CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 20-829

PHARMACOLOGY REVIEW(S)

JAN 28 1998

DIVISION OF PULMONARY DRUG PRODUCTS
REVIEW OF PHARMACOLOGY AND TOXICOLOGY DATA
(Addendum to Original Pharmacology Review dated 12/1/97)

NDA No. 20-829 and 20,830

Information to be Conveyed to Sponsor: Yes ( ), No (X)

Reviewer: Shannon P. Williams, Ph.D.

Date Addendum Completed: January 28, 1998

Sponsor: Merck & Co., Inc., West Point, PA

Drug Names: *Generic:* montelukast sodium; *Commercial:* Singulair™;
Code: MK-0476, MK-476 and L-706,631

Background: The labeling review contained in the Original Pharmacology reviews for NDAs 20829 and 20830 (Reviews by Shannon Wiliams, Ph.D., dated 12/1/97) contained an error in the section pertaining to montelukast's effect on fertility in male rats. In this regard, the 800 mg/kg dose, which had no effects on fertility in male rats, was incorrectly stated to be 80 times the maximum recommended daily oral dose in adults on a mg/m^2 basis. The 800 mg/kg dose is actually 650 times the maximum recommended daily oral dose in adults on a mg/m^2. This correction has been incorporated in to labeling recommendations for the package insert.

/S/ 1/28/98

Shannon Williams, Ph.D.,
Pharmacologist

/S/ Jan 29 1998

c.c. Original NDA 20-829 and 20-830
HFD-570/Division File for NDA 20-829 and 20-830
HFD-570/C.J. Sun
HFD-570/C.S.O.
HFD-570/Shannon Williams

DEC 1 1997

DIVISION OF PULMONARY DRUG PRODUCTS
REVIEW OF PHARMACOLOGY AND TOXICOLOGY DATA

Original Review

NDA No. 20-829 and 20,830 **Submission date:** 21 FEB 97

Information to be Conveyed to Sponsor: Yes (X), No ( )

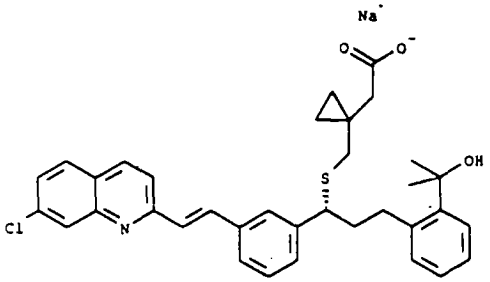
Reviewer: Shannon P. Williams, Ph.D. **Date Review Completed:** 01 DEC 97

Sponsor: Merck & Co., Inc., West Point, PA

Drug Names: *Generic:* montelukast sodium; *Commercial:* Singulair™;
Code: MK-0476, MK-476 and L-706,631

Chemical Name: [R-(E)]-1-[[[1-[3-[2-(7-chloro-2-quinolinyl)ethenyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]thio]methyl]cyclopropane acetic acid, monosodium salt.

Structure:



Empirical Formula: $C_{35}H_{35}ClNNaO_3S$ (M.W. = 608.18)

Drug Product Formulation: 10 mg Tablet

Ingredient	Core Tablet (mg)	Film Coating (mg)
Montelukast sodium (free acid equivalent)	**10.4 (10.0)**	
Hydroxypropyl cellulose		
Microcrystalline cellulose		
Lactose monohydrate		
Croscarmellose sodium		
Magnesium stearate		
Hydroxypropyl methylcellulose		
Titanium Dioxide		
Red Iron Oxide		
Yellow Iron Oxide		
Carnauba Wax		
Total Tablet Weight (mg)		

Drug Product Formulation: 5 mg ChewableTablet

Ingredient	mg/Tablet
Montelukast sodium (free acid equivalent)	**5.2 (5.0)**
Mannitol, USP	
Microcrystalline cellulose, NF	
Croscarmellose sodium, NF	
Hydroxypropyl cellulose, NF(EXF)	
Artificial Cherry Falvor, NF	
Magnesium stearate, NF	
Aspartame, NF	
Red Ferric Oxide, NF	
Total Tablet Weight	

Excipients, Degradants and Impurities: The proposed levels of all excipients, in both the adult tablet and the children's chewable tablet, occur at levels well within the ranges of those used in other currently approved drug products. Thus, there are no nonclinical issues with the proposed excipients in either the adult or pediatric formulation.

Class: cysteinyl leukotriene $CysLT_1$ receptor antagonist

Indication: prophylaxis and chronic treatment of asthma in adult and pediatric patients 6 years of age and older.

Route: Oral

Pediatric Dose 5 mg (tablet) once daily, In a 25 kg child this is 0.25 mg/kg, 6.2 mg/m^2.

Adult Dose: 10 mg (tablet) once daily in adults, In a 50 kg adult this is 0.2 mg/kg, 7.4 mg/m^2.

Related INDs/NDAs/DMFs:

Previous Review(s), Date(s) and Reviewer(s): This NDA has not been reviewed previously. Relevant reviews of related INDs are listed below:

Preclinical Studies Submitted and Reviewed in this NDA:

Note: Unless otherwise specified, studies were submitted to IND

ACUTE TOXICITY STUDIES	[Ref. No.]	Vol./pp.
Acute Oral Toxicity Study In Mice	TT#92-2820	1.30/H-61
PHARMACOKINETIC STUDIES		
Absorption and Disposition of MK-0476 in Mice, Rats & Monkeys*	[G-2]	29/G88
[^{14}C]MK-0476: Distribution in Blood and Octanol/Buffer Partition*	[G-4]	29/G199
Gastrointestinal Absorption Sites of [^{14}C]MK-0476 in Male Rats**	[G-5]	29/G206
Plasma Levels of MK-0476 After 14-Day Repeated Oral Administration of MK-0476 in Male Rats**	[G-6]	29/G216
Plasma Protein Binding of [^{14}C]-MK- 0476*	[G-7]	29/G216
Tissue Distribution of Radioactivity in Rats After Oral Administration of [^{14}C]MK-0476*	[G-8]	29/G239
Whole Body Autoradiography in Rats**	[G-9]	29/G247
Comparison of Metabolic Profiles of MK-0476 in Human, Monkey, Rat and Mouse*	[G-12]	29/G387
Hepatic Microsomal Metabolism of MK-0476 in Mouse, Rat, Monkey and Human*	[G-15]	29/G456
LOCAL TOLERANCE		
Dermal Irritation Study in Rabbits TT #94-2649		30/H-20 30/H-30
Ocular Irritation Study in Rabbits TT #94-4269		30/H-35
Dermal Irritation Study In Rabbits TT #92-2819		30/H-72 30/H-98
Ocular Irritation Study In Rabbits TT #92-4269		30/H-126
SPECIAL TOXICITY		
Oral/Subcutaneous Immunogenicity Study in Guinea pigs TT #95-9805		31/Q-87

Previously Reviewed Preclinical Studies Submitted in this NDA:

STUDY TYPE/REFERENCE NO.	Vol./pp.	Reviewer	Date of Rev.
PHARMACOLOGY STUDIES / F1- F26	28/F22-F373		
ANCILLARY PHARMACOLOGY			
Behavior and other effects in BKTO mice [F-25]	28/F-25	Choi	13 JUL 92
Cardiovascular and autonomic effects in anesthetized dogs [F-25]	28/F-25	Choi	13 JUL 92
Renal effects in conscious dogs [F-25]	28/F-25	Choi	13 JUL 92
Respiratory effects in conscious dogs [F25 and F-26]	28/F-25	Choi	13 JUL 92
Gastric acid secretion in chronic gastric fistula dogs: [F-25]	28/F26	Williams	02 JUN 97
	28/F26	Choi	13 JUL 92
PHARMACOKINETIC STUDIES			
Physiological Disposition in Rats and Monkeys/[G-1]	29/G-39	Choi	13 JUL 92
SINGLE DOSE TOXICITY			
PO Tox. Study in Mice TT #91-2787	7/A-17	Choi	13 JUL 92
IV Tox. Study in Mice TT #91-2788	7/ A-17	Choi	13 JUL 92
PO Tox. Study in Rats TT #91-2789	7/ A-17	Choi	13 JUL 92
IV Tox. Study in Rats TT #91-2790	7/ A-17	Choi	13 JUL 92
PO TK Study in Mice. TT #92-113-0	7/ A-33	Williams	02 JUN 97
PO TK Study in Rats. TT #92-031-0	7/ A-98	Williams	02 JUN 97
MULTIPLE DOSE TOXICITY			
5-Wk PO Tox. Study In Monkeys TT #91-121-0	8/B-90	Choi	13 JUL 92
14-Wk PO Tox. Study In Monkeys TT #92-611-0	9/B-402	Williams	24 OCT 96
53-Wk PO Tox. Study In Monkeys TT #92-650-0	9-10/B-701	Williams	24 OCT 96
14-Wk PO Tox. In Infant Monkeys TT #94-9003	11/B-1232	Williams	24 OCT 96
5- Wk PO Tox. Study In Rats TT #91-120-0	11-12/B-1362	Choi	13 JUL 92
Range-Find (8-Day PO)Tox. in Rats TT #92-609-0	12/B-1799	Williams	02 JUN 97
14- Wk PO Tox Study In Rats TT #92-610-0	12-13/B-1820	Williams	24 OCT 96
14-Wk PO Tox./TK Study In Rats TT #92-098-0	15-16/B-3107	Williams	24 OCT 96
53-Wk PO Tox/TK Study In Rats TT #92-651-0	13-15/B-2375	Williams	24 OCT 96
16-Day PO TK Study In Rats TT #93-054-0	16/B-3585	Williams	02 JUN 97
Range-Find (8-Day PO)Tox. in Mice TT #92-070-0	16/B-3703	Williams	02 JUN 97
14-Wk PO Tox. Study in Mice TT #93-001-0	17/B-3738	Williams	02 JUN 97
5-Wk PO TK Study in Mice TT #93-034-0	17/B-3907	Williams	02 JUN 97
16-Day IV Tox. Study In Rats TT #93-144-0	17/B-4013	Choi*	27 JUL 95
16-day IV Tox Study In Monkeys TT #93-145-0	17/B-4145	Choi*	27 JUL 95
17 day IV Irritation Study In Monkeys TT #94-629-0	17/B-4371	Choi*	27 JUL 95

Choi* Studies reviewed under

Previously Reviewed Preclinical Studies Submitted in this NDA: (CONT...)

REPRODUCTIVE TOXICITY	Vol./pp.	Reviewer	Date of Rev.
PO Rng-find in (non-pregnant) Rabbits TT #92-716-2	19/C-48	Williams	02 JUN 97
PO Range-find in (pregnant) Rabbits TT #92-716-1	19/C-86	Williams	02 JUN 97
PO Develop. Tox. Study in Rabbits TT #92-716-0	19/C-160	Williams	02 JUN 97
MK-0476: PO TK in Pregnant Rabbits. TT#93-738-0	19/C-228	Williams	02 JUN 97
MK-0476: PO TK Study in Pregnant Rats w/		Williams	02 JUN 97
Secretion in Milk. TT#93-740-0	22/C-1282	Williams	02 JUN 97
PO Range-find Study in Rats TT #92-717-1	19/C-280	Williams	02 JUN 97
PO Develop. Tox. Study in Rats TT #92-717-0	20/C-403	Williams	02 JUN 97
PO Fertility In Female Rats TT #92-723-0	20-21/C-500	Williams	02 JUN 97
PO Fertility In Male Rats TT #93-706-0	21/C-990	Williams	02 JUN 97
PO Late Gestation & Lactation in Rats TT #93-728-0	21-22/C-1136	Williams	02 JUN 97
GENETIC /MUTAGENIC TOXICITY			
	23/D-31	Williams	24 OCT 96
	23/D-31	Williams	24 OCT 96
	23/D-85	Williams	24 OCT 96
V79 M. cell Range-Find Cytotox. study TT #93-8550	23/D-141	Williams	24 OCT 96
	23/D-141	Williams	24 OCT 96
	23/D-194	Williams	24 OCT 96
Rat Hepatocyte Rng-Find Cytotoxicity TT #91-8375	23/D-240	Williams	24 OCT 96
	23/D-240	Williams	24 OCT 96
	23/D-289	Williams	24 OCT 96
CHO Rng-Find Cytotox. and Solubility TT #91-8881	23/D-349	Williams	24 OCT 96
In Vitro Chrom. Aberrat. in CHO Cells TT #91-8882	23/D-349	Williams	24 OCT 96
In Vitro Chrom. Aberrat. in CHO Cells TT #94-8638	24/D-420	Williams	02 JUN 97
Mouse Bone Marrow Chrom. Aberrat. TT #93-8681	24/D-487	Williams	24 OCT 96
Mouse Bone Marrow Chrom. Aberrat. TT #93-8696	23/D-487	Williams	24 OCT 96
ONCOGENIC/CARCINOGENIC POTENTIAL			
106-Week PO Carc. Study In Rats TT #93-078-0	25-26/ E-19	Williams	24 OCT 96
92-Week PO Carc. Study In Mice TT #93-110-0,-1	26-27/E-557	Williams	24 OCT 96
SPECIAL TOXICITY			
Oral Phototoxicity Study in Mice TT #91-2722	31/Q-20	Choi	13 JUL 97
PO Enzyme Induction Study in Mice [H-50] TT #91-			
061-0,-4	31/Q-28	Choi	13 JUL 97
Oral Enzyme Induction Study In Rats TT #91-074-0	31/Q-42	Choi	13 JUL 97
	31/Q-60	Choi*	27 JUL 95
	31/Q-60	Choi*	27 JUL 95
Oral Drug Interaction Study In Mice TT #92-2651	31/Q-67	Choi	13 JUL 92

Choi* Studies reviewed under

Note: Portions of this review were excerpted directly from the sponsor's submission.

PHARMACOLOGY

Table 1 (below and succeeding page) presents a tabulated summary of the studies which investigated the pharmacodynamic activity of montelukast.

Table 1 Biological Activities of Montelukast

Table 1 cont...

Summary of Pharmacodynamic Activity:

Submitted studies on the pharmacodynamic activity of montelukast included receptor binding studies, effects on contraction of isolated tissues, effects on agonist-induced bronchoconstriction, effects on antigen-induced bronchoconstriction and other miscellaneous pharmacological studies. Receptor binding studies using guinea-pig lung, sheep lung, and human differentiated U937 cell membranes, and studies on isolated guinea-pig trachea showed that monteluksast is a potent and selective competitive antagonist of the Cys LT_1 receptor. Potency and selectivity at the Cys LT_1 receptor was also demonstrated in *in vivo* studies wherein montelukast, administered by i.v., aerosol or oral route inhibited leukotriene

D_4-induced bronchoconstriction in guinea pigs, squirrel monkeys, and to a lesser extent in conscious sheep following aerosol administration. Montelukast was devoid of such inhibitory activity against a variety of other bronchoconstrictors including: histamine, arachidonic acid, serotonin, and acetylcholine in anesthetized guinea pigs.

A role for the Cys LT_1 receptor in antigen or ascaris-induced bronchoconstriction was demonstrated in in vivo models including: antigen-induced dyspnea in inbred rats, ascaris-induced bronchoconstriction in conscious squirrel monkeys and ascaris-induced early and late phase bronchoconstriction in conscious sheep, wherein montelukast was shown to be a potent and selective antagonist of the induced bronchoconstriction.

Thus, collectively the availabie pharmacodynamic studies suggest that Montelukast may have therapeutic value in treating human diseases such as bronchial asthma.

SAFETY PHARMACOLOGY

Previously submitted ancillary pharmacological studies included effects of montelukast on cardiovascular, renal, gastric, respiratory or CNS and behavioral parameters, where no significant activities were noted at the doses tested. The said studies have been reviewed previously (See attached Pharmacology Reviews by Dr . Choi dated 7/13/92 and by Dr. Williams dated 02 JUN 97) are more fully discussed in the Overall Summary and Evaluation Section of the Document.

PHARMACOKINETICS AND TOXICOKINETICS

Methods: The absorption, distribution, metabolism and excretion (ADME) of montelukast were studied in mice, rats, and rhesus monkeys following oral and iv dosing with Radio-labeled [^{14}C]-montelukast ([^{14}C]MK-0476, Batch # L-706,631-003S009), and Unlabeled MK-0476 Batch # L-706,631-001M035). The concentration of montelukast in plasma was measured Radioactivity in tissues, urine, bile and feces was determined
The limit of detection was 0.031 µg/ml. Initially conducted pharmacokinetic studies in rats and monkeys (Ref. [G-H]) were previously reviewed (See Pharmacology Review of . Although some variability between the two studies was observed, in general reported results were similar between the two studies. Thus, this review will focus on the more recent study submitted, unless indicated. Unless stated otherwise, male animals were used in the ADME studies. The animals were fasted overnight prior to oral absorption studies.

Results:
Absorption:
Oral:
Table 2 below presents a tabulated summary of the pharmacokinetics of montelukast following oral administration in rats, mice, monkeys and humans.

Table 2 Pharmacokinetics of Montelukast in Laboratory Animals and Humans After Oral Administration (Mean ± S.D.) (Sponsor's Summary Table G-2, NDA 20-829 Vol. 29 pp G-9)

Species	Dose (mg/kg)	$AUC_{0-\infty}$ (μg•min/ml)	Cmax (μg/ml)	Tmax (min)	F (%)	$t_{1/2}$[c] (min)
Rat (n=3-4)	5	100 ± 58.7	1.27 ± 1.36	22.5 ± 8.66	29.6 ± 17.3	80.2 ±12.8[c]
	25	580 ± 235	2.82 ± 1.61	37.5 ± 8.66	34.2 ± 13.8	176 ±122[c]
	50	1106 ± 325	4.35 ± 1.68	67.5 ± 26.0	32.6 ± 9.57	
	200	4795 ± 3628	15.7 ± 16.5	80.0 ± 34.6	35.3 ± 26.7	
Mouse [a]	5	180	0.940	30	45.6	
	25	705	3.72	60	35.7	
	50	2411	14.4	60	61.0	
	200	9004	34.2	120	57.0	
Monkey (n=4)	5	736 ± 191	4.54 ± 2.08	78.8 ± 48.0	36.2 ± 9.42	152 ± 29.4[c]
	25	5360 ± 2557	21.6 ± 4.32	67.5 ± 35.7	52.8 ± 25.2	457 ± 55.9[c]
	50	7795 ± 2751	27.3 ± 11.6	101 ± 37.5	38.4 ± 13.6	
	150	35456 ± 12405	60.4 ± 15.9	90.0 ± 60.0	58.2 ± 20.4	
Human [b] (n=6)	0.126	146 ± 26.5	0.385 ± 0.085	220 ± 49.0	67.3 ± 12.9	

[a] Mean plasma concentrations from three animals at each sampling time were used in the estimation of pharmacokinetic parameters.
[b] Clinical dose 10 mg (mean body weight = 79.3 kg)
[^{14}C]MK-0476, Batch # L-706,631-003S009; Unlabeled MK-0476 # L-706,631-001M035
[c][Ref. G-1] Reviewed by Y.S. Choi in pharmacology review dated 7-13-92

The Data in Table 2 above show that Montelukast was rapidly absorbed in rats and mice with increased Tmax indicative prolonged absorption seen with dose increases from 5 to 200 mg/kg in both species . Absorption was somewhat slower in monkeys and humans and in monkeys the rate of absorption appeared independent of the dose. Bioavailability (F%) although somewhat variable appeared to be independent of dose at the doses tested being around 30-35% in rats, 36-60 % in mice and monkeys, with greatest bioavailability (67%) observed in humans. In rats and mice increases in doses produced roughly proportional increases in Cmax and AUC values over the doses of 5 to 200 mg/kg. In monkeys increasing doses produced roughly proportional increases in AUC values, with less than proportional increases in Cmax values over a dose range of 5 to 150 mg/kg. Comparisons between species showed that similar doses resulted in greatest exposure (Cmax and AUC) in

monkeys, relative to rats and mice ,where exposure was comparable. Elimination half-life values following oral dosing were not provided in the study from which the nonclinical data in Table 2 was derived [Ref G-2]. However, previously conducted pharmacokinetic studies in rats and monkeys (Reviewed by Y.S. Choi in pharmacology review dated 7-13-92) reported elimination half-life values of 80.2 ± 12.8 and 176 ± 122 min in rats and 152 ± 29.4 and 457 ± 55.9 min in monkeys following oral administration of montelukast at doses of 5 and 25 mg/kg, respectively [Ref. G-1].

