KG/DAY
LIVE FETUSES/LITTERS EXAMINED	158/17	147/18	152/17	147/17
DEAD FETUSES/LITTERS EXAMINED	1/ 1	0	0	1/ 1
FETUSES WITH MALFORMATIONS %, LM ±S.D.	0	0	1	1
LITTERS WITH MALFORMATIONS (%)	0.00± 0.00	0.00± 0.00	0.74± 3.0	0.74± 3.0
FETUSES WITH VARIATIONS %, LM ±S.D.	0	0	1 (5.9)	1 (5.9)
LITTERS WITH VARIATIONS (%)	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.53± 2.2
PLACENTAL MORPHOLOGY				
NO. ABNORMAL PLACENTAS/TOTAL EXAMINED*	0/158 (3)	0/147 (2)	0/152 (4)	0/147 (3)
LITTERS WITH VARIATIONS (%)	0	0	0	1 (5.9)
TYPE AND NUMBER OF FETAL ALTERATIONS ± LM				
		CLASS		
OMPHALOCELE (M)	0		1 (0.74)	1 (0.74)
PETECHIAL HEMORRHAGE OF SKIN (V)	0	0	0	1 (0.53)

(LM) = LITTER MEAN (M) = MALFORMATION (V) = VARIATION
* = NUMBERS IN PARENTHESES REPRESENT PLACENTAS FROM DEAD FETUSES OR LATE RESORPTIONS.

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TABLE 6. MK-0462/L-783,540: ORAL DEVELOPMENTAL TOXICITY STUDY IN RABBITS. TT #97-705-0
SUMMARY OF VISCERAL EXAMINATION OF FETUSES

TREATMENT GROUP:	CONTROL	5 MG/KG/DAY	10 MG/KG/DAY	50 MG/KG/DAY
THORACIC AND ABDOMINAL EXAMINATION				
LIVE FETUSES/LITTERS EXAMINED	158/17	147/18	152/17	147/17
DEAD FETUSES/LITTERS EXAMINED	1/ 1	0	0	1/ 1
FETUSES WITH MALFORMATIONS %, LM ±S.D.	4	1	3	2
LITTERS WITH MALFORMATIONS (%)	2.7 ± 5.1	0.56± 2.4	2.0 ± 4.4	1.5 ± 4.2
FETUSES WITH VARIATIONS %, LM ±S.D.	4 (24)	1 (5.6)	3 (18)	2 (12)
LITTERS WITH VARIATIONS (%)	8	9	5	20
FETUSES WITH VARIATIONS %, LM ±S.D.	6.0 ± 11	5.6 ± 10	3.0 ± 4.9	14 ± 21
LITTERS WITH VARIATIONS (%)	5 (29)	6 (33)	5 (29)	8 (47)
CORONAL EXAMINATION				
LIVE FETUSES/LITTERS EXAMINED	158/17	147/18	152/17	147/17
FETUSES WITH MALFORMATIONS %, LM ±S.D.	0	0	0	0
LITTERS WITH MALFORMATIONS (%)	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00
FETUSES WITH VARIATIONS %, LM ±S.D.	0	0	0	0
LITTERS WITH VARIATIONS (%)	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00
TYPE AND NUMBER OF FETAL ALTERATIONS ± LM				
		CLASS		
ABNORMAL ORIGIN SUBC. ART. (M)	1 (0.53)	0	1 (0.74)	0
HYPOPLASTIC KIDNEY (M)	0	0	1 (0.74)	0
HYDRONEPHROSIS (M)	0	0	1 (0.74)	0
DISPLACED KIDNEY (M)	0	0	1 (0.74)	0
RETROCAVAL URETER (M)	3 (2.2)	0	1 (0.49)	2 (1.5)
AGENESIS OF GALLBLADDER (M)	0	1 (0.56)	0	1 (0.74)
AZYGOS VEIN VARIATION (V)	1 (0.74)	0	0	0
PALE KIDNEY (V)	0	0	1 (0.74)	1 (0.74)
SLIGHTLY DISPLACED KIDNEY (V)	0	0	1 (0.74)	0
GALLBLADDER REDUCED IN SIZE (V)	0	0	1 (0.53)	0
MISSING CAUDATE LOBE OF LUNG (V)	0	2 (1.1)	1 (0.53)	0
VARIATION IN LUNG LOBATION (V)	5 (4.0)	7 (4.5)	2 (1.1)	17 (12)
CYST (V)	1 (0.65)	1 (0.56)	0	2 (1.1)
DISCOLORED LIVER (V)	0	0	1 (0.59)	0
HEMORRHAGIC FOCUS ON LIVER (V)	1 (0.65)	1 (0.56)	0	1 (0.53)