Additional repeat dose pharmacokinetic testing in rats [Ref. G-6] showed similar pharmacokinetic parameters: Cmax [0.563 to 0.875 μg/ml], Tmax [40-55min], AUC [80-117 μg·min/ml], and t1/2 [87-105 min] on days 1, 8, and 14 of dosing, indicating that repeated oral administration of MK-0476 (5 mg/kg) for 2 weeks had no significant effects on its pharmacokinetic handling in rats [Ref. G-6].

Other studies conducted in rats investigated the mechanism behind the low bioavailability (33%) in rats in relation to potential intestinal metabolism and/or first pass metabolism. In vitro studies using an isolated rat intestinal loop preparation showed that introduction of a 1 mg (3-4 mg/kg) dose of [^{14}C]-montelukast directly into the jejunum underwent negligible intestinal metabolism [Ref. G-2]. In contrast, studies which compared the steady-state drug concentrations in systemic plasma during portal or femoral vein infusion (4 mg/min estimated the first pass metabolism to be 27% [Ref. G-2]. Thus, considering the hepatic first-pass extraction, the extent of absorption for montelukast in rats was estimated to be 50%, in rats, suggesting the low bioavailability was due to the combination of incomplete absorption and hepatic first-pass elimination.

Additional studies in rats investigated the gastrointestinal (GI) absorption site(s) of montelukast [Ref. G-5] and possibility of enterohepatic circulation [Ref. G-1]. In studies on the site of absorption, injection of [^{14}C]montelukast (5 mg/kg) into the pre-ligated duodenum, jejunum, or ileum of rats resulted in substantial plasma concentrations (0.3-0.4 mg/ml) at 15 min after dosing, whereas no plasma concentrations were observed after direct dosing in the ligated stomach. Other studies which investigated the possibility of interohepatic circulation showed that iv. administration of montelukast (5 mg/kg) into intact and bile duct-cannulated rats resulted in comparable plasma AUC values for montelukast in both models, suggesting that enterohepatic circulation of montelukast was negligible in rats [Ref. G-1].

I.V.

Table 3 (succeeding page) presents a tabulated summary of the Pharmacokinetics of montelukast in rats mice monkeys and humans following iv administration.

Table 3 Pharmacokinetics of Montelukast in Laboratory Animals and Humans After I.V. Administration (Mean ± S.D.) (Sponsor's Summary Table G-1 NDA 20-829 Vol. 29. pp. G-7)

Species	Dose (mg/kg)	$AUC_{0-\infty}$ (μ·min/ml)	CLp (ml/min/kg)	T½ (min)	Vd_{ss} (L/kg)
Rat	2	136 ± 21.9	15.0 ± 2.27	68.9 ± 25.1	0.467 ± 0.143
(n=4)	5	290 ± 44.9	17.6 ± 3.04	98.0 ± 45.9	0.923 ± 0.489
	10	830 ± 136	12.3 ± 1.91	97.4 ± 36.8	0.681 ± 0.340
Mouse [a]	2	158	12.7	63.6	0.906
	5	482	10.4	81.6	0.812
	10	949	10.5	81.7	0.840
Monkey	2	812 ± 163	2.54 ± 0.481	135 ± 20.5	0.199 ± 0.055
(n=4)	5	2508 ± 653	2.09 ± 0.487	123 ± 38.5	0.162 ± 0.057
	10	3634 ± 453	2.79 ± 0.383	123 ± 46.5	0.185 ± 0.050
Human [b]	0.038	61.9 ± 11.9	0.636 ± 0.127	276 ± 66.6	0.143 ± 0.018
	0.114	197 ± 17.0	0.585 ± 0.075	327 ± 10.6	0.132 ± 0.013
(n=6)	0.227	458 ± 83.6	0.513 ± 0.094	322 ± 11.8	0.122 ± 0.011

[a] Mean plasma concentrations from three animals at each sampling time were used in the estimation of pharmacokinetic parameters.
[b] Clinical i.v. dose (mean body weight = 79.3 kg)

Following i.v. administration, the plasma concentrations of montelukast declined in a polyphasic manner in mice, rats and monkeys. The plasma clearance CLp was relatively constant across doses in all species and averaged 15 ml/min/kg for rats, 11 ml/min/kg for mice, and 2.5 ml/min/kg for monkeys versus 0.578 ml/min/kg in humans. Likewise terminal elimination half-life values (t1/2) averaged 88, 76, and 127 min in rats, mice and monkeys, respectively, whereas elimination was somewhat prolonged in humans (mean t1/2 = ~5 hr following single iv doses ranging from 0.38 to 0.227 mg/kg . Volumes of distribution at steady state (Vd_{ss}) in rats (range 0.467 to 0.923 L/kg) and mice (0.85 L/kg) were approximately equal to that of body water (0.6 L/kg) suggesting that the drug was distributed to the tissues. Steady state Volumes of Distribution were less in monkeys and man ranged from 0.122 to 0.199 L/kg (approximately equal to the blood + extracellular fluid volumes 0.271 l/kg) and suggest a more limited distribution to tissues. The calculated mean blood clearance (CLB) was 25 ml/min/kg for the rat, 16 ml/min/kg for the mouse and 4.1 ml/min/kg for the monkey. The pharmacokinetic parameters (CLP, t½, and Vd_{ss}) remained relatively constant up to an i.v. dose of 10 mg/kg in the animal species studied and following iv doses ranging from 0.38 to 0.227 mg/kg in humans.

Distribution:

In Vitro Plasma Protein binding:

The extent of [^{14}C]MK-0476 (2 to 250 μg/ml; Batch # L-706,631-004S007, specific activity = 21.22 μCi/mg and # L-706,631-004S009, specific activity = 13.58 μCi/mg) binding to

plasma protein was determined in in vitro studies using blood samples from CD-1 mice Sprague Dawley rats, rhesus monkeys, and Humans. These studies showed that [^{14}C]MK-0476 was extensively bound to plasma proteins from all species, with the unbound fraction only amounting to 0.3% for mice at (10 and 50 μg/ml), 0.9% in rats and 0.4% in plasma from monkeys and human [Ref. G-7]. Further studies showed that [^{14}C] Montelukast bound to both albumin and α1-acid glycoprotein.. Binding to albumen was unsatrurable, with a constant unbound fraction (0.2%), at concentrations up to 250 μg/ml, whereas binding to α1-acid glycoprotein was saturated at a concentration of 10 μg/ml. Collectively, these studies suggest binding to albumin, may account for the majority of the extensive plasma protein binding, especially at higher drug concentrations.

In Vitro Partition Between Erythrocytes and Plasma:
In vitro studies examined the distribution of [^{14}C]MK-0476 (2 to 250 μg/ml; Batch # L-706,631-004S007, specific activity = 21.22 μCi/mg and # L-706,631-004S009, specific activity = 13.58 μCi/mg) between blood cells and plasma and the partitioning of [^{14}C]MK-0476 (2-250 μg/ml) between
Results from these studies showed that the blood/plasma concentration ratio (Cblood/Cplasma) ranged from in rats, monkeys and man at all concentrations tested. In addition, the experiments revealed log P values of the concentration ratios which ranged form at the concentrations tested. These latter findings indicated that MK-0476 was fairly hydrophobic.

Tissue Distribution:
The tissue distribution of [^{14}C]Montelukast-derived radioactivity was evaluated in rats using scintillation counting following sample combustion after an i.v. administration of [^{14}C]Montelukast (5 mg/kg i.v., [Ref. G-1]). The distribution of [^{14}C]montelukast-derived radioactivity was also assessed using whole body autoradiography following single oral doses of 10 mg/kg in male rats [Ref.G-8] and after single oral doses of 5 mg/kg dose in male, nonpregnant and pregnant (Day 18 of gestation) female rats [Ref.G-9].

Table 4 (Succeeding page) presents a tabulated summary of the radioactive equivalents (μg/g or μg/ml) observed in rat tissues at 1 to 24 hours following administration of the [^{14}C]montelukast i.v. dose.

Table 4 **Radioactive Equivalents (μg/g or μg/ml) of [^{14}C]Montelukast in the Tissues of Rats Receiving 5 mg/kg i.v. (Mean ± SD; n=3) [Sponsors Table 17 Ref. G-1 Vol. 29 pp. G-65]**

	Hours After Dose			
Tissue	**1 Hour**	**2 Hours**	**4 Hours**	**24 Hours**
Liver	7.73 ± 0.869	4.70 ± 0.911	2.67 ± 0.704	0.392 ± 0.025
Kidney	3.76 ± 0.253	1.98 ± 0.274	0.808 ± 0.218	0.108 ± 0.007
Mesenteric Lymph Nodes	3.18 ±2.31	1.56 ± 0.474	1.23 ± 0.503	0.092 ± 0.024
Pancreas	2.41 ± 0.840	0.847 ± 0.205	1.26 ± 0.817	0.100 ± 0.013
Fat	2.01 ± 0.221	0.900 ± 0.189	0.380 ± 0.020	0.076 ± 0.011
Heart	1.71 ± 0.267	0.877 ± 0.092	0.396 ± 0.071	0.161 ± 0.012
Adrenals	1.53 ± 0.240	0.855 ± 0.191	0.442 ± 0.089	0.414 ± 0.258
Plasma	1.22 ± 0.249	0.641 ± 0.097	0.371 ± 0.040	0.041 ± 0.002
Lung	0.939 ± 0.221	0.410 ± 0.052	0.256 ± 0.033	0.139 ± 0.034
Bladder	0.813 ± 0.147	0.416 ± 0.097	0.240 ± 0.092	0.101 ±0.022
Skin	0.703 ± 0.119	0.314 ± 0.038	0.190 ± 0.089	0.072 ± 0.028
Muscle	0.652 ± 0.099	0.271 ± 0.020	0.130 ± 0.020	0.085 ± 0.031
Spleen	0.560 ± 0.065	0.387 ± 0.036	0.219 ± 0.042	0.136 ± 0.012
Testes	0.307 ± 0.021	0.247 ± 0.036	0.129 ± 0.009	0.030 ± 0.004
Red Blood Cells	0.166 ± 0.031	0.083 ± 0.016	0.051 ± 0.011	0.041 ± 0.002
Brain	0.117 ± 0.004	0.136 ± 0.005	0.085 ± 0.017	0.142 ± 0.027
Gastrointestinal Tract (Tissue and Contents, % of Dose)				
Stomach	0.515 ± 0.432	0.280 ± 0.153	0.391 ± 0.298	0.277 ± 0.296
Small Intestine	64.8 ± 6.74	79.5 ± 4.23	40.0 ±11.7	1.05 ± 0.238
Large Intestine	0.195 ± 0.036	0.108 ± 0.042	15.0 ± 7.63	0.860 ± 0.739
Cecum	0.175 ± 0.040	1.18 ±1.75	30.6 ± 6.45	1.90 ± 0.512
Urine				0.468 ± 0.122
Feces				81.1 ± 2.40

[^{14}C]montelukast-derived radioactivity was widely distributed in rats following i.v. administration [Ref. G-1]. The majority of the radioactivity was recovered in the GI tract and feces, indicating that biliary excretion was the major route of elimination in rats. Tissues with radioactivity greater than plasma at 1 hr after dosing included: liver, kidney mesenteric lymph nodes, pancreas, fat, heart, and adrenals, with only a trace amount detected in brain. Radioactivity in all tissues declined with time, and the remaining radioactive equivalents in tissues were very low at 24 hr postdose.

Table 5 (Succeeding page) presents a tabulated summary of the radioactive equivalents (μg/g or μg/ml) observed in rat tissues at 1 to 24 hours following oral administration of the 10 mg/kg [^{14}C]-montelukast dose.

Table 5 **Radioactive Equivalents (μg/g or μg/ml) of [^{14}C]Montelukast in the Tissues of Rats Receiving 10 mg/kg p.o. (Mean ± SD; n=3) [Sponsors Table 2 Ref. G-8 Vol. 29 pp. G-239]**

	Hours After Dose		
Tissue	**1 Hour**	**6 Hours**	**24 Hours**
Small Intestine	85.9 ± 39.7	27.2 ± 6.29	1.68 ± 0.74
Stomach	29.53 ± 17.5	8.27 ± 4.14	1.14 ± 1.43
Liver	20.3 ± 16.2	6.65 ± 3.15	0.77 ± 0.09
Large Intestine	15.4 ± 1.28	24.9 ± 15.8	1.31 ± 0.27
Cecum	4.02 ± 0.54	67.7 ± 27.1	3.95 ± 0.84
Kidney	2.56 ± 1.20	1.95 ± 0.45	0.21 ± 0.07
Mesenteric Lymph Nodes	5.42 ±2.30	4.0 ± 0.98	0.24 ± 0.05
Pancreas	4.18 ± 1.79	2.84 ± 1.40	0.19 ± 0.07
Fat	1.20 ± 0.61	0.86 ± 0.26	0.16 ± 0.03
Heart	1.44 ± 0.83	0.88 ± 0.39	1.08 ± 0.69
Adrenals	1.81 ± 0.58	0.54 ± 0.47	0.33 ± 0.15
Plasma	1.23 ± 0.75	0.49 ± 0.13	0.07 ± 0.02
Lung	1.62 ± 1.25	1.51 ± 1.84	0.39 ± 0.27
Bladder	0.49 ± 0.15	0.45 ± 0.17	0.10 ± 0.03
Skin	0.41 ± 0.11	0.30 ± 0.05	0.10 ± 0.05
Muscle	0.40 ± 0.16	0.34 ± 0.04	0.05 ± 0.02
Spleen	0.61 ± 0.18	0.52 ± 0.37	0.16 ± 0.07
Testes	0.38 ± 0.26	0.39 ± 0.16	0.05 ± 0.02
Red Blood Cells	0.19 ± 0.11	0.15 ± 0.02	0.06 ± 0.04
Brain	0.12 ± 0.06	0.84 ± 0.70	0.68 ± 0.54
Gastrointestinal Tract (Tissue and Contents, % of Dose)			
Stomach	33.2 ± 17.4	4.40 ± 2.89	1.38 ± 2.30
Small Intestine	46.0 ± 15.5	27.3 ± 14.8	0.711 ± 0.373
Large Intestine	4.63 ± 2.25	13.0 ± 14.1	9.94 ± 4.22
Cecum	1.02 ± 0.597	48.8 ± 25.0	10.2 ± 3.46
Urine			0.458 ± 0.048
Feces			106 ± 20.7
GI Contents + Excreta	84.9 ± 3.78	98.3 ± 12.3	128 ± 25.3
Total Recovery	111 ± 10.6	111 ± 13.7	130 ± 25.2

The data in table 5 above show a pattern of distribution following oral dosing which was qualitatively similar to that seen following i.v. dosing. At 1 hr after administration of the 10 mg/kg oral dose, tissues which had levels of [^{14}C]montelukast-derived radioactivity greater than that in plasma included: in descending order small intestine, stomach, liver, large

intestine, mesenteric lymph nodes, pancreas ,cecum, kidney, adrenal, lung and heart, with only trace amounts detected in the brain and red blood cells. Radioactivity declined over time with only ~1% remaining in the tissues at 24 hr after dosing. Essentially all of the radioactivity was recovered in the feces and only 0.5% recovered in the urine [Ref. G-8].

The second autoradiographic study in which a 5 mg/kg oral dose of [^{14}C]montelukast was administered, male and nonpregnant female rats showed essentially the same pattern of distribution outlined above (i.e. highest contents observed in intestinal contents, gastric contents, mesenteric lymph nodes, bile and liver). At 96 hr after dosing only the intestinal contents of the male showed detectable levels of radioactivity.

Finally, in an autoradiographic study in pregnant rats, the distribution of [^{14}C]-MK-0476-derived radioactivity to fetoplacental tissues was investigated following administration of a 5 mg/kg p.o. dose. At 1 hr postdose, radioactivity of stronger intensity than maternal blood was detected in the ovary and mammary glands, while intensity in the placenta, fetal membrane, uterus, fetal liver, clitoral gland, fetus and amniotic fluid was weaker than that of maternal blood. At 24 hr, radioactivity in the fetoplacental tissues was barely detectable. The pattern of distribution of radioactivity in tissues other that fetoplacental tissues was similar to that seen in non pregnant females.

Metabolism:
The metabolic profiles of MK-0476 were determined in plasma and bile samples from humans (100 mg or 50 mg, p.o.), monkey (plasma only, 50 mg/kg p.o. or 10 mg/kg i.v.), rat (20 mg/kg i.v., or 200 mg/kg p.o.) and mouse (50 or 100 mg/kg p.o.) were determined

Additional in vitro hepatic microsomal metabolism studies using hepatic microsomal preparations from mice, rats, monkeys and humans were conducted in order to determine the in vitro metabolism of MK-0476 as well as to identify the monooxygenase system and P-450 isoforms responsible for MK-0476 metabolism [Ref. G-15].

In all species the unchanged MK-0476 accounted for the majority of the observed plasma radioactivity. Human plasma also contained low levels of the diastereometric 21- hydroxy metabolites and 36-hydroxy metabolites. Plasma metabolites identified in mice included: the diasteromeric 21 (S)-hydroxy (M5a, L-772,146), 21(R)-hydroxy (M5b, L772-145), and 36-hydroxy metabolites (M6a, L-775,066 and M6b, L-775,065, absolute stereochemistry unknown). Monkey plasma also contained the diastereometric 21- and 36-hydroxy metabolites and in rats all metabolites except the 36-hydroxy (M6a) metabolite were observed. Finally rat and mouse plasma showed both a sulfoxide (M1) and a phenol (M3 partial structure) metabolite, whereas only the M1 was evident from monkeys and neither metabolite was evident in human plasma

Comparison of profiles of bile from human (50 mg p.o.), rat (20 mg/kg i.v. or 200 mg/kg p.o.) and mouse (50 mg/kg p.o.) revealed the following species specific differences: The major metabolite in human bile was the dicarboxylic acid (M4), whereas in

rat and mouse bile, the acyl glucuronide conjugant (M1) was the major metabolite, although trace quantities of the M4 metabolite were detectable in both rat and mouse bile using LC-MS/MS. Other metabolites common to all species included a sulfoxide (M1), a phenol (M3 partial structure) and the diastereomeric 21-(M5a and M5b) and the 36-hydroxyl metabolites (M6a and M6b).

Figure 1 below presents a qualitative summary of the metabolic profiles of MK-0476 in plasma and bile form humans, monkeys, rats and mice following in vivo dosing.

In vitro studies on the hepatic microsomal metabolism of MK-0476 showed four prominent metabolites were common to humans (adult and pediatric), mice , rats, and monkeys. These metabolites were identified as the acyl glucuronide (M1), sulfoxide, (M2), 21-hydroxylated (M5), and 36-hydroxylated (M6) metabolites. The rank order of acyl glucuronidation (M1) was: Mouse > monkey > rat > human, whereas the formation of the (M2 + M5 + M6) metabolites appeared comparable in rats, humans, and monkeys, but was somewhat less in

mice (See Table 6, below). There were no significant differences in the metabolic profiles in human microsomes from adults (age = 49±8) versus pediatrics (age = 9±3). Additional kinetic studies also indicated that acyl glucuronidation was more prevalent in rodents relative to humans.