(LM) = LITTER MEAN (M) = MALFORMATION (V) = VARIATION

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TABLE 7. MK-0462/L-783,540: ORAL DEVELOPMENTAL TOXICITY STUDY IN RABBITS. TT #97-705-0
SUMMARY OF SKELETAL EXAMINATION OF FETUSES (EXCLUDING OSSIFICATION DATA)

TREATMENT GROUP:	CONTROL	5 MG/KG/DAY	10 MG/KG/DAY	50 MG/KG/DAY
TORSO AND LIMB EXAMINATION				
LIVE FETUSES/LITTERS EXAMINED	158/17	147/18	152/17	147/17
DEAD FETUSES/LITTERS EXAMINED	1/1	0	0	1/1
FETUSES WITH MALFORMATIONS	2	1	4	2
%, LM ±S.D.	1.5 ± 4.2	0.62 ± 2.6	2.5 ± 6.2	1.6 ± 4.6
LITTERS WITH MALFORMATIONS (%)	2 (12)	1 (5.6)	3 (18)	2 (12)
FETUSES WITH VARIATIONS	22	9	29	18
%, LM ±S.D.	15 ± 16	6.6 ± 10	18 ± 14	12 ± 14
LITTERS WITH VARIATIONS (%)	12 (71)	7 (39)	13 (76)	9 (53)
HEAD EXAMINATION				
LIVE FETUSES/LITTERS EXAMINED	158/17	147/18	152/17	147/17
DEAD FETUSES/LITTERS EXAMINED	1/1	0	0	1/1
FETUSES WITH MALFORMATIONS	0	0	0	0
%, LM ±S.D.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
LITTERS WITH MALFORMATIONS (%)	0	0	0	0
FETUSES WITH VARIATIONS	0	0	0	0
%, LM ±S.D.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
LITTERS WITH VARIATIONS (%)	0	0	0	0
TYPE AND NUMBER OF FETAL ALTERATIONS & LM CLASS				
THORACIC VERTEBRA MALFORMATION (M)	2 (1.5)	1 (0.62)	1 (0.49)	1 (0.59)
CAUDAL VERTEBRA MALFORMATION (M)	0	0	1 (0.65)	0
FUSED RIB (M)	1 (0.74)	1 (0.62)	0	1 (0.98)
STERNBRAL MALFORMATION (M)	0	0	2 (1.4)	0
CERVICAL VERTEBRA VARIATION (V)	0	0	1 (0.74)	0
CERVICAL RIB (V)	0	0	4 (2.4)	4 (2.3)
REDUCED 13TH RIB (V)	22 (15)	9 (6.6)	26 (16)	13 (8.7)
STERNBRAL VARIATION (V)	0	0	0	1 (0.59)

(LM) = LITTER MEAN (M) = MALFORMATION (V) = VARIATION

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TABLE 8. MK-0462/L-783,540: ORAL DEVELOPMENTAL TOXICITY STUDY IN RABBITS. TT #97-705-0
SUMMARY OF FETAL OSSIFICATION DATA

TREATMENT GROUP:	CONTROL	5 MG/KG/DAY	10 MG/KG/DAY	50 MG/KG/DAY
TORSO AND LIMB EXAMINATION				
LIVE FETUSES/LITTERS EXAMINED	158/17	147/18	152/17	147/17
FETUSES WITH INCOMPLETE OSSIFICATION	39	34	29	37
%, LM ±S.D.	23 ± 20	22 ± 17	18 ± 22	24 ± 25
LITTERS WITH INCOMPLETE OSSIFICATION (%)	11 (65)	15 (83)	10 (59)	12 (71)
NUMBER OSSIFIED SACROCAUDAL VERTEBRAE				
LITTER MEAN ±S.D.	19.8 ± 0.4	19.6 ± 0.4 ^a	19.7 ± 0.4 ^a	19.6 ± 0.4
HEAD EXAMINATION				
LIVE FETUSES/LITTERS EXAMINED	158/17	147/18	152/17	147/17
FETUSES WITH INCOMPLETE OSSIFICATION	0	0	0	0
%, LM ±S.D.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
LITTERS WITH INCOMPLETE OSSIFICATION (%)	0	0	0	0
SITE AND NUMBER OF FETUSES WITH INCOMPLETE OSSIFICATION & LM				
INCOMP. OSS. LUMBAR VERTEBRA	3 (1.9)	2 (2.4)	2 (1.2)	0
INCOMP. OSS. STERNBRAL	14 (8.2)	18 (11)	8 (5.3)	11 (6.9)
INCOMP. OSS. METACARPAL	26 (15)	15 (8.9)	21 (12)	29 (19)
INCOMP. OSS. PELVIC BONE	1 (0.74)	1 (0.56)	2 (1.4)	5 (2.7)
INCOMP. OSS. TALUS/CALCANEUS	0	1 (0.62)	3 (2.0)	7 (4.3)

(LM) = LITTER MEAN
^a = SEE INDIVIDUAL TABLE FOR EXCLUSIONS.

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E.2.g. MK-0462/L-783,540: Oral Developmental Toxicity Study in Rats

(GLP; Report: TT #97-706-0; Vol. 34)

Conducted by: MRL, West Point, PA

Study Dates: 2/2/97 - 2/28/97

Summary:

The developmental toxicity of RIZ in combination with the degradant L-783,540 was assessed in rats by orally administering doses of 0, 2, 10 and 100 mg/kg/day on days 6-17 of gestation (n = 18 mated females per dose). Dams were C-sectioned on day 21.

There were no deaths, abortions or treatment-related effects on pregnancy parameters. Decreased weight gain and food consumption were observed in HD dams during the treatment period. Fetal body weights were slightly but significantly reduced in the HD group. No other treatment-related effects on embryofetal development/morphology were evident.

The NOAEL in this study for maternal and developmental toxicity was 10 mg/kg. The degradant did not introduce any additional reproductive toxicity to rats.

Methods:

Animals: Crl:CD(SD)BR Rat; 10 wks;
N: 25/group
Dosages: 0, 2, 10, 100 mg/kg/day (RIZ Lot: 004B024; calcd. as base).

Doses were the same as those used in the rat teratology study of RIZ alone.

Regimen: once daily on GD 6-17; all animals sacrificed on GD 21
Route: oral (gavage) in water (10 ml/kg)

Parameters: *Maternal* - clin signs, body wt, food cons, preg/non-preg, corpora lutea, implants, resorptions, live/dead fetuses, necropsy (thoracic, pelvic, abdominal)
Fetuses - body wt, external exam, visceral exam (1/3 of total), skeletal exam (alizarin red)

Results:

Maternal - There were no mortalities, treatment-related clinical signs, or treatment-related effects on pregnancy parameters (sponsor Table 4). Body weight gain in the HD group was significantly reduced by 14.5% during the treatment period (GD6-18; sponsor Fig.1). Food consumption was also reduced in HD (sponsor Fig. 2).