Table 6. Formation of MK-0476 metabolites by liver microsomes from mice, rats, monkeys and humans[a] (Sponsor's Table 1 Ref. G-15 Vol. 29 pp. G-471)

	Formation of Metabolites (pmol/min/mg protein)						
Species	M1	M2a	M2b	M5a	M5b	M6a/b	M6a:M6b[b]
Mouse	675	13.7	21.1	1.77	6.06	2.16	13:87
Rat	486 ± 135	50.7 ± 15.5	93.4 ± 28.4	2.33 ± 0.69	8.81 ± 2.76	3.51 ± 0.88	27:73
Monkey	611 ± 91	37.4 ± 11.8	52.1 ± 13.6	3.78 ± 0.91	12.6 ± 5.01	3.47 ± 0.59	33:67
Human	251 ± 72	56.9 ± 49.2	68.8 ± 45.6	5.66 ± 5.58	13.1 ± 11.8	5.80 ± 2.93	73:27

[a] Data represent mean, n=2 for mouse or mean ± SD, n=3 for rat and monkey, n=6 (M1) or 12 (M2a to M6a/b) for human

[b] Ratio of disatereomers of 36-hydroxy- MK-0476 was determined by

Marker studies which identified the human P-450 isoforms responsible for MK-0476 metabolism showed that the microsomal CYP3A4 isoform catalyzed the diastereomeric sulfoxidation and 21-hydroxylation, whereas the CYP2C9 isoform selectively formed the 36-hydroxylated metabolites. Finally the rank order of percent contribution of oxidative metabolism to total in vitro metabolism of MK-0476 ($\sum V_{max}/K_m$) was: monkey (20%) = rat (20%) > human (17%) > mouse (1.3%).

Excretion:

Table 7 (below) presents a tabulated summary of the of the recovery of [^{14}C]Montelukast-derived radioactivity following i.v. dosing in rats (5 mg/kg) and monkeys (2 mg/kg) and following oral dosing in humans (100 mg/kg).

Table 7. Recovery of [^{14}C]Radioactivity in Urine and Feces in Rats, Monkeys and Humans (Mean ± SD) (Sponsor's Summary Table G-6 Vol. 29 pp. G31)

Species	Dose of [^{14}C]Montelukast	% of Dose	
		Feces	Urine
Rat (n=5)	5 mg/kg, i.v.	82.4 ± 8.89	0.71 ± 0.14
Monkey (n = 4)	2mg/kg, i.v.	88.0 ± 1.95	0.33 ± 0.03
Human (n = 6)	100 mg, p.o.	86.3 ± 3.65	0.118 ± 0.045

The data in table 7 (preceding page) show that almost essentially all of the administered radioactivity was excreted in the feces of rats monkeys and humans following oral and i.v.

dosing. Biliary excretion was demonstrated to play a major role in the elimination of MK-0476. This was demonstrated by the extensive recovery of [^{14}C]-montelukast derived radioactivity in the feces following i.v. administration in rats and monkeys [Ref. G-1]. Biliary excretion was also directly demonstrated using bile duct cannulated rats where essentially all of the radioactivity was recovered in the bile in 6 hr following administration of MK-0476 (20 mg/kg i.v.) [Ref. G-1] . Additional analysis of the metabolic profile of MK-0476 in bile from rats indicated that unchanged drug accounted for only 4% of the dose. Thus while the unchanged parent compound accounts for the majority of circulating radioactivity, montelukast is extensively metabolized in the liver prior to its excretion in the bile and subsequent elimination in the feces in rats.

In humans recovery of radioactivity in the feces was > 86% following a single oral dose of 100 mg [^{14}C]montelukast, with about 80% of the circulating radioactivity accounted for by the unchanged parent compound [Ref. G-13, Vol. 29 pp. G397]. However, qualitative analysis of human bile following administration of a 50 mg p.o. dose showed that MK-0476 was extensively metabolized to a major and several minor metabolites. These findings suggest that montelukast is extensively metabolized by the liver and the majority of the metabolites are excreted in the bile, with only low levels of metabolites observed in plasma. Thus, the studies in humans indicated that hepatic metabolism followed by biliary excretion plays a significant role in the elimination of montelukast and its metabolites, as was true for the other species studied.

ADME SUMMARY

Absorption following oral dosing with montelukast rapid in rats and mice at low doses (at 5 mg/kg, Tmax =22 and 30 min) but increased to 1.3 and 1.5 hr at top doses of 200 mg/kg. More prolonged absorption was seen in monkeys (Tmax =68 min to 1.7 hr over a dose range of 5 to 150 mg/kg) and humans (Tmax = 3.7 hr at a 10 mg/ day dose). Oral bioavailability of MK-0476 was greatest in humans (67%) followed by mouse and monkey (25-61%) and lowest in rats (33%).

Following i.v. dosing plasma concentrations declined in a multiexponential fashion in rats, mice, and monkeys with respective t½ values of 88, 76, and 127 min versus ~5 hr in humans. Plasma clearance is essentially constant up to 10 mg/kg i.v. in all animal species, with greatest clearance observed in rat (15 ml/min/kg) followed by mouse (11 ml/min/kg), monkey (2.5 ml/min/kg), and human (0.58 ml/min/kg). The Vd_{ss} of montelukast varied from 0.85 L/kg for mice (close to total body water) to 0.13 L/kg for humans (approximately equal to blood volume + extracellular fluid volume). Montelukast binds extensively (>99%) to plasma proteins (primarily albumin) and preferentially distributes to plasma component of blood (blood/plasma ratio ~ 0.65) in rats monkeys and man. [^{14}C]montelukast-derived radioactivity was widely distributed following i.v. and oral dosing in rats with liver, kidney, mesenteric lymph nodes, pancreas, fat, heart and adrenals showing levels greater than plasma and little radioactivity in brain and red blood cells. Autoradiographic studies in pregnant rats also showed that in addition to the aforementioned tissues, radioactivity also distributed to the ovaries and mammary glands at levels which exceeded that in blood.

Montelukast was extensively metabolized in humans, mice, rats, and monkeys with the majority of metabolites excreted in bile and feces, with only low levels of metabolites observed in plasma. Major metabolic pathways included: (a) acyl glucuronidation, (b) sulfoxidation, (c) hydroxylation of the isopropylphenyl moiety, (d) further oxidation of the 36-hydroxy metabolite to a dicarboxylic acid, and (e) hydroxylation at the 21-position. Diastereomers of the sulfoxide, 21- and 36-hydroxy and the dicarboxylic acid analogs were common to all species. In rodents, acyl glucuronidation is predominant, while in humans, oxidation at the 36-position is favored. The Cytochrome P-450 isoforms: CYP3A4 (catalyzes sulfoxidation and 21-hydroxylation) and CYP2C9 (selectively forms the 36-hydroxylated metabolites) were exclusively responsible the formation of oxidative metabolites. Biliary excretion was shown to be the the major route of elimination of montelukast and its metabolites in rat, monkey and human, with less than 1% recovery in the urine after i.v. or oral administration.

Interspecies comparisons of PK data (i.v. and oral) and metabolic handling are contained in (Table 2 page 9 and Table 3 page 11) and Figure 1 page 15 of this review, respectively. These comparisons showed that the pharmacokinetic and metabolic handling of montelukast was qualitatively comparable in most species tested.

TOXICOLOGY

Single Dose

Exploratory Acute Oral Toxicity of L-706,631-002P in Female Mice

(TT#92-2820 Vol. 30, pp. H-61)

Methods: This study was conducted in order to attempt to find the approximate lethal $dose_{50}$ of L-706,631-002P, a process intermediate and dicyclohexylamine salt of MK-0476. L-706,631-002 (lot #7), was dissolved in 0.5% aqueous methylcellulose and administered to female mice via gavage at doses of 320, 800, and 2000 mg/kg (n = 3 mice/group) and at a dose 5000 mg/kg (n = 1). Mice were observed for mortality and clinical signs of drug effects for seven days after dosing.

Results: L-706,631-002P produced clinical signs of ataxia, decrease activity, bradypnea, ptosis, (2000 mg/kg only) and clonic convulsions (5000 mg/kg only) from 30 min through day 3 after dosing. Death occurred within 30 min to day 3 in the mouse at the 5000 mg/kg dose and in 1 of 3 mice at the 2000 mg/kg dose. The minimum lethal dose was 2000 mg/kg and the maximum nonlethal dose was 800 mg/kg. The LD_{50} for L-706,631-002 was probably between 2000 and 5000 mg/kg in mice.

In conclusion, L-706,631-002P, a process intermediate and dicyclohexylamine salt of MK-0476, was well tolerated in mice at acute doses up to 800 mg/kg, p.o., but produced death preceded by signs of CNS toxicity at higher doses of 2000 and 5000 mg/kg.. The LD50 for L-706,631-002P was between 2000 and 5000 mg/kg in mice.

LOCAL TOLERANCE

Exploratory Dermal Irritation Study in Rabbits TT #94-2649 (Vol. 30, pp.H-20)

Study Dates: 26 APR 94 - 15 AUG 94
Testing Lab: Merck Research Laboratories, West Point, PA
Test Articles: L-706,631-001M (lot # 24)
GLP: The study was designated as exploratory and thus was not accompanied by a signed GLP statement.

Methods: The potential for dermal irritative effects of L-706,631 was determined in rabbits (2male and 1 female) following dermal application of 500 mg (neat powder) on a 5 cm^2 dorsal site (covered with a gauze pad, moistened with 0.5ml of saline, and wrapped with an occlusive dressing) for 24 hours. Treatment sites were examined at 24 hr intervals and results were recorded according to the Draize scoring system (J. Pharmacol. Exp. Therap. 82:377-390)

Results: L-706,631 produced well defined erythema in all rabbits after 24 hours of treatment, becoming very slight on days 3 and 4 and resolving by day 8. Thus, the results of this study indicated that L-706,631 is a mild dermal irritant in rabbits.

Study Dates: 26 MAY 94 to 06 OCT 94
Testing Lab: Merck Research Laboratories, West Point, PA
Test Articles: L-706,631-001M024
GLP: The study was not accompanied by a signed GLP statement.

Methods:

Results: L-706,631 produced no with only a weak effect on (0.419 ± 0.035 versus 0.056 ± 0.002 in control) such that final irritancy scores were 6.3 for L706,631 and 1.2 for controls. Thus, L-706,631 was classified as a mild irritant to the eye.

Exploratory Primary Eye Irritation Study in Rabbits TT #94-4269 (Vol. 30 pp.H-35)

Study Dates: 26 MAY 94 to 26 OCT 94
Testing Lab: Laboratories Merck

Test Articles: L-706,631 001M (Batch 024)
GLP: The study was not accompanied by a signed GLP statement, but was stated to be conducted in accordance with SOP.

Methods: MK-0476 (100 mg of the sodium salt powder) was placed in the conjunctival sac of the left eye of rabbits; the right eye was untreated. The eye lids were then held together for 20 sec. Ocular reactions were scored based on Draize scoring system at 15, 120 minutes, 24, 48, and 72 hours, 4 and 7 days after instillation.

Results: MK-o476 produced slight conjunctival redness and very slight to slight chemosis of bulbar conjunctiva in all rabbits at 15 to 120 min after dosing. slight corneal opacity (3 of 3 rabbits and slight iritis (2 of 3 rabbits) were also noted. The corneal opacity was increased in severity at the 24 hr time period and remained unchanged at the subsequent 48 hr time period. Most of the ocular reactions regressed in 2 of three rabbits by 72 hr after dosing but, not in the third which was killed for humane reasons due to persistent ocular signs. Maximum Draize scores ranged from ' and occurred from 120 min to 48 hr after dosing in all three rabbits. Based on these findings the powder of MK-0476 was classified as severely irritating to the eyes of rabbits.

Dermal Irritation Study with L-706,631-002P (a process intermediate and dicyclohexylamine salt of MK-0476) in Rabbits TT #92-2819 (Vol. 30 pp.H-72)

Study Dates: 21 SEP 92 to 24 MAY 95
Testing Lab: Merck Research Laboratories, West Point, PA
Test Articles: L-706,631-002P (lot # 7)
GLP: The study was exploratory in nature and thus not accompanied by a signed GLP statement.

Methods:, The potential for dermal irritative effects of L-706,631-002P, a process intermediate and dicyclohexylamine salt of MK-0476 was determined in rabbits (1 male and 2 females) following dermal application of 500 mg on a 5 cm^2 dorsal site (moistened with 0.5ml of saline, covered with a gauze pad then wrapped with an occlusive dressing) for 24 hours. Treatment sites were examined at 24 hr intervals and results were recorded according to the Draize scoring system (J. Pharmacol. Exp. Therap. 82:377-390)

Results: L-706,631-002P, a process intermediate and dicyclohexylamine salt of MK-0476 produced no signs of dermal irritation throughout the 8 day observation period. Thus, the results of this study indicated that L-706,631-002P had no dermal irritant effects in rabbits.

Study Dates: June 95
Testing Lab: Laboratories Merck

Test Articles: L-706,631-002P007 a process intermediate and dicyclohexylamine salt of L-706,631.
GLP: The study was exploratory in nature and thus was not accompanied by a signed GLP statement. However, the study was stated to be conducted in accordance with SOP.

Methods:

Results: L-706,631-002P007 produced (7.75 ± 1.26 versus 0.000 ± 1.73 in controls), but had no effects on permeability such that final irritancy scores were 8.4 for L706,631 and 0.7 for controls. Thus, L-706,631-002P007 was classified as a mild irritant to the eyes.

Exploratory Primary Eye Irritation Study with L-706,631-002P in Rabbits TT #92-4269 (Vol. 30 pp.H-126)

Study Dates: 02 NOV 92 to 15 JUN 95
Testing Lab: Laboratories Merck

Test Articles: L-706,631-002P; Batch #007
GLP: The study was exploratory in nature and thus was not accompanied by a signed GLP statement. However, the study was stated to be conducted in accordance with SOP.

Methods: L-706,631-002P, a process intermediate and dicyclohexylamine salt of MK-0476, (100 mg of the powder) was placed in the conjunctival sac of the left eye of rabbits; the right eye was untreated. The eye lids were then held together for 20 sec. Ocular reactions were scored based on Draize scoring system at 15, 120 minutes, 24, 48, and 72 hours, 6 and 7 days after instillation.

Results: L-706,631-002P produced evidence of irritation which included moderate to severe conjunctival redness and slight to severe discharge of the treated eyes, very slight to severe chemosis and slight to severe discharge were seen in all rabbits. Two rabbits showed iris congestion was noted in two rabbits and was associated with very slight edema of the cornea in one. Evidence of ocular irritant effects were first observed at the 15 min time point and were maximal (i.e. maximal Draize scores 1 to 20) between 120 and 48 hours after dosing. Evidence of irritation persisted in 2 of the three rabbits at the 72 hour time point, but were resolved in all rabbits by Day 6 after dosing. According to the interpretation of eye irritation tests by Kay and Calendra[1], L-706,631-002P was classified as mildly irritating to the eyes of rabbits.

SUMMARY OF STUDIES ON LOCAL TOLERANCE:
Dermal and ocular studies on the local tolerance to the montelukast sodium bulk drug showed it to be mildly irritating to the skin of rabbits.
assays also indicated that montelukast had mild ocular irritant potential, whereas Draize scores indicated it to be severely irritating to the eyes of rabbits.

In additional studies reviewed herein, the local tolerance of L-706,631-002P, a process intermediate and dicyclohexylamine salt of MK-0476 was assessed. Results from these studies showed that L-706,631-002P had no dermal irritant effects in rabbits, but was classified as a mild irritant to the eyes based on the results of both the and on the basis of Draize scores from in vivo studies, wherein it was administered directly to the eyes of rabbits.

Oral/Subcutaneous Immunogenicity Study in Guinea Pigs TT #95-9805 (Vol.31 pp.Q-87)
Study Dates: 16 MAR 95 to 21 JUN 95
Testing Lab:
Test Articles: MK-0476; L-706,631-001M (lot # 042)
GLP: The study was accompanied by a signed GLP statement.

Methods: The potential immunogenicity of MK-0476 was studied in guinea pigs

[1] Kay J.H. and Calandra J.C.,; Interpretation of Eye Irritation Tests. J. of the Society of Cosmetic Chemists. 13: 281-289, 1962

Results:

Guinea pigs immunized with MK-0476 (1 or 10 mg/animal) orally or (1 mg/animal) subcutaneously showed no systemic anaphylactic type reactions after intravenous challenge with MK-0476 (1 mg/animal). In contrast, challenge with the positive control produced severe systemic anaphylactic reactions in 4 of the 5 animals tested resulting in death in 3 of the said animals. Finally, neither MK-0476 nor . conjugate induced systemic reactions in two negative control animals.

Likewise, the Four-hour failed to demonstrate a reaction in in naive guinea pigs receiving intradermal injections of from guinea pigs administered either the MK-0476 (1 or 10 mg/animal) solution orally or the MK-0476 (1 mg/animal) emulsions subcutaneously when challenged intravenously with MK-0476 (1 mg/animal). Only 3 of the 5 sera from positive controls had of 2^6 or 2^{13}. However, 2 sera from two animals with mild and severe reactions did not show a positive reaction. from negative control animals showed no reaction.

In conclusion, under the conditions of this assay, montelukast did not induce active systemic or passive cutaneous anaphylaxis in guinea pigs.

OVERALL SUMMARY AND EVALUATION

Montelukast sodium is a potent and highly selective leukotriene D_4 (LTD_4) antagonist which acts at the structurally specific, high affinity Cys LT_1 receptor. Currently the NDA applications 20-829 and 20-830 propose to market montelukast (Singulair ™, 10 mg oral tablet and 5 mg oral chewable tablet) for the prophylaxis and chronic treatment of asthma in adults and pediatric patients 6 years of age and older.

In support of the current application the Sponsor has submitted preclinical studies including: in vitro and in vivo pharmacology, ancillary pharmacology, ADME studies in rats, mice, and monkeys including placental and milk transfer studies in rats and/or rabbits; acute single

dose oral and i.v. toxicity studies in rats and mice; subacute toxicity testing in adult monkeys (5 and 14 week oral toxicity; 16 day i.v. toxicity and 17 day i.v. irritation studies), infant monkeys (14 week oral toxicity) rats (5 and 14 week oral toxicity and 16 day i.v. toxicity), and mice (5 and 14 week oral toxicity); chronic 53 week oral toxicity testing in rats and monkeys, reproductive toxicology studies (Segment I oral fertility studies in male and female rats, Segment II oral developmental toxicity studies in rats and rabbits and a Segment III oral late gestation and lactation study in rats); Genetic toxicity/mutagenic potential studies

Carcinogenicity studies (106-week oral study in rats, and 92-week oral study in mice); Local tolerance studies (Dermal irritation in rabbits; and Ocular irritation study in rabbits) and special toxicity studies (Phototoxicity in mice, enzyme induction in mice and rats, in whole blood and washed RBCs from rat, human and dog; drug interaction studies in mice; and immunognenicity studies in guinea pigs).

Pharmacodymamics: Montelukast's potency and selectivity at the Cys LT_1 receptor was demonstrated in in vitro pharmacology studies including: receptor binding studies on guinea-pig lung, sheep lung and human dU937 cell membranes, and studies on isolated guinea-pig trachea. In vivo potency and selectivity have also been demonstrated in in vivo studies where montelukast administered by the i.v., aerosol or oral route inhibited leukotriene D_4-induced bronchoconstriction in the guinea-pig (ED_{50} = 0.001 mg/kg, i.v.; and 13 nM nebulizer concentration), squirrel monkey (ED_{50} ~ 0.01 mg/kg p.o.), and to a lesser degree in conscious sheep when administered by aerosol. Montelukast was devoid of any activity against a variety of other bronchoconstrictors in anesthetized guinea pigs and did not inhibit the binding of 5-lipoxygenase activating protein (FLAP).

In vivo pharmacology studies also demonstrated a role for Cys LT_1 receptor activation, in the mediation of antigen-induced bronchoconstriction, wherein montelukast inhibited antigen-induced dyspnea in inbred rats (ED_{50} = 0.032 mg/kg p.o.), ascaris-induced bronchoconstriction in conscious squirrel monkeys (ED_{50}~0.03 mg/kg p.o), allergen induced bronchoconstriction in allergic conscious Squirrel monkeys (0.1 mg/kg p.o.) and ascaris-induced early and late phase bronchoconstriction in conscious sheep (1 mg/kg i.v. loading dose + 8 ug/kg/min i.v. infusion).

Montelukast administered to dogs in doses up to 10 mg/kg i.v. or 20 mg/kg p.o. was devoid of deleterious effects on cardiovascular, autonomic, renal, gastrointestinal, or respiratory functions and produced no significant behavioral changes in mice at oral doses up to 100 mg/kg. Thus, there were no significant ancillary pharmacological effects at the doses tested, which would raise concern for the currently proposed marketing of singulair.

Clinical studies which have demonstrated that Cys LT1 antagonists can effectively block antigen-induced bronchoconstriction, exercise-induced bronchoconstriction, and aspirin-

induced bronchoconstriction in asthmatic subjects[2,3,4,5] portend a role for mediators such as LTD4 and LTE4 in the pathogenesis of asthma. Thus, montelukast may have therapeutic value as a LTD4 receptor antagonist in the treatment of asthma.

Pharmacokinetics: Montelukast has relatively poor oral bioavailability in rats (33%), but greater bioavailability in humans (67%) followed by mouse and monkey (25-61%). In rats, Montelukast distributed primarily to liver, kidney, mesenteric lymph nodes, pancreas, fat, heart and adrenals, with little distribution in brain and red blood cells. Montelukast crossed the placental barrier and entered the fetal circulation in rats and rabbits and significant amounts of MK-0476 transferred into the milk of lactating rats. Montelukast extensively binds (>99%) to plasma proteins (primarily albumin) in all species. Montelukast undergoes extensive oxidative metabolism in the liver, with the majority of its metabolites excreted in the bile and only low levels of metabolites observed in plasma.. Its metabolic profiles were qualitatively similar across rats, mice, monkeys, and man, with major metabolites (M1-M6) formed via the following metabolic pathways: (a) acyl glucuronidation (M1), (b) sulfoxidation (M2), (c) hydroxylation of the isopropylphenyl moiety (M3) , (d) further oxidation of the 36-hydroxy metabolite (M6) to a dicarboxylic acid (M4), and (e) hydroxylation at the 21-position (M5). The major metabolite in bile from rats and mice was the acyl glucuronide conjugant (M1) whereas a dicarboxylic acid (M4) was the major biliary metabolite in humans. In vitro studies using human liver microsomes showed oxidative metabolism was catalized mainly by cytochrome P450 enzymes and involved the P450 isoform, CYP3A4 in the sufoxidation (M2a/b) and 21-hydroxylation (M5a/b) of MK-0476 and the CYP2C9 isoform for the 36-hydroxylation pathway (M6a/b). Biliary excretion was the major route of elimination of Montelukast and metabolites in rat, monkey and human, with less than 1% recovery in the urine after i.v. or oral administration. Elimination appeared multiexponential in rats, mice, and monkeys with respective t½ values of 88, 76, and 127 min versus ~5 hr in humans.

Acute Toxicity: Acute oral and i.v. toxicity of Montelukast was evaluated in rats and mice. In rats and mice oral doses ≥ 1250 mg/kg produced signs of CNS toxicity including decreased activity and ptosis in mice and decreased activity, salivation and soft stool in rats. The minimum lethal oral dose in mice and rats was > 5000 mg/kg and the oral NOEL was

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[2] Rasmussen JB *et al.* Leukotriene D_4-receptor blockade inhibits the immediate and late bronchoconstrictor responses to inhaled antigen in patients with asthma. J. Allergy Clin Immunol 1992;90:193-201.

[3] Manning PJ *et al.* Inhibition of exercise-induced bronchoconstriction by MK-571, a potent leukotriene D_4-receptor antagonist. N Engl J Med 1990;323:1736-1739.

[4] Christie PE *et al.* The potent and selective sulfidopeptide leukotriene antagonist, SK&F 104353, inhibits aspirin-induced asthma. Am Rev Respir Dis 1991;144:957-958.

[5] Dahlén B, *et al.* The leukotriene antagonist MK-0679 improves baseline pulmonary function and blocks aspirin-induced airway obstruction in aspirin-sensitive asthmatics. Am Rev Respir Dis 1992;145 (suppl):A15.

625 mg/kg. The maximum non lethal i.v. dose was 78 mg/kg in rats and 156 mg/kg in mice, with 100% mortality seen at the next higher doses of 156 and 312 mg/kg in rats and mice, respectively. Clinical signs of CNS toxicity (decreased activity, bradypnea, ataxia, convulsions and/or gasping) preceded death following i.v. dosing in both species. A subsequent acute toxicokinetic study in mice demonstrated a plateau for systemic exposure in mice at approximately 800-1200 mg/kg.

Subacute and Chronic Toxicity: Subacute toxicity studies were conducted in mice, rats, and monkeys, with chronic toxicity studies conducted in rats and monkeys. Primary targets organs of toxicity included the GI tract in mice, rats, and monkeys and the bone marrow in monkeys.

In mice, abdominal distention and/or cecal dilation and mortality occurred after oral dosing at levels of 800 and 1200 mg/kg for 5-weeks and at doses ≥200 mg/kg for 14 weeks. The NOAEL in the 14-week study in mice was 50 mg/kg (approximately 250 and 200 times, respectively, the proposed adult and pediatric doses on mg/kg basis). In single dose pharmacokinetic studies in mice oral doses of 200 mg/kg resulted in a mean AUC values of 9904 μg·hr/ml, whereas AUC values at the NOAEL dose (50 mg/kg) averaged 2411 μg·hr/ml.

In rats, treatment-related mortality occurred at doses of 800 mg/kg in one of two 14-week studies and at doses ≥200 mg/kg in a 1 year study. GI toxicity at these doses was evidenced grossly as dose-dependent dilation of the whole or part of the GI tract. However, histological correlates of ulcer of the nonglandular mucosa, moderate dilation of the cecum, atrophy of the duodenal villi, and typhlitis, were seen only in a limited number of the animals which died. Other target organs of toxicity in rats which died or were sacrificed early included: pancreas (zymogen granule depletion/cytoplasmic rarefaction), adrenals (hemorrhage/degeneration of zona granulosa), lung (edema/congestion) and lymphoid system (lymphoid atrophy of spleen, lymph nodes and thymus). Pharmacokinetic analysis in 3-month oral toxicity study in rats suggested that saturation of absorption occurred at doses 200-400 mg/kg (AUC range from 163 to 215 μg·hr/ml). Animals which died in both the 3-month and 1-year toxicity studies showed comparable toxicity profiles, however death at the 200 mg/kg dose in the 1 year study versus 800 mg/kg in the 14 month study suggested that there is a duration-dependent component to the observed toxicity. The NOAEL in the 1-year rat study was 50 mg/kg. In the 3-month study in rats the 50 mg/kg dose resulted in mean exposures ($AUC_{0\text{-}24h}$) of 58.5 and 37.9 μg·hr/ml in males and females, respectively.

In adult monkeys, treatment related mortality was seen at doses of 450 mg/kg after 3 months and at 300 mg/kg after 1 year of dosing. At the 450 mg/kg dose in the 3 month study, two males which were euthanized showed GI findings of necrosis of the glandular fundic cells and depletion of mucinous cells in the stomach, and congestion, erosions and atrophy of the villi and/or mucosa of the intestines). However, GI disturbances other than soft stools and diarrhea were not seen in monkeys in the subsequent 1-year study. Toxicity to the hematopoietic system (hypoplasia/degeneration of erythropoietic tissue in the bone marrow)

were seen in high dose monkeys which died or were euthanized in both the 3 month and 1-year studies. Multiple organ toxicity observed in monkeys which died included effects on: spleen (congestion, lymphoid depletion, sinusoid congestion, artery and/or fibrinoid necrosis), lymph nodes (edema and inflammatory cell infiltration of the sinusoids and/or fibrinoid arterial necrosis), thymus (lymphoid necrosis), pancreas (focal depletion of zymogen granules), adrenals (degeneration of the zona glomerulosa and/or zona glomerulosa, and single cell necrosis, and congestion of the zona fasciculata), kidneys (hyaline cast, degeneration and single cell necrosis and epithelial cell vacuolation of the tubules), and heart (focal agonal hemorrhage and focal degeneration of the myocardium). Exposures in monkeys at doses ranging form 50 to 450 mg/kg were reduced by about 50% after 13 weeks of dosing versus Day 1 values, suggestive of possible enzyme induction or reduced absorption with repeat dosing in monkeys. The NOAEL for adult monkeys in the 1-year oral toxicity study was 150 mg/kg. The 150 mg/kg dose was shown to result in mean exposures (AUC-values) of 626.7 μg·hr/ml (after 13-weeks of repeated dosing in monkeys). Although treatment-related mortality/euthanasia occurred at a dose of 450 in the 3-month study and at a dose of 300 mg/kg in the 1-year study, determination of a possible duration dependent component to the toxicity in monkeys was not possible, since the next highest dose tested in both studies (150 mg/kg, p.o.) was the NOAEL in each.

A three month toxicity study in infant monkeys (4 weeks of age) showed a toxicity profile comparable to that seen in adults (i.e. GI Toxicity including epithelial vacuolation of the glandular mucosa, chronic enteritis, and colitis in a high dose female which was euthanized and gastritis in 2 high dose males). However, infants may be more sensitive to the toxicity, since one infant monkey required euthanasia at an oral dose of 150 mg/kg, whereas in the 3-month study in adult monkeys, euthanasia was performed on two monkeys at an oral dose of 450 mg/kg. Thus, the NOAEL for infant monkeys in the 3-month oral toxicity study was 50 mg/kg versus 150 mg/kg in the 3-month study in adults.

The other most noteworthy finding observed in various studies were increases in ALT: (2-3.6 fold) in rats in a 7-day oral range finding study at doses of 800 and 1600 mg/kg and transiently ($\leq$ 2 fold increases) in rats given doses of 100 and 200 mg/kg in a 5-week oral toxicity study. However in the latter 5-week study, observed elevations in ALT returned to control levels during the 5th week of treatment. Increases in ALT were also observed sporadically in other 3-month oral studies in rats at doses 200 mg/kg. However, correlative elevations in other liver enzymes AST and ALK were not consistently observed. In addition, elevated ALT levels were not observed in 1-year studies in rats at doses up to 400 mg/kg and no evidence of histological alterations have been correlated with these biochemical changes in any of the studies mentioned. In the 3-month and 1-year studies in monkeys, animals which died also showed increased AST and ALT levels. However, histological correlates to these findings were limited to very slight hepatocellular vacuolation observed in one high dose female which was euthanized in the 1-year study. Thus collectively, the available studies in rats and monkeys provided little evidence for toxicologically significant hepatotoxic effects in either species.

Reproduction: Effects of MK-0476 on fertility were studied in rats of both sexes. In males. MK-0476 at doses up to 800 mg/kg, p.o. (~4800 mg/m^2, approximately 650 times the proposed adult dose on mg/m^2 basis) produced no treatment related effects on fertility or reproductive performance. In females, oral doses of 200 mg/kg (~1200 mg/m^2, approximately 160 times the proposed adult dose on mg/m^2 basis) produced slight reductions in the fertility (15.8%) and fecundity (13.7 %) indexes. The next lower dose 100 mg/kg (~600 mg/m^2. approximately 80 times the proposed adult and pediatric doses on mg/m^2 basis) had no effects on fertility or fecundity in female rats.

Teratogenicity studies were carried out in rats and rabbits. In rats, oral (gavage) administration of MK-0476 (L-706,631) at doses up to 400 mg/kg/day (~2400 mg/m^2; 300 times the proposed adult dose on mg/m^2 basis) and in rabbits, at doses up to 300 mg/kg/day (~3600 mg/m^2, approximately 490 times the proposed adult dose on mg/m^2 basis) during the period of organogenesis produced no evidence of embryo-fetal toxicity or teratogenicity. An overall increased fetal incidence of incomplete ossification of the pelvic bone along with reduced fetal weights and/or increases in fetal deaths was attributable to severe maternal toxicity (protracted fasting and/or blood in bottom of cage pan) in two dams at a dose of 300 mg/kg in rabbits. No evidence of maternal toxicity was observed at the next lower dose tested, 100 mg/kg/day (~1200 mg/m^2, approximately 160 times the proposed adult dose on mg/m^2 basis).

Potential developmental effects in the F1 generation were assessed in a Segment III oral late gestation and lactation study in rats. MK-0476 at doses up to 200 mg/kg (~1200 mg/m^2; 160 times the proposed adult dose on mg/m^2 basis) had no toxicologically significant effects on pup survival, pre-weaning growth, or F1 development.

Genotoxicity: MK-0476 (L-706,631) tested negative for mutagenic/clastogenic activity in the following assay systems:

Tumorigenicity: MK-0476 was administered to mice and to rats The maximum dose tested in rats was one at which maximal absorbtion was demonstrated, whereas in mice it was MTD. Both studies were acceptable (see attached CAC comments). No statistically significant or dose related increases in the incidence of any tumor type was detected in either mice or rats under the conditions Thus, MK-0476 was regarded as negative for tumorigenic activity in both the mouse and rat In mice, the NOEL of 100 mg/kg = 300 mg/m^2 and is approximately 40 and 50 times the maximum proposed daily dose in adults and pediatric patients on a mg/m^2 basis. In rats the NOEL of 200 mg/kg = 1200 mg/m^2 and is

approximately 160 and 190 times, respectively, the maximum proposed daily therapeutic dose in adults and pediatric patients on an mg/m^2 basis.

Local tolerance: Dermal and ocular studies on the local tolerance to the montelukast sodium bulk drug showed it to be mildly irritating to the skin of rabbits. also indicated that montelukast had mild irritant potential, whereas Draize scores indicated it to be severely irritating to the eyes of rabbits. However, the potential for irritant effects of the bulk drug are not considered to be a risk for the patient population and the formulated therapeutic product used via the intended route of administration.

In additional studies reviewed herein, the local tolerance of L-706,631-002P, a process intermediate and dicyclohexylamine salt of MK-0476 was assessed. Results from these studies showed that L-706,631-002P had no dermal irritant effects in rabbits, but was classified as a mild irritant to the eyes based on the results of both the and on the basis of Draize scores from in vivo studies wherein it was administered directly to the eyes of rabbits.

Special Toxicity: Evaluations of montelukast's potential for phototoxic effects, hemolyisis in vitro, induction hepatic P-450 or peroxisomal enzyme activity in mice or rats, drug interactions in mice and immunogenic effects in guinea pigs was evaluated in a series of studies.

Tests for phototoxicity in mice showed that montelukast, at doses up to 500 mg/kg p.o., was not phototoxic for UVA, UVB or visible light spectra.

In vitro tests for hemolytic potential using rat, dog, and human blood showed that Montelukast produced hemolysis in vitro in blood from all three species. Hemolysis in human blood was observed at MK-0476 concentrations as low as 47 µg/ml in whole blood and 13 µg/ml in washed red blood cells. However, no hemolysis was reported in i.v. studies in rats or monkeys after 2 weeks of i.v. doses up to 0 .72 mg/kg in rats and up to 0.36 mg/kg.

MK-0476's effects on, and/or potential to induce either P450-linked 7-ethoxy-4-trifluoromethylcoumarin 0-deethylase (EFCOD) or hepatic fatty acyl CoA-oxidase activity (FACO; a non-P450 hepatic peroxisomal enzyme) were assessed in mice and rats following 4 days of repeated oral dosing. In mice, MK-0476 (400 mg/kg/day, p.o.) inhibited P450-mediated EFCOD activity (38-55%), but had no significant effects on liver weights or FACO activity. In rats oral doses of MK-0476 (400 and 800 mg/kg; p.o.) produced no effects on liver weights, EFCOD or FACO activities. These studies showed no evidence of P450 or peroxisomal enzyme induction after 4 days of repeated dosing in rats (at doses of 400 and 800 mg/kg, p.o.) or mice (at doses of 400 mg/kg p.o.). However, specific assessments of MK-0476's potential to induce either CYP3A4 [involved the sufoxidation (M2a/b) and 21-hydroxylation (M5a/b) of MK-0476 in humans] or CYP2C9 [involved in the 36-hydroxylation (M6a/b) of MK-0476 in humans] P450 isoforms, were not conducted.

Studies using immunized guinea pigs showed that intravenous challenge with MK-0476 (1 mg/animal) did not induce active systemic or passive cutaneous anaphylaxis.

Drug interaction studies in mice showed that montelukast (5000 mg/kg p.o.) produced no additive or synergistic toxicological effects when given at 1 hr post doing with prednisone (500 mg/kg; 500 times the clinical dose), theophylline (30 mg/kg; 5 times the clinical dose), or salbutamol (40 mg/kg; 500 times the clinical dose).

In conclusion, the pharmacology, pharmacokinetics and toxic potential of montelukast sodium has been evaluated extensively in multiple in vitro and in vivo studies. Treatment related gastrointestinal disturbances were observed in mice, rats and monkeys and potential hematopoietic toxicity was observed in monkeys following repeated in subacute and chronic toxicity testing. However, NOAELs observed in all repeat dose toxicity studies demonstrated wide margins of safety relative to the proposed therapeutic doses for all observed toxicity.

MK-0476 (L-706,631) showed no potential for mutagenic/clastogenic activity

Further, MK-0476 (L-706,631) showed no evidence of tumorigenic activity

The potential of montelukast sodium for reproductive toxicity was characterized in rats and/or rabbits, at high multiples over the proposed clinical range. Results from these studies revealed only mild effects on fertility and fecundity in female rats, but no evidence of embryofetal toxicity, teratogenicity in either species or on pup survival, preweaning growth or F1 development in rats. The NOAELs for effects on fertility and fecundity provided wide margins of safety compared to the proposed therapeutic dose.

Collectively, adequate preclinical testing and demonstration of wide safety margins for the observed toxicity, indicate no preclinical issues related to the proposed therapeutic indication. Such that from a preclinical standpoint the applicationis recommended for approval.

Excipients, Degradants and Impurities: The proposed levels of all excipients, in both the adult tablet and the children's chewable tablet, occur at levels well within the ranges of those used in other currently approved drug products. Thus, there are no nonclinical issues with the proposed excipients in either the adult or pediatric formulation.

The safety of the proposed limits for degradants of the both the adult and pediatric drug products , as well as that for impurities in the drug substance

was qualified based on adequate exposure and margins of safety between the maximum amounts to be administered clinically and amounts administered at the NOAEL in

OVERDOSAGE

No mortality occurred following single oral doses up to 5000 mg/kg in mice (approximately 2000 times the maximum recommended daily oral dose in adults and 2400 times the maximum recommended daily oral dose in children, on a mg/m^2 basis) and rats (approximately 4100 times the maximum recommended daily oral dose in adults and 4800 times the maximum recommended daily oral dose in children, on a mg/m^2 basis). Mortality occurred at an i.v. dose of 156 mg/kg in rats (approximately 130 times the maximum recommended daily oral dose in adults and 150 times the maximum recommended daily oral dose in children, on a mg/m^2 basis) and at an i.v. dose of 312 mg/kg in mice (approximately 130 times the maximum recommended daily oral dose in adults and 150 times the maximum recommended daily oral dose in children, on a mg/m^2 basis).

No specific information is available on the treatment of overdosage with SINGULAIR. In chronic asthma studies, SINGULAIR has been administered at doses up to 200 mg/day to patients for 22 weeks and in short-term studies, up to 900 mg/day to patients for approximately a week without clinically important adverse experiences.

It is not known whether montelukast is dialyzable by peritoneal- or hemodialysis.

Note: Calculations of mg/m^2 doses used for comparison to clinical doses in the preclinical sections of the labeling are provided in table 8 (Succeeding page).

Table 8. Calculations for labeling revisions.

Drug: **Singulair 10 mg tablets and 5 mg chewable tablets**

	age	mg/dose	# daily doses	mg/day	kg	mg/kg	factor	mg/m²
Pediatric	6	5	1	5	20	0.25	25	6.25
Adult	>12	10	1	10	50	0.20	37	7.40

	route	mg/kg/d	conv. factor	mg/m²	Dose Ratio Adults	Dose Ratio Children	Rounded Dose Ratio Adults	Rounded Dose Ratio Children
Carcinogenicity:								
mouse	p.o.	100	3	300	40.54	48.00	40	50
rat	p.o.	200	6	1200	162.16	192.00	160	190
Reproduction and Fertility:								
rat	po ♂ Noel	800	6	4800	648.65	N/A	650	N/A
rat	p.o. ♀	200	6	1200	162.16	N/A	160	N/A
rat	p.o. ♀ Noel	100	6	600	81.08	N/A	80	N/A
Teratogenicity:								
rat	p.o. ♀ Noel	400	6	2400	324.32	N/A	320	N/A
rabbit	p.o. ♀ Noel	300	12	3600	486.49	N/A	490	N/A
rabbit	p.o mat tox	300	12	3600	486.49	N/A	490	N/A
rat	p.o Seg.III	200	6	1200	162.16	N/A	160	N/A
Overdosage:								
mouse	p.o.	5000	3	15000	2027.03	2400.00	2000	2400
mouse	lethal I.v	312	3	936	126.49	149.76	130	150
mouse	non lethal iv	156	3	468	63.24	74.88	65	75
rat	p.o.	5000	6	30000	4054.05	4800.00	4100	4800
rat	lethal I.v	156	6	936	126.49	149.76	130	150
rat	non lethal iv	78	6	468	63.24	74.88	65	75
Other:	(Describe studies here)							
monkey	1-year p.o.	150	12	1800	243.24	288.00	240	290
rat	1-year p.o.	50	6	300	40.54	48.00	40	50
monkey	14-wk infant	50	12	600	81.08	96.00	80	95

RECOMMENDATION

The NDAs: for SINGULAIR are approvable from a preclinical standpoint, with incorporation of the suggested revisions for the labeling sections entitled: *Carcinogenesis, Mutagenesis, and Impairment of Fertility, Pregnancy Category, Nursing Mothers, and OVERDOSAGE* as indicated above.

/S/ 12/1/97

Shannon Williams, Ph.D.,
Pharmacologist

/S/ Dec. 1, 1997

c.c. Original NDA 20-829 and 20-830
HFD-570/Division File
HFD-570/C.J. Sun
HFD-570/A. Trontell
HFD-570/C.S.O.
HFD-570/Shannon Williams
HFD-570/M. Himmel

cc:
NDA 20-829/20-830/Division file
HFD-570/MO/Honig/Kwong
HFD-570/Pharm/Williams
HFD-570/Chem/Leak/Poochiakian
HFD-570/PM/Kuzmik
HFD-530/Boring

OCT 3 1 1997

DIVISION OF PULMONARY DRUG PRODUCTS
REVIEW OF PHARMACOLOGY AND TOXICOLOGY DATA
Chemistry Consult/ Review for Safety of Impurities

NDA: 20-829 and 20830

Submissions for NDA 20-829 and NDA 20830 covered in this review:

NDA 20-829 Singulair®Tablets		NDA 20-830 Singulair® Chewable Tablets	
Submission Date	**Date received**	**Submission Date**	**Date received**
30 JUL 97 (BC)	31 JUL 97	31 JUL 97 (BC)	01 AUG 97
16 OCT 97 (BP)	17 OCT 97	16 OCT 97 (BP)	17 OCT 97
29 OCT 97 (BP)	30 OCT 97	29 OCT 97 (BP)	30 OCT 97

Information to be conveyed to the Sponsor: Yes( ) No (X)

Reviewer: Shannon Williams, Ph.D.

Date of Consult Request: 26 SEP 97 **Date of Consult Review:** 31 OCT 97

Sponsor: Merck & Co., Inc., West Point, PA

Drug Name: Oral MK-0476 (Oral L-706,631)

Related INDs/NDAs/DMFs:

Class: Leukotriene D_4/E_4 receptor antagonist

Indication: Chronic Asthma

Clinical Formulation: 10 mg tablet for adults and 5 mg chewable tablet for children 6 years of age

Review

Dr. John Leak (reviewing Chemist for montelukast) has currently requested that the safety of the proposed levels of degradants in the final drug products and the proposed levels of impurities in the drug substance and be evaluated. Table 1 (succeeding page) presents a tabulated summary of the proposed limits for degredation products in the drug products and impurities in the drug substance as well as the threshold limits for each requiring qualification.

Table 1. **Proposed Limits for Degredation Products in the Drug Products (10 mg adult tablet and 5 mg pediatric chewable tablet) and Impurities in the Drug Substance which safety evaluation was requested.**

Degradation Products in the Drug Product:
The guideline published in the Federal Register (Vol. 62, 5/19/97, p 27454) on impurities in new drug products, indicate that degradation products which occur at levels > 1.0% for maximum daily doses of <10 mg and > 0.5% for maximum daily doses of 10 to 100 mg require qualification. The safety of the proposed limits for degradation products in the drug products are evaluated below, according to this guideline.

The levels of the at the proposed limits of the thresholds which require qualification of degradation products (0.5% for the 10 mg tablet and 1.0% for the 5 mg/kg tablet.

The maximum limits proposed for the degradation product were for the 10 mg tablet (NDA 20829) and for the 5 mg chewable tablet (NDA 20830). These represent a total daily intake of the for the 10 mg tablet of 0.002 mg/kg for a 50 kg adult and a total daily intake of 0.005 mg/kg for the 5 mg chewable tablet in a 20 kg child.

In a 3 month oral toxicity study in rats (Report No.TT#92-610-0) three lots (M013, M014, and M015) were used which contained 0.12, 0.07, and 0.12% of the degradation product. The NOAEL for the study was 400 mg/kg, and Batch No. M014 which contained the least amount of (0.07%) was used for qualification of the degradation product.

Calculation:
NOAEL x % degradation product = total daily amount administered at the NOAEL
400 mg/kg x 0.0007 =0.28 mg/kg total daily amount of administered. The total daily intake in adults (0.002 mg/kg) and children (0.005mg/kg) are 140 and 56 times less than that given to rats at the NOAEL in the 3-month toxicity study and are well above the safety margin of 10 needed for qualification.

Impurities in the Drug Substance:
The guidelines published in ICH topic Q3A, Impurities in New Drug Substances, indicate that impurities in the drug substance which occur at levels < 0.1% for maximum daily doses of ≤2 g/day require qualification. The safety of the proposed limits for impurities in the drug substance are evaluated below, according to this guideline.

The proposed maximum limit for the impurity in the drug substance was . This limit represents a total daily intake of 0.0006 mg/kg for a 50 kg adult taking the 10 mg tablet and a total daily intake of 0.00075 mg/kg for a 20 kg child taking the 5 mg chewable tablet.

In a 14-week oral toxicity study in mice (Report No.TT#93-001-0) 1 lot (M021) was used which contained impurity. The NOAEL for the study was 50 mg/kg.

Calculation:
NOAEL x % degradation product = total daily amount administered at the NOAEL
50 mg/kg x 0.0026 = 0.13 mg/kg total daily amount of administered. The total daily intake in adults (0.0006 mg/kg)and children (0.00075 mg/kg) is 217 and 173 times less than that given to mice at the NOAEL in the 14-week oral toxicity study, respectively, and are well above the safety margin of 10 needed for qualification.

The maximum limits proposed for the degradation product in the drug substance was These represent a total daily intake of the for the 10 mg tablet of 0.0004 mg/kg for a 50 kg adult and a total daily intake of 0.0005 mg/kg for the 5 mg chewable tablet in a 20 kg child.

In a 3 month oral toxicity study in rats (Report No.TT#92-610-0) three lots (M013, M014, and M015) were used which contained 0.12, 0.07, and 0.12% of the degradation product. The NOAEL for the study was 400 mg/kg, and Batch No. M014 which contained the least amount of (0.07%) was used for qualification of the degradation product.

Calculation:
NOAEL x % degradation product = total daily amount administered at the NOAEL
400 mg/kg x 0.0007 = 0.28 mg/kg total daily amount of administered. The total daily intake in adults (0.0004mg/kg) and children (0.0005mg/kg) are 700 and 560 times less

than that given to rats at the NOAEL in the 3-month toxicity study, respectively, and are well above the safety margin of 10 needed for qualification.

The maximum limit proposed for the impurity in the drug substance was . This limit represents a total daily intake of for the 10 mg tablet of 0.0004 mg/kg for a 50 kg adult and a total daily intake of 0.0005 mg/kg for the 5 mg chewable tablet in a 20 kg child.

In a three month oral toxicity study in infant monkeys (Report No.TT#94-9003) 1 lot (M027) was used which contained 0.06% of the impurity. The NOAEL for the study was 50 mg/kg.

Calculation:
NOAEL x % degradation product = total daily amount administered at the NOAEL
50 mg/kg x 0.0006 = 0.03 mg/kg total daily amount of administered.
The total daily intake in adults (0.0004 mg/kg) and children (0.0005 mg/kg) is 75 and 60 times less than that given to infant monkeys at the NOAEL in the 3-month toxicity study, respectively, and are well above the safety margin of 5 needed for qualification.

- The maximum limit proposed for the impurity in the drug substance was . This limit represents a total daily intake of for the 10 mg tablet of 0.0004 mg/kg for a 50 kg adult and a total daily intake of 0.0005 mg/kg for the 5 mg chewable tablet in a 20 kg child.

In a 1 year oral toxicity studies in rats (Report No.TT#92-651-0) 5 lots (M0019, M020, M021, M023, and M024) were used which contained from 0.06% to 0.18% of the impurity, except for batch No. M021, in which the impurity was not quantified. Information regarding the duration of use for batch No. M021 in the 1 year toxicity study in rats was requested from the Sponsor via a telecommunication on Friday October 3, 1997. Additional information provided by the Sponsor (Submission dated 10/29/97) indicated that batch No 21 was used continuously for the period of week 14 day 5 through week 25 day 3 of treatment. Thus, continuous use of the other batches which contained a minimum of 0.06% of the for a duration ≥ 3 months was assured. The NOAEL for the rat study was 50 mg/kg, and the minimum amount of the 0.06%, was used for qualification.

Calculation:
NOAEL x % degradation product = total daily amount administered at the NOAEL
50 mg/kg x 0.0006 = 0.03 mg/kg total daily amount of
administered. Thus the total daily amount of the impurity at the NOAEL (given to rats for periods ≥ 3months) in the 1-year toxicity study is 75 times greater than the maximum daily intake (0.0004 mg/kg) in adults and 60 times greater than the maximum daily intake (0.0005 mg/kg) in a 20 kg child. This difference is well above the safety margin of 10 needed for qualification.

The level of the at the proposed limits of do not exceed the threshold for requiring qualification of impurities in the drug substance (0.1%).

- The maximum limit proposed for the impurity in the drug substance was . This limit represents a total daily intake of of 0.0004 mg/kg for the 10 mg tablet in a 50 kg adult and 0.0005 mg/kg for the 5 mg chewable tablet in a 20 kg child.

In a 1-year oral toxicity studies in rats (Report No.TT#92-651-0) 5 lots (M0019, M020, M021, M023, and M024) were used, which contained from 0.09% to 0.23% of the impurity. The NOAEL for the study was 50 mg/kg, and the minimum amount of the impurity, 0.09%, was used for qualification.

Calculation:
NOAEL x % degradation product = total daily amount administered at the NOAEL
50 mg/kg x 0.0009 = 0.045 mg/kg total daily amount of administered. Thus, the minimum total daily amount of the) at the NOAEL in the 1-year toxicity study in rats (0.045 mg/kg) is 113 times greater than the maximum daily intake (0.0004mg/kg) in adults and 90 times greater than the maximum daily intake (0.0005 mg/kg) in children. This difference is well above the safety margin of 10 needed for qualification.

SUMMARY AND EVALUATION:

Review of proposed limits for the two degradation products identified in the drug substance indicated that: 1) the proposed limits for the the threshold level which would require qualification and 2) adequate exposure and margins of safety for the degradation product (beyond that needed for qualification) were demonstrated in a 3 month oral toxicity study in rats (Report No.TT#92-610-0). Other impurities in the drug substance whose proposed limits required qualification included: Qualification of these impurities was demonstrated through adequate exposure and margins in toxicity studies of 3 months or greater duration as follows: for , a 14-week oral toxicity study in mice (Report No.TT#93-001-0); for , a 3 month oral toxicity study in rats (Report No.TT#92-610-0); for a three month oral toxicity study in infant monkeys (Report No.TT#94-9003); and the and the all were qualified using a 1-year oral toxicity study in rats (Report No.TT#92-651-0). The proposed limits for the impurity did not exceed the threshold limit for qualification of impurities in the new drug substance and thus did not require qualification.

In conclusion, the safety of the proposed limits for degradants of the both singuilar drug products as well as those for impurities in the drug substance which required qualification, was demonstrated based on adequate exposure and margins of safety between the maximum amounts to be administered clinically and amounts asministered at the NOAEL in previously conducted preclinical toxicity studies of three months or longer duration.

RECOMMENDATION:

The safety of proposed limits for degradants and impurities in the drug products and drug substances for Singulair was demonstrated by adequate exposures achieved in previous nonclinical toxicity studies of 3 months duration or longer. Thus the proposed specifications are acceptable form a preclinical standpoint

/S/ 10/31/97

Shannon P. Williams, Ph.D.
Pharmacologist

/S/

Oct 31, 1997

c.c.
Original NDA 20-829 and 20-830
HFD-570/Division File/NDA
HFD-570/C.J. Sun
HFD-570/M.O./Trontell
HFD-570/C.S.O./B. Kuzmik
HFD-570/S.P. Williams
HFD-570/C.M.C./J. Leak

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 20-829

STATISTICAL REVIEW(S)

Statistical Review and Evaluation

NDA #: 20-829 and 20-830

AUG 16 1997

Applicant: Merck

Name of Drug: Singulair (Montelukast Sodium) 10mg Tablets and 5mg Chewable Tablets

Indication: Treatment of Symptoms of Asthma

Documents Reviewed: Volumes 1.1,1.2,1.96-1.114 of NDA 20-830 dated February 21, 1997

This review pertains to 3 studies in the treatment of asthma, two in adults (Studies 20 and 31) and the other in children 6 to 14 years old (Study 49); 2 studies in exercise induced asthma, one in adults (Study 42) and the other in children (Study 40) ; and one corticosteroid sparing trial in adults (Study 46). The 5mg montelukast chewable tablets were used in the studies in children. The study reports were presented in both submissions and, therefore, only the jackets of one submission were reviewed.

The medical officer of this submission is P. Honig, M.D. (HFD-570), with whom this review was discussed.

This review will mainly focus on the primary efficacy variables. The results of the secondary efficacy variables will be mentioned briefly to highlight the consistency of efficacy.

Methods of analyses were discussed in the sponsor's data analysis plans. The sponsor followed these plans in their study reports.

I. Study 20

A. Study Description and Method of Analysis

This study was an international multi-center, randomized, double blind, parallel group study in nonsmoking asthmatic patients 15 years of age or over with a FEV$_1$ between 50 and 85% of predicted normal and demonstrating reversibility of at least 15% with beta-agonist. Up to 25% of the patients were allowed concomitant use of theophylline.

There was a 2-week placebo run-in period, a 12-week treatment period and, for a subset of the patients, a 3-week placebo wash-out period. (Other non-placebo patients could go into a 9-month double-blind extension.) The purpose of the placebo washout period was to see how Montelukast patients responded when taken off drug. Patients during the placebo run-in period had to have a predetermined level of daytime symptoms (biweekly total score of at least 64) and daytime and nighttime beta-agonist use (weekly

average of at least one puff per day).

Clinic visits were every three weeks during the 12 week treatment period. An additional clinic visit was scheduled after three weeks for those patients who went into the placebo washout period. Spirometry measurements were obtained between 6 and 9 AM of each visit, approximately 8 to 10 hours after the previous bedtime dose.

Four daytime asthma symptom scores were assessed, at bedtime and before taking medication, on 7 point scales:

- How often did you experience asthma symptoms today?

0	1	2	3	4	5	6
None of the time						All of the time

- How much did your asthma symptoms bother you today?

0	1	2	3	4	5	6
Not at all bothered						Severely bothered

- How much activity could you do today?

0	1	2	3	4	5	6
More than usual activity						Less than usual activity

- How often did your asthma affect your activity today?

0	1	2	3	4	5	6
None of the time						All of the time

The daily daytime symptom score was determined by averaging the daily scores for the four questions. The average daytime symptom score for the visit was determined by averaging the daily symptom scores over all days between two consecutive visits.

Randomization was done by stratified randomization in each center. The two strata were theophylline users and non-users. Blocked randomization was used with a block size of 7 (three montelukast patients and two of both placebo and beclomethasone.) Patients without concurrent theophylline use were assigned the smallest patient numbers, while patients with concurrent theophylline were assigned the largest patient number available.

The primary efficacy variables were daytime asthma symptom scores and FEV_1 both averaged over the whole treatment period. Both efficacy variables had to be significant to declare efficacy. The primary efficacy variables were analyzed by an analysis of

variance with factors: treatments, centers and strata (theophylline users or non-users). Treatment-by-center and treatment-by-stratum interaction were tested by supplementary analyses.

B. Results

Eight hundred and ninety-five patients (257 placebo, 387 montelukast, and 251 beclomethasone) were randomized at 38 centers in 19 countries. About 10% of the patients were taking theophylline.

The 15 patients of study center 020-030 were not included in the intent-to-treat analyses because of Good Clinical Practice compliance issues. [This reviewer reran the primary analyses including this center. The exclusion of this center had negligible effect on the results of the study.] A further 10 patients were excluded from the intent-to-treat analysis of FEV_1 and 19 patients from the intent-to-treat analysis of daytime asthma symptom score because they either did not have baseline scores or on-treatment data.

The treatment groups were comparable at baseline in demographic and baseline efficacy variables.

Table 1 contains the percent changes from baseline for FEV_1 and p-values comparing treatments (average over the treatment period). Table 2 contains the mean average changes in daytime asthma symptom scores and the p-values comparing treatments. Montelukast was significantly better than placebo but less effective than beclomethasone for these parameters.

Significant results for both efficacy variables, not shown here, were also seen at the last on-treatment clinic visit.

Significance of montelukast over placebo and beclomethasone over montelukast were seen in most secondary efficacy variables, global evaluations and quality of life assessments.

The treatment-by-center and treatment-by-stratum interactions were not significant ($P>0.05$) for both primary efficacy variables. The treatment-by-gender interaction was also not-significant for these variables.

C. Reviewer's Comments

This study showed efficacy of Montelukast in adults.

II. Study Protocol 31

A. Study Description and Method of Analysis

This study was similar to study 20 with the following exceptions. It did not contain Beclomethasone. Up to 25% of the patients were allowed concomitant use of inhaled corticosteroids rather than theophylline. Randomization was by blocked randomization in each center with a block size of ten (6 montelukast patients and 4 placebo patients).

B. Results

There were 681 randomized patients (273 placebo and 408 montelukast) at 52 U.S. centers who entered the study. About 23% of the patients were taking inhaled corticosteroids.

All randomized patients (N=2, one in each group) from center 031-028 were excluded from the intent-to-treat analyses because case report forms could not be verified (the center lost their copies and all source documents). These patients are not included in the 681 patients listed above. A further 5 patients were excluded from the intent-to-treat analysis of FEV_1 and 8 patients from the intent-to-treat analysis of daytime asthma symptom score because they either did not have baseline scores or on-treatment data.

The treatment groups were comparable at baseline in demographic and baseline efficacy variables.

Table 3 contains the average percent changes from baseline for FEV_1 over the whole treatment period and p-values comparing treatments. Table 4 contains the mean changes in daytime asthma symptom scores over the whole treatment period and the p-values comparing treatments. Montelukast was significantly better than placebo for these primary efficacy parameters.

Significant results for both efficacy variables, not shown here, were also seen at the last on-treatment clinic visit.

Significance of montelukast over placebo were seen in most secondary efficacy variables, global evaluations and quality of life assessments.

The treatment-by-center interaction was not significant ($P>0.05$) for both primary efficacy variables. The treatment-by stratum interaction was significant for daytime symptom score. The patients on corticosteroids showed only a small difference between treatments with a change of -0.24 for placebo and -0.29 for montelukast. Both users of corticosteroids and non-users showed comparable increases in FEV_1, however. The treatment-by-gender interaction was significant for FEV_1. Here the interaction

was a quantitative interaction with more increase over placebo in males 9.5% than females 7.2%.

C. Reviewer's Comments

This study showed efficacy in adults. If a patient is taking corticosteroid, efficacy might be limited to FEV_1, no effect in daytime asthma systems was demonstrated.

III. Study Protocol 49

A. Study Description and Method of Analysis

This study was similar to study 20 with the following exceptions. It was in children 6- to 14- years of age rather than adults. It was only 8 weeks rather than 12 weeks. This study used the 5-mg chewable tablets rather than the 10-mg tablets used with adults. Up to 40% of the children were allowed to continue on inhaled corticosteroids. The stratification factor was therefore corticosteroids use or non-use. The primary efficacy variable was defined to be FEV_1 only rather than both FEV_1 and daytime asthma score.

The daytime asthma score was defined differently also. The patient answered each of the following questions (based on symptoms since arising) by circling the most appropriate number:

- How much of the time did you have trouble breathing today?

None of the time	A little of the time	Some of the time	A good bit of the time	Most of the time	All of the time
0	1	2	3	4	5

- How much did your asthma bother you today?

Did not bother me	Bothered me a little	Bothered me somewhat	Bothered me a good deal	Bothered me very much	Bothered me as much as possible
0	1	2	3	4	5

- How much of the time did your asthma limit your activity today?

None of the time	A little of the time	Some of the time	A good bit of the time	Most of the time	All of the time
0	1	2	3	4	5

B. Results

There were 336 patients (135 placebo and 201 montelukast) randomized into the trial. About 37% of the patients were on inhaled corticosteroids.

The treatment groups were comparable at baseline in demographic and baseline efficacy variables.

Five patients (2 placebo and 3 Montelukast) from center 049-032 were excluded because of significant deviations from good clinical practice. An additional 4 patients were excluded from the analysis of FEV_1 and an additional two patients from the analysis of asthma symptom scores because they either did not have baseline scores or on-treatment data.

Table 5 contains the percent changes from baseline for average FEV_1 and p-values comparing treatments. Montelukast was significantly better than placebo for this primary efficacy parameters. Table 6 contains the mean changes in average daytime asthma symptom scores and the p-values comparing treatments for this analysis. This difference was not significant. It should be emphasized that this was not a primary efficacy parameter in this study.

C. Reviewer's Comments

The evidence for efficacy is weaker here than in the adult studies. Since the FEV_1 measurements are at about 8 to 10 hours after dosing while the daytime asthma scores are at near the end of dosing interval, no end of dosing interval efficacy is demonstrated here. Less efficacy was seen in daytime asthma score in inhaled corticosteroid users (placebo mean change -0.11, Montelukast mean change -0.14) than in nonusers (placebo mean change -0.13, Montelukast mean change -0.22). Since the proportion of inhaled corticosteroid users was higher in this study than in the adult study (Study 31), this also may have caused the lack of overall efficacy in this parameter. [The daytime asthma scores are not equivalently defined, however.]

Some efficacy was seen in secondary measures: total daily b-agonist use and clinic assessed AM PEFR but not in nocturnal assessments and patient assessed AM PEFR.

IV. Study 42 - Exercise Induced Asthma

A. Study Design and Method of Analysis

This was a multi-center, placebo controlled, randomized, double blind, parallel group exercise challenge study with a one week

placebo run-in period, a 12 week treatment period, and a two week placebo washout period.

Two exercise challenges were held during the placebo run-in period. The patient had to demonstrate a post-exercise fall of at least 20% at both challenges. Exercise challenges were also done at weeks 4, 8 and 12 of treatment and after 2 weeks of placebo washout. The exercise challenge after two weeks of placebo washout was to test for persistence of effect.

The exercise challenge had a two minute or more warm up to obtain a targeted heart rate of 80 to 90% of age predicted maximum. This targeted heart rate was maintained for 6 minutes.

Spirometry was performed immediately after exercise and at 5, 10, 15,30, 45 and 60 minutes. If by 60 minutes the patient had not returned to within 5% of the pre-exercise level, an FEV_1 measurement was obtained at 75 minutes, and, if necessary, at 90 minutes. If the patient had still not returned to within 5% of the pre-exercise FEV_1, then rescue beta-agonist was given.

The primary efficacy variables in this study were $AUC_{0-60min}$ and Maximum Percent Fall in FEV_1. The sponsor considered $AUC_{0-60min}$ primary, while the medical officer considered Maximum Percent Fall in FEV_1 most important.

The primary analyses was endpoint changes from baseline with last value carried forward. To calculate $AUC_{0-60min}$ the last spirometry value at the clinic assessment was also carried forward.

The $AUC_{0-60min}$ was calculated as area below the pre-exercise FEV_1. If the FEV_1 went above pre-exercise FEV_1, no positive area was added.

The primary endpoints were analyzed by an analysis of variance with factors treatment and center. Treatment-by-center interaction was assessed in supplementary analyses.

The sponsor also analyzed $AUC_{0-60min}$ and Maximum Percent Fall in FEV_1 with a repeated measures (Weeks 4,8 and 12) mixed model.

B. Results

There were 110 patients (56 placebo and 54 montelukast) who entered the trial. The treatment groups were comparable at baseline in demographic and baseline efficacy variables.

Four patients(two in each treatment group) were excluded from the intent-to-treat analysis of the primary efficacy variables because they either had no baseline values or no on-treatment values and hence no changes from baseline could be obtained.

The table below shows the mean changes from baseline for the week 12 endpoint analysis of $AUC_{0-60min}$ of FEV_1. Montelukast showed significantly less decrease than placebo in the hour after exercise.

Analysis of $AUC_{0-60min}$ of FEV_1 (week 12 endpoint)
(Intent-to-treat)

		Mean(%*min)	Change from baseline at week 12		
Treatment	N	Baseline	Mean	SD	P-value
Placebo	54	1540.0	-99.2	983.4	
Montelukast	52	1397.6	-630.0	783.1	0.001

The table below shows the mean changes from baseline for the week 12 endpoint analysis of maximum percent fall in FEV_1. Montelukast showed significantly less of a fall in FEV_1 than placebo after exercise.

Maximum Percent Fall in FEV_1 (Week 12 endpoint)
(Intent-to-treat)

		Mean (%)	Change from baseline at week 12		
Treatment	N	Baseline	Mean	SD	P-value
Placebo	54	38.3	-5.90	14.61	
Montelukast	52	36.45	-14.12	12.56	0.003

The repeated measures analysis found no difference between the slope of the two treatments but a difference in intercept for both primary endpoints. The slope for both treatments looked to be zero, which means that the treatment difference at weeks 4, 8 and 12 were effectively constant and significant.

Fifty percent (26/52) of Montelukast patients were protected against a 20% drop in FEV_1 compared to 37% (20/54) of the placebo patients. This difference is not significant (p=0.177, binomial test).

Two weeks after cessation of treatment the montelukast parameter values approached the placebo values but did not exceed them. The

protection has worn off by two weeks after treatment.

C. Reviewer's Comments

This study showed an effect on AUC FEV_1 and max percent fall in FEV_1 but only 50% of the patients were protected against a 20% fall in FEV_1 on Montelukast. Whether such a protection percentage is adequate must be left to clinical judgement.

V. Study 040 - Exercise Induced Asthma

A. Study Design and Method of Analysis

This was a two period, randomized, double-blind, crossover exercise challenge study comparing montelukast 5-mg chewable tablet with placebo in children 6 to 14 years of age. There was a three day treatment period with the exercise challenge at the end of the third day. The exercise challenge was done 20 to 24 hours post-dose. There was a 4-day washout period between treatments.

Children were exercised on a treadmill for 6 minutes at a workload calculated to increase the patient's heart rate to approximately 160 to 190 beats per minute. This workload was used on all exercise challenges for that patient.

$AUC_{0\text{-}60\ min}$ and Maximum FEV_1 percent fall from pre-exercise challenge FEV_1 were analyzed by an analysis of variance with factors for centers, sequence, subjects within center-by-sequence, period and treatment.

B. Results

There were 27 children who entered the study. Two patients on placebo during the second period dropped out and did not perform an exercise challenge. Therefore the primary efficacy analyses included only 25 patients who took both treatments.

The table below provides the treatment means and p-values comparing treatments for the primary efficacy variables. Montelukast provided more protection against fall in FEV_1 than placebo.

Variable	Placebo Mean(SD) n=25	Montelukast Mean(SD) n=25	P-value
$AUC_{0\text{-}60\ min}$ FEV_1 (%*min)	-589.72 (705.27)	-264.60 (271.56)	0.013
Maximum % Fall	-26.11 (13.93)	-18.27 (12.54)	0.009

Sixty percent of the children were protected against a 20% drop in FEV_1 on Montelukast compared to only 40% while on placebo. This difference is not significant using McNemar's test.

No period or carryover effects were detected (P>0.05).

C. Reviewer's Comments

This study showed an effect on AUC FEV_1 and max percent fall in FEV_1 but only 60% of the patients were protected against a 20% fall in FEV_1 on Montelukast. Whether such a protection percentage is adequate must be left to clinical judgement.

VI. Study 046 - Corticosteroid Sparing Study

A. Study Description and Method of Analysis.

This was a high-dose inhaled corticosteroid study to investigate the ability of Montelukast to allow tapering of inhaled corticosteroids in asthmatic patients. It was a multi-center, double-blind, randomized, parallel group study with a one month single-blind placebo period where patients were tapered once or twice (at two week intervals) while maintaining FEV_1 at 90% or greater of their run-in baseline value (pre-study visit and visit 1 average). If the FEV_1 fell below 90%, the inhaled cortico-steroid was increased. The purpose of this run-in period was to handle the situation that the dose of corticosteroids that the patient was using might be higher than the patient needed to control his asthma.

Patients entered a pre-randomization baseline period during which baseline values of FEV_1, daytime symptom score and total daily inhaled beta-agonist use were determined. These three parameters were used to determine whether the patient's inhaled cortico-steroid dose would be tapered during the double-blind period.

The patients who entered the study were stratified into high and low dose groups with separate randomizations in each group within a center.

The inhaled corticosteroid tapering criteria depended upon a composite clinical score determined over the clinic visit for FEV_1 or the last 7 days for the two diary components. If pre-beta-agonist $FEV_1 \geq 90\%$ of pre-randomized baseline then 1 point was scored. If daytime symptom score $\leq 120\%$ of pre-randomized baseline, another point was added. If beta-agonist use $\leq 135\%$ of pre-randomized baseline, another point was added. If the composite score was 3, inhaled corticosteroid was tapered. If the composite score was 2, the dose was maintained. If the composite score was 0 or 1, the dose of corticosteroid was increased. The taper dose or dose increase in puffs/day were proportional to the

dosage of the inhaled corticosteroids in puffs per day that the patient was currently taking.

The primary efficacy variable was last dose of inhaled corticosteroid as a percent change from pre-randomized baseline dose. Since the patients were using a variety of inhaled corticosteroids, this variable is independent of the dosage of corticosteroid. (It is also why the dose increase or dose taper were proportional to the current dose taken.) This percent was analyzed by an analysis with factors for treatment, stratum and center. The treatment-by-stratum and treatment-by-center interactions were assessed in supplementary analysis and found to be not significant.

B. Results

The table below provides the mean percent changes in last tolerated dose of inhaled corticosteroids and p-value comparing treatments. Montelukast was able to reduce the inhaled corticosteroid dosages significantly more than placebo.

Percent Change from baseline Last Tolerated dose of inhaled corticosteroids (Intent-to-treat)

		Mean (mcg/day)	Percent Change from pre-randomized baseline		
Treatment	N	Baseline	Mean	SD	P-value
Placebo	113	1078.8	30.27	67.37	
Montelukast	112	975.9	46.73	62.22	0.046

C. Reviewer's Comments

This study demonstrated that Montelukast would provide some steroid tapering.

The tapering criteria allowed a patient to be slightly worse and still have the dosage of inhaled corticosteroid reduced. This may partially explain why the placebo patients were able to further reduce their inhaled corticosteroid from their baseline level even with the run-in tapering period.

VII. Overall Conclusions

Studies 20 and 31 showed efficacy of Montelukast in adults in AUC FEV$_1$ and daytime asthma score averaged over the treatment period.

Study 49 showed efficacy for AUC FEV_1 in children 6- to 14-years of age.

Both studies 31 and 49 showed almost no efficacy in daytime symptom score if patients were taking corticosteroid. This difference was not seen in AUC FEV_1. Both corticosteroid users and non-users increased their AUC FEV_1.

Both exercise challenge studies (Studies 40 and 42) showed efficacy for AUC FEV_1 and Maximum percent fall in FEV_1. However, only 50 to 60% of the patients were protected against a fall of 20% in FEV_1.

Montelukast showed steroid sparing ability in Study 46 where the mean reduction from baseline of corticosteroid dosage was 47% for Montelukast and 30% for placebo.

/S/

James R. Gebert, Ph.D.
Mathematical Statistician HFD-715

Concur: Dr. Wilson /S/ 8/8/97

Dr. Nevius 8/16/97

This review contains 12 pages of text and 6 pages of tables.

cc:
Orig NDA 20-829
NDA 20-830
HFD-570
HFD-570/Dr. Honig
HFD-570/Ms. Trout
HFD-715/Div. File
HFD-715/Dr. Gebert
HFD-715/Dr. Wilson

Table 1

Analysis of FEV1

Study 20

(Intention-To-Treat Approach)

Treatment	N	Mean (L)		Percent Change From Baseline			
		Baseline	Treatment Period	Mean	SD	LS Mean	95% CI for Mean
Placebo	249	2.21	2.23	1.07	15.87	0.71	(-2.27, 3.69)
Montelukast	375	2.16	2.32	7.49	17.01	7.35	(4.61, 10.08)
Beclomethasone	246	2.10	2.38	13.30	19.72	13.12	(10.06, 16.18)

Comparison Between Treatments	p-Value	LS Mean	95% CI for Difference
Montelukast vs Placebo	<0.001	6.64	(3.89, 9.38)
Beclomethasone vs Placebo	<0.001	12.41	(9.39, 15.44)
Montelukast vs Beclomethasone	<0.001	-5.78	(-8.53, -3.02)

p-Value For Effect	
Treatment	<0.001
Study center	<0.001
Stratum	0.751

Root MSE of Percent Change = 17.02

Table 2

Analysis of Daytime Symptom Score
Study 20

(Intention-To-Treat Approach)

Treatment	N	Mean (Score)		Change From Baseline			
		Baseline	Treatment Period	Mean	SD	LS Mean	95% CI for Mean
Placebo	245	2.40	2.14	-0.26	0.74	-0.17	(-0.30, -0.05)
Montelukast	372	2.35	1.85	-0.49	0.81	-0.41	(-0.53, -0.29)
Beclomethasone	244	2.38	1.68	-0.70	0.80	-0.62	(-0.75, -0.49)

Comparison Between Treatments	p-Value	LS Mean	95% CI for Difference
Montelukast vs Placebo	<0.001	-0.24	(-0.35, -0.12)
Beclomethasone vs Placebo	<0.001	-0.44	(-0.57, -0.31)
Montelukast vs Beclomethasone	<0.001	0.21	(0.09, 0.33)

p-Value For Effect	
Treatment	<0.001
Study center	<0.001
Stratum	0.410

Root MSE of Change = 0.73

Table 3

Study 31

Analysis of FEV1

(Intention-To-Treat Approach)

Treatment	N	Mean (L)		Percent Change From Baseline			
		Baseline	Treatment Period	Mean	SD	LS Mean	95% CI for Mean
Placebo	270	2.54	2.64	4.22	12.67	3.21	(1.45, 4.96)
Montelukast	406	2.47	2.78	13.05	13.84	12.10	(10.60, 13.61)

Comparison Between Treatments	p-Value	LS Mean	95% CI for Difference
Montelukast vs Placebo	<0.001	8.90	(6.84, 10.96)

p-Value For Effect	
Treatment	<0.001
Study center	0.359
Stratum	0.012

Root MSE of Percent Change = 13.28

Table 4

Analysis of Daytime Symptom Score

Study 31

(Intention-To-Treat Approach)

		Mean (Score)		Change From Baseline			
Treatment	N	Baseline	Treatment Period	Mean	SD	LS Mean	95% CI for Mean
Placebo	269	2.49	2.32	-0.18	0.59	-0.17	(-0.25, -0.08)
Montelukast	404	2.51	2.10	-0.41	0.69	-0.39	(-0.47, -0.32)

Comparison Between Treatments	p-Value	LS Mean	95% CI for Difference
Montelukast vs Placebo	<0.001	-0.23	(-0.33, -0.13)

p-Value For Effect	
Treatment	<0.001
Study center	0.119
Stratum	0.357

Root MSE of Change = 0.65

Table 5

Analysis of FEV_1

Study 49

(Intention-To-Treat Approach)

Treatment	N	Mean (L)		% Change From Baseline			
		Baseline	Treatment Period	Mean	SD	LS Mean	95% CI for Mean
Placebo	131	1.85	1.93	4.16	10.74	3.58	(1.29, 5.87)
Montelukast	196	1.85	2.01	8.71	12.54	8.23	(6.33, 10.13)

Comparison Between Treatments	p-Value	LS Mean	95% CI for Difference
Montelukast vs Placebo	<0.001	4.65	(1.92, 7.38)

p-Value For Effect	
Treatment	<0.001
Study center	0.849
Stratum	0.370

Root MSE of % Change = 12.05

Table 6

Analysis of Daytime Symptom Score
Study 49
(Intention-To-Treat Approach)

		Mean (Score)		Change From Baseline			
Treatment	N	Baseline	Treatment Period	Mean	SD	LS Mean	95% CI for Mean
Placebo	132	1.26	1.14	-0.12	0.55	-0.09	(-0.19, 0.02)
Montelukast	197	1.28	1.09	-0.19	0.58	-0.16	(-0.25, -0.07)

Comparison Between Treatments	p-Value	LS Mean	95% CI for Difference
Montelukast vs Placebo	0.273	-0.07	(-0.20, 0.06)

p-Value For Effect	
Treatment	0.273
Study center	0.714
Stratum	0.265

Root MSE of Change = 0.57

STATISTICAL REVIEW AND EVALUATION
STABILITY STUDY

Date: DEC 15 1997

NDA Number:	20-830 and 20-829
Applicant:	Merck
Name of Drug:	Singulair® Chewable Tablets and Singulair® Tablets
Statistical Reviewer:	Girish Aras Ph. D. (HFD-715)
Chemistry Reviewer:	John Leak Ph. D. (HFD-570)
Document Reviewed:	Stability Report, dated March 18, October 29, November 26 and December 4, 97
Date of Consult	October 6, 97

I. Introduction

The sponsor submitted 18 and 12 months of stability data for 3 developmental batches (MR-3230, MR-3239 and MR-3251) on March 19, 97 for bottles and blisters, respectively, for 10 mg tablets and 18 months of stability data for bottles and blisters for 5 mg tablets on a 3.5" diskette for Singulair® Chewable Tablets and Singulair® Tablets stored at [redacted] The data were also submitted in the document referenced above. Based on their analyses, the sponsor has proposed expiration periods of 24 and 12 months for bottles and blisters, respectively, for 10 mg Singulair® Chewable Tablets and 24 months for bottles and blisters, respectively, for 5 mg Singulair® Tablets.

The sponsor's data described above is on three developmental batches only. The sponsor recently submitted 6 month data (November 26, 97) for one commercial batch MR-3339.

II. Stability Parameters

The following list of stability parameters with specification was used to evaluate the stability for 10 mg Singulair® Chewable Tablets and 5 mg Singulair® Tablets.

Table 1. Specifications

III. Reviewer's Analyses

The reviewer analyzed the data submitted by the sponsor on three developmental batches using the FDA stability program. The data from the commercial batch are not adequate to perform a valid statistical analysis. In addition, according to chemistry reviewer, these data cannot be combined with the data from the developmental batches. The conditions under which they were produced are different. Hence only the developmental batches were analyzed for this review. The FDA recommended test schedule is to test the product every 3 months during the first year, and every 6 months, thereafter. However, this schedule was not followed for some of the parameters as described in the remarks for the tables below. As there was only one data point for the HDPE bottle the sponsor has not submitted adequate data for a statistical analysis. The reader is cautioned that the statistical methods used for prediction beyond the testing period are valid only under the assumptions that the conditions of the experiment remain unchanged and linearity of the fitted equation holds for that period.

The predicted expiry given in tables below are not necessarily due to crossing of the 95% confidence band with the specification limits, but could be due to Biometrics program's convention of not extrapolating maximum predicted expiry beyond 4 times the study period. As mentioned before, the tables are based solely on the data from the developmental batches.

The data from the commercial batch, though far from adequate, falls inside the prescribed specification limits. By inspection, the individual values do not appear to differ from the developmental batches, though occasional differences can be noted, perhaps due to higher initial values for some variables in the commercial batches. However, even these entries are well below the specification limits. A statistical judgment on prediction and extrapolation based on commercial batches has to be delayed till adequate data is generated on at least 3 batches for 12 or 18 months.

Table 2. Statistical Summary for Stability Batches of 10 mg Singulair® Chewable Tablets

Packaging Type	Analysis Parameter	Model*	Least Favorable Batch**	Predicted Expiry (Months)
14ozHDPE	Degradation Products	-	-	-
	Sulfoxide	Common Slope		
	Cis-isomer	Not Combined		
	Dissolution	Combined		
	Moisture	Not Combined	MR-3230	
30mlHDPE	Degradation Products			
	Sulfoxide	Common Slope		
	Cis-isomer	Not Combined		
	Dissolution	Common Slope		
	Moisture	Combined		
75HDPE90	Degradation Products			
	Sulfoxide	Common Slope		
	Cis-isomer	Not Combined		
	Dissolution	Combined		
	Moisture	Not Combined	MR-3230	
75HDPE30	Degradation Products			
	Sulfoxide	Combined		
	Cis-isomer	Combined		
	Dissolution	Common Slope		
	Moisture	Common Slope	MR-3239	
Blisters	Degradation Products			
	Sulfoxide	Combined		
	Cis-isomer	Combined		
	Dissolution	Common Slope		
	Moisture	Not Combined		

* Models: Combined = Common slopes and common intercepts
Common Slopes = Common slopes but separate intercepts
Not Combined = Separate slopes and separate intercepts

** Least Favorable Batch = Stability Batch with the shortest predicted expiry
NA = Not Applicable

- Data available only at 6 month-intervals and not at 3 month-intervals during first year, as requested in the FDA guideline.

Table 3. Statistical Summary for Stability Batches of 5 mg Singulair® Tablets

Packaging Type	Analysis Parameter	Model*	Least Favorable Batch**	Predicted Expiry (Months)
14ozHDPE	Degradation Products	-	-	-
	Sulfoxide	Combined	NA	
	Cis-isomer	Not Combined	NA	
	Dissolution	Not Combined	MR-3276	
	Moisture	Combined		
30mlHDPE	Degradation Products			
	Sulfoxide	Combined	NA	
	Cis-isomer	Not Combined	NA	
	Dissolution	Not Combined	MR-3276	
	Moisture	Common Slope		
75HDPE90	Degradation Products			
	Sulfoxide	Combined	NA	
	Cis-isomer	Not Combined	NA	
	Dissolution	Not Combined	MR-3276	
	Moisture	Combined		
75HDPE30	Degradation Products			
	Sulfoxide	Combined	NA	
	Cis-isomer	Not Combined	NA	
	Dissolution	Common Slope	MR-3276	
	Moisture	Combined		
Blisters	Degradation Products			
	Sulfoxide	Combined		
	Cis-isomer	Combined		
	Dissolution	Common Slope		
	Moisture	Combined		

* Models: Combined = Common slopes and common intercepts
Common Slopes = Common slopes but separate intercepts
Not Combined = Separate slopes and separate intercepts

** Least Favorable Batch = Stability Batch with the shortest predicted expiry
NA = Not Applicable

- Data available only at 6 month-intervals and not at 3 month-intervals during first year, as requested in the FDA guideline.

IV. Conclusion

Given the acceptability of using the developmental batches for the assessment of stability, the overall stability data support expiry dates proposed by the sponsor for 75mL HDPE bottles, 90 and 30 tablet count. They are 24 months for 10 mg and and 5 mg formulations in bottles. Package types, 14oz. HDPE and 30 mL (4 tablets) HDPE bottles support similar expiry periods, however there are no data at 3 and 9months. The package type HPPE bottle does not have adequate data points to support extrapolation beyond the period of experiment, 12 months.

The data for Blister packaging support the sponsor's proposed expiry dates. They are 12 and 24 months for 10 mg and 5 mg blisters, respectively.

Extrapolation beyond the testing period is based on the assumptions that the condition of the experiment remains unchanged and the linearity of the fitted equation holds for that period.

/S/

Girish Aras, PhD

Concur: Dr. Karl Lin /S/ 2/15/97

cc:
Orig. NDA 20-829 and 20-830
HFD-570 / Division File
HFD-570 / JLeak
HFD-715 / Division File, Chron
HFD-715 / GAras, KLin

[redacted]

STATISTICAL REVIEW AND EVALUATION
CARCINOGENICITY

IND #:	**Date:** AUG 12 1996
Applicant:	Merck Research Laboratories
Name of Drug:	Montelukast Sodium
Documents Reviewed:	2-29-96 Vol 35.20-35.22
	5-22-96 Supporting Statistical Analysis Datasets & Documentation
Statistical Reviewer:	B Bono, M.S.
Pharmacologist:	S Williams, Ph.D.
Key Words:	Peto, trend test, adjusted *p*-values, adjusted α-levels

Text in italics is from the Investigational New Drug Application submitted by the sponsor.

Summary of Review

- There are no statistically significant *p*-values from the trend test in either of the two animal studies provided that:
  - the α-level of a "rare" tumor is 0.025 and the α-level of a "common" tumor is 0.005, and
  - a pancreatic islet adenoma is a "common" tumor among rats.

- The pairwise comparisons of the control with the low and high dose groups for hepatocellular carcinoma liver tumors among male rats is not statistically significant provided that:
  - the α-level of a "common" tumor is 0.01, and
  - a hepatocellular carcinoma liver tumor is "common" among rats.

- The pairwise comparisons of the control with the middle and high dose groups for pancreatic islet adenoma tumors among male rats is not statistically significant provided that:
  - the α-level of a "common" tumor is 0.01, and
  - a pancreatic islet adenoma tumor is "common" among rats.

- Greater than 50% of the animals in both studies were still alive between weeks 80-90, thus there was adequate exposure of the drug to study tumor incidence.

- Using the log-rank test, the survival rates were not found to be statistically significantly different among the dose groups in either of the two animal studies.

I. Background

Two animal carcinogenicity studies (one in rats, and one in mice) were included in this IND submission. These two studies were intended to assess the oncogenic potential of Montelukast Sodium (MK-0476) in rats and mice when administered orally for two years. The design of these

studies is summarized below.

Study Number	Species	Duration	Doses (mg/kg)
93-110-0	CD-1 (ICR)BR Mouse	92 weeks	0, 0, 25, 50, 200/100*
93-078-0	CD-1 Rat	105 weeks	0, 0, 50, 100, 200

* Due to a treatment-related decrease in body weight gain, the dose level for the high dose group was reduced from 200 to 100 in drug week 10 for both the male and female mice.

In both studies, male and female animals were assigned at random to one of five treatments groups which included two controls and three graded doses of MK-0476 (Mice: 25, 50, 200/100 mg/kg/day; Rats: 50, 100, and 200 mg/kg/day). In the mouse study, due to a treatment-related decrease in body weight gain, the dose level for the high dose group was reduced from 200 to 100 in drug week ten for both the male and female mice. In both studies, the sample size for each sex was 50 for each of two control groups and 50 for each MK-0476 dosage group. The control groups were combined in the analyses to give each study a combined control group size of 100. However, one rat was mis-sexed and excluded from the study in week three resulting in a male rats' combined control group size of 99. Treatment was administered orally (gavage) daily for a period of approximately 92 weeks for the mice and 105 weeks for the rats with terminal necropsy on all remaining animals performed during weeks 92 and 105, respectively, of the mice and rat studies.

Palpable tumors are those which were detected prior to the death or terminal sacrifice of the animal. A nonpalpable tumor was termed "lethal" if classified by the pathologist as a cause of the animal's death (or moribund status leading to an unscheduled sacrifice).

II. Analysis

The sponsor and reviewer analyzed palpable, nonpalpable-lethal and nonpalpable-nonlethal tumors separately, then combined the results using Peto et al. procedures. For a particular tumor type of interest, the incidence data can be summarized in a 2×D table, where D is the number of dose groups. The first row contains the numbers of animals with the tumor of interest, and the second row contains the numbers of animals without the tumor. However, this summary table can be misleading. If the drug causes animals to die early by some non-cancer related cause, fewer animals will be at risk for tumors in the higher dose groups. Thus, even if the drug also increases the tumor rate, the overall incidence of that tumor in the high dose groups may be smaller than in the control groups. To adjust for the effect that potential differential mortality between the dose groups has on tumor occurrence, the Peto method breaks up study time into several discrete intervals. The intervals used in both studies were: 0-52 weeks, 53-78 weeks, 79-92 weeks, 93-104 weeks, and over 104 weeks. The data can thus be represented by several 2×D tables, one for each time interval.

The dose groups can also be assigned weights in the statistical analysis to test various hypotheses.

For example, using weights of 0, 1, ... D gives the trend test, which is sensitive to a linear dose effect. Using equal weights (1, 1, 1, 1) gives a test of association between dose and tumor rate without specifying the form of the relationship. Weight can also be made equal to the actual doses given. Finally, choosing weights close to the actual biological effect of the doses will result in the most sensitive test, but in practice this effect is not known. Linear weights or the dose weights are often used.

For the tumor type of interest, each tumor is classified as "fatal", "non-fatal" or "observed before sacrifice or death". This is not a biological classification but a statistical classification. *P*-values are calculated for the three classes separately, and then combined to yield a single *p*-value for the tumor type. Both exact and asymptotic *p*-values can be calculated for tumor type where all of the tumors found were either fatal, non-fatal or observed early. If for a particular tumor type, more than one of the three classes were detected, only asymptotic *p*-values are available. Clearly, when available, the exact *p*-values are preferable.

One-sided *p*-values may be more appropriate than two-sided, since they are more conservative and we are only interested in whether increased doses *increase* tumor incidence.

One hundred forty-one (141) distinct sex/organ/tumor type combinations were found in the two studies. Using an α-level of .05 to determine significance would yield a high false positive rate.

Since so many sex/organ/tumor type combinations are present, a simple application of a .05 decision rule does not appropriately control the overall false positive rate. It has been suggested by Dr. Karl Lin and Dr. Mohammad Rahman[1] that if the tumor is "rare" the cutoff should be .025 and if the tumor is "common" the cutoff should be .005. (Tumors are defined as rare or common using historical control data or the control group in the study being analyzed. The usual practice at FDA is to classify a tumor as common if it occurs in the control group at an incidence of greater than 1%.) Using simulation tests on CD-1 rats and CD(BR) mice, Lin and Rahman found that the overall false positive rate resulting from the use of the α-levels .025 and .005 in the tests for linear trend in a two-species-two-sex study is about 10%. These false-positive rates are judged by the Center for Drug Evaluation and Research as the most appropriate in a regulatory setting.

For pairwise comparisons, the levels of significance are .05 and .01 for a rare and common tumor, respectively.

[1]Lin, KK and MA Rahman (1995), "False Positive Rates in Tests for Linear Trends in Tumor Incidences in Animal Carcinogenicity Studies of New Drugs", unpublished report, Division of Biometrics, CDER, FDA, Rockville, MD.

III. Discussion

Dose Weights

As discussed above, it is the usual practice to use either dose weights or linear weights in the analysis of carcinogenicity data. The applicant used dose weights in their analyses. Recall that the mice in the high dose group received 200 mg/kg/day in the first 10 weeks of the study and 100 mg/kg/day after week 10. In the analyses of the mouse study, the applicant selected the 200 mg/kg/day as the highest dose (instead of the 100 mg/kg/day or an intermediate dose). According to the sponsor, the 200 mg/kg/day dose is:

> *"...the most conservative choice for the male and female mice since it maximizes the differences among the three scales used in the Tukey trend test, and, therefore, will have the greatest chance of obtaining statistical significance..."*
>
> *VOL35.22, page E-1090*

It is assumed that the applicant used the word "conservative" to mean "has the greatest chance of obtaining statistical significance" in the trend tests. Assuming no true tumor trend, the statement is true based on a simulation study conducted by Robert Condon of the Center for Veterinary Medicine at FDA. Additionally, assuming a non-linear tumor trend, the dose weights using the 200 mg dose will also have the greatest chance of obtaining statistical significance. However, assuming a linear tumor trend, the dose weights using the 100 mg dose as the highest dose will have the greatest chance of obtaining statistical significance. Thus, when looking at the Type I error rate, the 200 mg dose is the choice that will have the greatest chance of obtaining statistical significance. However, when looking at power, the most "conservative" choice will depend on the linearity of the true tumor trend.

In the absence of any information about the actual tumor trends for each individual tumor, the *p*-values in this review reflect a linear dose trend; i.e., the dose groups were given the values (0, 1, 2, 3) in the equations.

Adjusted *P*-values

As described above, an α-level of .05 is not appropriate because there are 141 unique sex/organ/tumor combinations. Instead of adjusting the α-level at which statistical significance is declared, the applicant adjusted the one-sided *p*-values using a procedure described by Heyse and Rom[2] and by Harter[3] and then used the usual .05 α-level to determine significance.

Using the adjusted *p*-values, the applicant found no statistically significant evidence of an

[2] Heyse, J.F., Rom, D., "Adjusting for Multiplicity of Statistical Tests in the Analysis of Carcinogenicity Studies", *Biometrical Journal* Vol. 30, 1988, 883-896.

[3] Harter, H.L., "Error Rates and Sample Sizes for Range Tests in Multiple Comparisons", *Biometrics* Vol. 13, 1957, 511-536.

increasing trend in the incidence of tumor-bearing mice or rats with increasing doses of MK-0476.

Sites In Which Only One Rat Was Observed With Tumor
The applicant's analysis only included sites for which at least two animals were observed with tumor. The applicant argues that statistical significance cannot be achieved for sites in which only one animal was observed with tumor. This is usually true. Since it is possible to find statistical significance, however unlikely, all sites where at least one animal was observed were analyzed in this review.

IV. Reviewer's Analyses and Results

The reviewer's analyses used Peto et al. procedures (described above). The results are on pages 7-9. For both male and female animals, an analysis was performed for each organ/tumor type combination even for cases where only one rat was observed with tumor. The first column in the tables is the sex group, followed by the tumor type and organ. Certain tissue types are labeled as "PRSUNDETER", which indicates that the primary site of the tumor was undetermined. The column labeled "Class" indicates whether the tumors were classified as fatal (FA), non-fatal (NF), observed before sacrifice or death (OB), or mixed (MI), meaning tumors fall into two or more of the former three classes. The incidence in each of the dose groups is shown, although, as discussed above, these may not always be meaningful because the drug may cause the animals to die early by some non-cancer related explanation. Asymptotic and exact *p*-values are given next, with both one-sided and two-sided *p*-values shown. (These are denoted by "Asymp1", "Exact1" and "Asymp2" and "Exact2".) Unlike the sponsor, the *p*-values presented in this review are the actual *p*-values, not adjusted *p*-values.

Since the highest dose in the mouse study was reduced from 200 mg/kg/day to 100 mg/kg/day during week 10 of the study, linear dose weights were used in the analyses of this study. To be consistent, linear dose weights were also used in the analyses of the rat study.

As described above, Dr. Karl Lin suggested that if the tumor is "rare" the α-level should be 0.025 and if the tumor is "common" the α-level should be 0.005. Using this rule, there are no statistically significant *p*-values from the trend test in either of the two animal studies.[4] This means that as dose increases linearly, there are no statistically significant increases in incidence of tumor. However, the animals in these studies were fed an "optimized diet" which is a modification of a restricted diet regimen; and according to the reviewing pharmacologist Dr. Shannon Williams, a restricted diet can suppress tumor formation. The applicant was asked to send historical control data from studies using this optimized diet and an ad lib diet to help determine which tumors are rare and which are common in this unusual situation. At the time of this review, the data were not available.

[4] The one-sided exact *p*-value for the male rats' pancreas islet adenoma tumors is 0.0149. According to the reviewing pharmacologist, this tumor is common, thus the *p*-value would need to be less than .005 to be considered statistically significant.

The pharmacologist requested pairwise comparisons between each dose level and the control group for five tumor type/organ site combinations in the rat study (page 10). Recall, for pairwise comparisons, the α-levels recommended by Lin are .01 and .05 for common and rare tumors, respectively. The only comparisons that may be statistically significant were the low dose versus control and the high dose versus control for the hepatocellular carcinoma in the liver (50 mg: p=0.0138; 200 mg: p=0.0394). However in this study, the control group's incidence was 2.02%. Recall that the usual practice at FDA is to classify a tumor as common if it occurs in the control group at an incidence of greater than 1%. Thus, the pharmacologist may want to study the historical control data to be submitted by the applicant to decide whether this p-value is statistically significant or not. The p-values of the middle and high dose group comparisons with placebo for Pancreatic Islet Adenoma tumors were .0301 and .0397 respectively. Pancreatic islet adenoma tumors are common, thus the p-values were not statistically significant. All of the other pairwise comparisons requested by Dr. Williams yielded p-values greater than .05.

The pharmacologist considered combining types of tumors within tissue type based on McConnell et al (1986)[5]. However, from inspection after grouping the tumors, it was apparent that there were no increasing tumor trends.

Survival

In the Guidance for Industry draft, it is stated that "a 50% survival rate of the 50 initial animals in the high dose group between weeks 80-90 of a two-year study will be considered as a sufficient number and adequate exposure."[6] For both the mouse and rat study, plots of survival demonstrate that greater than 50% of the high dose group animals were still alive during weeks 80-90 (page 11).

As discussed above, the trend test used in the applicant's and reviewer's analyses take into account any potential difference in survival rates. Nevertheless, Kaplan-Meier plots and log-rank tests were used to determine if the survival rates among the different dose groups were similar (page 11). Neither the plots nor the log-rank test p-values show any statistically significant evidence of a difference in survival among the dose groups.

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[5] McConnell, EE, HA Solleveld, JA Swenberg and GA Boorman. "Guidelines for Combining Neoplasms for Evaluation of Rodent Carcinogenesis Studies", *Journal of National Cancer Institute* 1986; 76:283-289.

[6] Guidance For Industry, "On Statistical Aspects of Design, Analysis, and Interpretation of Animal Carcinogenicity Studies."

P-values from the Trend Test

Mouse Study

Sex	Tumor Type	Tissue	Class	C	L	M	H	Asymp2	Exact2	Asymp1	Exact1
F	ADENOMA	LUNG	NF	8	9	2	11	0.1116	0.1149	0.0558	0.0671
M	HEMANGIOMA	TESTIS	NF	0	0	0	1	0.1204	0.2030	0.0602	0.2030
M	HEMANGIOMA	SPLEEN	NF	0	0	0	1	0.1204	0.2030	0.0602	0.2030
M	HEMANGIOSARCOMA	LYMPHNODE	NF	0	0	0	1	0.1204	0.2030	0.0602	0.2030
F	FIBROSARCOMA	SKIN	OB	0	2	1	2	0.1216	0.1302	0.0608	0.0928
M	PAPILLOMA	SKIN	OB	0	0	0	1	0.1227	0.2000	0.0614	0.2000
F	ADENOMA	ADRENACORT	NF	0	0	0	1	0.1248	0.2018	0.0624	0.2018
F	LYMPHOMA	PRSUNDETER	MI	9	3	7	8	0.1628	NA	0.0814	NA
M	ADENOMA	LUNG	NF	17	12	10	12	0.2633	0.2735	0.1317	0.1473
M	ADENOMA	THYRFOLLIC	NF	0	2	0	1	0.3026	0.4445	0.1513	0.2313
M	ADENOMA	PITUITARY	NF	0	0	1	0	0.3156	0.3438	0.1578	0.3438
M	LIPOSARCOMA	LIVER	NF	0	0	1	0	0.3156	0.3438	0.1578	0.3438
F	ADENOCARCINOMA	SMAINTESTI	NF	0	1	0	1	0.3341	0.4018	0.1671	0.2400
M	ADENOMA	PANCREAISL	NF	0	0	1	0	0.4864	0.7871	0.2432	0.3861
M	ADENOMA	PROSTATE	NF	0	0	1	0	0.4864	0.7871	0.2432	0.3861
M	HEMANGIOMA	PERITONEUM	NF	0	0	1	0	0.4864	0.7871	0.2432	0.3861
M	SERTOLICELLTUMOR	TESTIS	NF	0	0	1	0	0.4864	0.7871	0.2432	0.3861
M	SQUAMOUSCELLCARCINOMA	EAR	NF	0	0	1	0	0.4864	0.7871	0.2432	0.3861
F	HISTIOCYTOMA	SKIN	NF	0	0	1	0	0.4956	0.8073	0.2478	0.4037
F	LEIOMYOSARCOMA	SMAINTESTI	NF	0	0	1	0	0.4956	0.8073	0.2478	0.4037
F	ADENOMA	PITUITARY	NF	1	2	2	1	0.5282	0.6050	0.2641	0.3221
M	ADENOMA	ADRENACORT	NF	5	3	2	4	0.5345	0.5568	0.2673	0.3050
F	ADENOMA	OVARY	NF	2	1	0	2	0.7026	0.8502	0.3513	0.4163
M	SPINDLECELLTUMOR	ADRENAL	NF	1	0	0	1	0.7046	0.8079	0.3523	0.4767
M	HEMANGIOSARCOMA	SKELETMUSC	OB	0	1	1	0	0.7057	0.8038	0.3528	0.4756
F	GRANULOSACELLTUMO	OVARY	NF	2	1	2	1	0.7809	0.8644	0.3904	0.4515
F	SPINDLECELLTUMOR	ADRENAL	NF	1	2	0	1	0.9340	1.0000	0.4670	0.4601
F	ADENOCARCINOMA	MAMMARGLAN	MI	3	5	1	2	0.9588	NA	0.5206	NA
M	LYMPHOMA	PRSUNDETER	MI	2	3	1	1	0.9484	NA	0.5258	NA
F	POLYP	UTERUS	NF	1	3	2	0	0.9407	1.0000	0.5297	0.5485
F	HISTIOCYTICSARCOM	PRSUNDETER	MI	1	3	1	0	0.9390	NA	0.5305	NA
M	POLYP	LARINTESTI	NF	1	0	1	0	0.9364	1.0000	0.5318	0.5947
M	ADENOMA	SMAINTESTI	NF	0	1	0	0	0.8719	1.0000	0.5641	0.6139
M	NEUROFIBROMA	PLEURA	NF	0	1	0	0	0.8719	1.0000	0.5641	0.6139
M	HISTIOCYTICSARCOM	PRSUNDETER	FA	0	1	0	0	0.8638	1.0000	0.5681	0.6000
F	ADENOCARCINOMA	EHARDERIGL	NF	0	1	0	0	0.8632	1.0000	0.5684	0.5963
F	ADENOMA	UTERUS	NF	0	1	0	0	0.8632	1.0000	0.5684	0.5963
F	HEMANGIOSARCOMA	UTERUS	NF	0	1	0	0	0.8632	1.0000	0.5684	0.5963
F	LEIOMYOSARCOMA	UTERUS	NF	0	1	0	0	0.8632	1.0000	0.5684	0.5963
M	HEMANGIOSARCOMA	LIVER	MI	1	1	1	0	0.7661	NA	0.6169	NA
F	ADENOMA	THYRFOLLIC	NF	1	1	1	0	0.7643	0.8334	0.6178	0.5034
F	HEPATOCELLULARADENOMA	LIVER	NF	4	3	3	1	0.7478	0.7962	0.6261	0.5678
F	ADENOCARCINOMA	LUNG	MI	6	3	5	1	0.6215	NA	0.6892	NA
F	ADENOMA	EHARDERIGL	NF	8	2	3	3	0.5945	0.6626	0.7028	0.6583
M	PHEOCHROMOCYTOMA	ADRENAL	NF	1	1	0	0	0.4030	0.5659	0.7985	0.6688
F	SARCOMA	UTENDOMETS	MI	3	0	2	0	0.3985	NA	0.8008	NA
M	HEMANGIOSARCOMA	SPLEEN	MI	1	1	0	0	0.3959	NA	0.8021	NA
M	ADENOMA	EHARDERIGL	NF	11	7	4	3	0.3656	0.4131	0.8172	0.7898
M	ADENOCARCINOMA	LUNG	MI	14	1	5	4	0.3359	NA	0.8321	NA
M	ADENOCARCINOMA	EHARDERIGL	NF	1	0	0	0	0.3084	0.6040	0.8458	0.5990
M	HEMANGIOMA	LYMPHNODE	NF	1	0	0	0	0.3084	0.6040	0.8458	0.5990
M	OSTEOMA	BONE	NF	1	0	0	0	0.3084	0.6040	0.8458	0.5990
M	POLYP	GALLBLADDE	NF	1	0	0	0	0.3084	0.6040	0.8458	0.5990
F	ADENOCARCINOMA	UTERUS	NF	1	0	0	0	0.3049	0.6055	0.8476	0.5963
F	ADENOMA	SMAINTESTI	NF	1	0	0	0	0.3049	0.6055	0.8476	0.5963
F	HEMANGIOMA	UTERUS	NF	1	0	0	0	0.3049	0.6055	0.8476	0.5963
F	HEMANGIOMA	SKIN	NF	1	0	0	0	0.3049	0.6055	0.8476	0.5963
F	HEMANGIOMA	SPLEEN	NF	1	0	0	0	0.3049	0.6055	0.8476	0.5963
F	HEMANGIOSARCOMA	LIVER	NF	1	0	0	0	0.3049	0.6055	0.8476	0.5963
F	LEIOMYOSARCOMA	OVARY	NF	1	0	0	0	0.3049	0.6055	0.8476	0.5963

F MENINGIOMA	BRAIN	NF	1	0	0	0	0.3049	0.6055	0.8476	0.5963
F OSTEOMA	BONE	NF	1	0	0	0	0.3049	0.6055	0.8476	0.5963
F OSTEOSARCOMA	PRSUNDETER	NF	1	0	0	0	0.3049	0.6055	0.8476	0.5963
F SEBACEOUSADENOMA	SKIN	NF	1	0	0	0	0.3049	0.6055	0.8476	0.5963
F TERATOMA	OVARY	NF	1	0	0	0	0.3049	0.6055	0.8476	0.5963
F BASALCELLTUMOR	SKIN	OB	1	0	0	0	0.3035	0.6000	0.8483	0.6000
M TRICHOEPITHELIOMA	SKIN	OB	1	0	0	0	0.3035	0.6000	0.8483	0.6000
M HEPATOCELLULARCARCINOMA	LIVER	MI	17	5	5	5	0.2523	NA	0.8738	NA
M HEPATOCELLULARADENOMA	LIVER	NF	14	8	7	3	0.2474	0.2516	0.8763	0.8584
F HEPATOCELLULARCARCINOMA	LIVER	NF	3	1	1	0	0.2462	0.2688	0.8769	0.8255
M FIBROSARCOMA	SKIN	MI	3	1	1	0	0.2184	NA	0.8908	NA
M ADENOCARCINOMA	SMAINTESTI	MI	2	1	0	0	0.1974	NA	0.9013	NA
M LIPOMA	SKIN	OB	2	0	0	0	0.1501	0.2786	0.9250	0.8358
M ADENOMA	TESTLEYDCE	NF	2	0	0	0	0.1487	0.2747	0.9256	0.8404
M LEUKEMIA	PRSUNDETER	NF	2	0	0	0	0.1487	0.2747	0.9256	0.8404
M POLYP	URINABLADD	NF	2	0	0	0	0.1487	0.2747	0.9256	0.8404
F POLYP	GALLBLADDE	NF	2	0	0	0	0.1458	0.2837	0.9271	0.8382
F ADENOACANTHOMA	MAMMARGLAN	MI	3	1	0	0	0.1037	NA	0.9481	NA
F LEIOMYOMA	UTERUS	NF	6	1	2	0	0.0917	0.1111	0.9542	0.9417
M HEMANGIOMA	LIVER	NF	4	0	0	0	0.0401	0.0470	0.9799	0.9753

Rat Study

Sex	Tumor Type	Tissue	Class	C	L	M	H	Asymp2	Exact2	Asymp1	Exact1
M	ADENOMA	PANCREAISL	NF	3	4	6	6	0.0212	0.0239	0.0106	0.0149
F	PAPILLOMA	STNONGLANM	NF	0	0	0	2	0.0364	0.0498	0.0182	0.0498
F	ADENOCARCINOMA	UTERUS	MI	0	1	1	2	0.0811	NA	0.0406	NA
F	ADENOMA	KIDNEY	NF	1	0	0	3	0.0897	0.1402	0.0448	0.0755
M	HEPATOCELLULARCARCINOMA	LIVER	MI	2	6	3	5	0.0902	NA	0.0451	NA
M	MESOTHELIOMA	HEART	FA	0	0	0	1	0.1237	0.2008	0.0618	0.2008
M	FIBROADENOMA	MAMMARGLAN	OB	0	0	0	1	0.1237	0.2008	0.0618	0.2008
F	GLIOMA	BRAIN	NF	0	0	0	1	0.1247	0.1923	0.0623	0.1923
M	ADENOCARCINOMA	LAINTESTCO	NF	0	0	0	1	0.1256	0.1964	0.0628	0.1964
M	ADENOMA	MAMMARGLAN	NF	0	0	0	1	0.1256	0.1964	0.0628	0.1964
M	HEMANGIOMA	SKELETMUSC	NF	0	0	0	1	0.1256	0.1964	0.0628	0.1964
M	PAPILLOMA	TONGUE	NF	0	0	0	1	0.1256	0.1964	0.0628	0.1964
F	MESOTHELIOMA	PERITONEUM	NF	0	0	0	1	0.1402	0.2256	0.0701	0.2256
F	SQUAMOUSCELLCARCINOMA	SKIN	NF	0	0	0	1	0.1402	0.2256	0.0701	0.2256
M	KERATOACANTHOMA	SKIN	OB	0	2	3	1	0.1779	0.2242	0.0890	0.1256
M	HISTIOCYTICSARCOM	PRSUNDETER	MI	1	0	0	2	0.2354	NA	0.1177	NA
F	POLYP	UTERUS	NF	5	4	8	4	0.2834	0.2867	0.1417	0.1656
F	ADENOMA	PANCREAISL	NF	1	1	1	2	0.2884	0.3523	0.1442	0.1962
M	GLIOMA	BRAIN	NF	0	0	2	0	0.3073	0.4011	0.1536	0.2265
F	ADENOMA	THYRFOLLIC	NF	0	0	2	0	0.3721	0.5661	0.1860	0.2623
F	HISTIOCYTICSARCOM	PRSUNDETER	OB	0	0	1	0	0.4927	0.8000	0.2464	0.4000
M	ADENOCARCINOMA	MAMMARGLAN	OB	0	0	1	0	0.4953	0.7992	0.2476	0.4016
M	ADENOMA	PANCREACIN	NF	0	0	1	0	0.5022	0.8095	0.2511	0.4167
M	HEMANGIOMA	LYMPHNODE	NF	0	0	1	0	0.5022	0.8095	0.2511	0.4167
M	THYMOMA	THYMUS	NF	0	0	1	0	0.5022	0.8095	0.2511	0.4167
F	ADENOCARCINOMA	PANCREAISL	NF	0	0	1	0	0.5292	0.7866	0.2646	0.4085
F	ADENOMA	PARATHYROI	NF	0	0	1	0	0.5292	0.7866	0.2646	0.4085
F	ADENOMA	LIVEBILDUC	NF	0	2	0	1	0.5450	0.6305	0.2725	0.3566
M	INTERSTITIALCELLTUMOR	TESTIS	NF	2	4	2	2	0.6150	0.6789	0.3075	0.3552
M	HEPATOCELLULARADENOMA	LIVER	NF	3	5	4	2	0.6336	0.6398	0.3168	0.3570
M	ADENOMA	SKSEBACEGL	OB	0	1	1	0	0.7175	0.8000	0.3587	0.4808
M	PAPILLOMA	SKIN	OB	1	0	0	1	0.7214	0.8000	0.3607	0.4828
M	PAPILLOMA	MOUTHLIP	OB	0	1	1	0	0.7238	0.7971	0.3619	0.4833
F	FIBROMA	SKIN	MI	1	1	0	1	0.7306	NA	0.3653	NA
M	GRANULARCELLTUMOR	BRAIN	NF	1	0	0	1	0.7327	0.7906	0.3664	0.4871
F	MELANOMA	EYEIRIS	NF	0	1	1	0	0.7698	1.0000	0.3849	0.4875
M	FIBROSARCOMA	SKIN	OB	1	0	1	0	0.7949	1.0000	0.3975	0.4878
F	FIBROSARCOMA	SKIN	OB	1	0	1	0	0.8109	1.0000	0.4054	0.4785
F	FIBROADENOMA	MAMMARGLAN	MI	30	14	19	14	0.8674	NA	0.4337	NA
F	PHEOCHROMOCYTOMA	ADRENMEDUL	NF	1	1	0	1	0.9094	1.0000	0.4547	0.4700
M	LIPOSARCOMA	KIDNEY	MI	2	0	1	1	0.9656	NA	0.4828	NA

F LYMPHOMA	PRSUNDETER	NF	1	0	1	0	0.9825	1.0000	0.4913	0.3902
M ADENOMA	PARATHYROI	NF	0	1	0	0	0.9194	1.0000	0.5403	0.6667
M HISTIOCYTICSARCOM	PROSTATE	FA	0	1	0	0	0.8606	1.0000	0.5697	0.5984
M MELANOMA	EYE	FA	0	1	0	0	0.8606	1.0000	0.5697	0.5984
M HEMANGIOSARCOMA	SKIN	OB	0	1	0	0	0.8606	1.0000	0.5697	0.5984
M SQUAMOUSCELLCARCINOMA	EAEXTERNAE	OB	0	1	0	0	0.8606	1.0000	0.5697	0.5984
M TRICHOEPITHELIOMA	SKIN	OB	0	1	0	0	0.8606	1.0000	0.5697	0.5984
M ACINAR-ISLET-CELLTUMOR	PANCREAS	NF	0	1	0	0	0.8497	1.0000	0.5752	0.5833
M ADENOCARCINOMA	LUNG	NF	0	1	0	0	0.8497	1.0000	0.5752	0.5833
M ADENOMA	LUNG	NF	0	1	0	0	0.8497	1.0000	0.5752	0.5833
M HEMANGIOSARCOMA	SPLEEN	NF	0	1	0	0	0.8497	1.0000	0.5752	0.5833
F ADENOMA	VAGCLITOGL	NF	0	1	0	0	0.8285	1.0000	0.5857	0.5915
M ADENOMA	THYRFOLLIC	NF	1	0	1	0	0.7881	1.0000	0.6060	0.5129
M MESOTHELIOMA	TESTUVAGIN	NF	1	1	1	0	0.7843	0.8205	0.6079	0.5129
F LEIOMYOSARCOMA	UTERUS	MI	0	2	0	0	0.7633	NA	0.6183	NA
M ADENOMA	ADRENACORT	NF	3	0	1	1	0.6679	0.7117	0.6660	0.5815
F ADENOCARCINOMA	PITUITARY	MI	3	1	1	1	0.6017	NA	0.6991	NA
F ADENOCARCINOMA	MAMMARGLAN	MI	19	7	2	10	0.4827	NA	0.7586	NA
M LIPOMA	SKIN	MI	1	2	0	0	0.4271	NA	0.7864	NA
M CARCINOMA	TPARAFOLLC	NF	4	1	1	1	0.4221	0.5183	0.7890	0.7297
M HISTIOCYTOMA	SKIN	MI	2	1	1	0	0.3983	NA	0.8009	NA
M ADENOCARCINOMA	PANCREAISL	MI	7	2	2	2	0.3848	NA	0.8076	NA
F ADENOMA	MAMMARGLAN	MI	3	2	0	1	0.3844	NA	0.8078	NA
M PHEOCHROMOCYTOMA	ADRENMEDUL	NF	8	3	3	2	0.3595	0.3820	0.8202	0.7867
M ADENOMA	EZYMBALGLA	NF	1	0	0	0	0.3337	0.6111	0.8332	0.5556
M TRANSITIONALCELLCARCINOMA	URINABLADD	NF	1	0	0	0	0.3337	0.6111	0.8332	0.5556
F GRANULARCELLTUMOR	BRAIN	NF	1	0	0	0	0.3112	0.6154	0.8444	0.5769
F HISTIOCYTICSARCOM	LIVER	FA	1	0	0	0	0.3035	0.6000	0.8483	0.6000
F LEIOMYOSARCOMA	LARINTESTA	FA	1	0	0	0	0.3035	0.6000	0.8483	0.6000
F FIBROSARCOMA	EYELID	OB	1	0	0	0	0.3035	0.6000	0.8483	0.6000
F KERATOACANTHOMA	SKIN	OB	1	0	0	0	0.3035	0.6000	0.8483	0.6000
F PAPILLOMA	MOUTHLIP	OB	1	0	0	0	0.3035	0.6000	0.8483	0.6000
M BASALCELLTUMOR	SKIN	OB	1	0	0	0	0.3015	0.5984	0.8493	0.6024
M FIBROSARCOMA	EARPINNA	OB	1	0	0	0	0.3015	0.5984	0.8493	0.6024
M ADENOMA	KIDNEY	NF	1	0	0	0	0.2937	0.5893	0.8532	0.6071
F ADENOCARCINOMA	KIDNEY	NF	1	0	0	0	0.2880	0.6037	0.8560	0.6220
F CARCINOMA	STNONGLANM	NF	1	0	0	0	0.2880	0.6037	0.8560	0.6220
F FIBROSARCOMA	EARPINNA	NF	1	0	0	0	0.2880	0.6037	0.8560	0.6220
F PAPILLOMA	SKIN	NF	1	0	0	0	0.2880	0.6037	0.8560	0.6220
M FIBROMA	SKIN	MI	8	2	0	3	0.2723	NA	0.8638	NA
M PAPILLOMA	URINABLADD	NF	2	1	0	0	0.2125	0.3066	0.8937	0.8182
M LYMPHOMA	PRSUNDETER	MI	5	3	0	1	0.1968	NA	0.9016	NA
F GRANULOSACELLTUMOR	OVARY	NF	2	0	0	0	0.1879	0.2821	0.9061	0.7900
F CARCINOSARCOMA	MAMMARGLAN	OB	2	0	0	0	0.1468	0.2786	0.9266	0.8393
M ADENOMA	PITUITARY	MI	40	15	19	13	0.1420	NA	0.9290	NA
F CARCINOMA	TPARAFOLLC	NF	2	0	0	0	0.1318	0.1913	0.9341	0.8585
F HEPATOCELLULARADENOMA	LIVER	NF	7	3	2	1	0.1289	0.1475	0.9355	0.9202
F ADENOMA	ADRENACORT	NF	4	4	0	0	0.1137	0.1204	0.9432	0.9258
F ADENOMA	PITUITARY	MI	69	30	33	27	0.0696	NA	0.9652	NA
M ADENOMA	TPARAFOLLC	NF	14	4	2	3	0.0459	0.0473	0.9771	0.9737
F ADENOMA	TPARAFOLLC	NF	13	3	3	2	0.0282	0.0309	0.9859	0.9840

Pairwise Comparisons of Neoplastic Findings in Rats

Male Rats

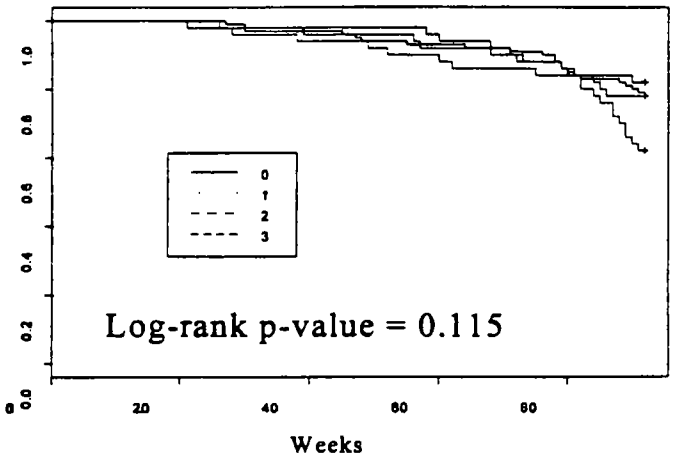
	Controls 1+2	50 mg	100 mg	200 mg
Total number of animals	99	50	50	50
Number of animals with tumor (*p*-value of pairwise comparison with control groups)				
Liver: Hepatocellular carcinoma	2	6 (0.0138)	3 (0.2171)	5 (0.0394)
Pancreas: Islet adenoma	3	4 (0.1471)	6 (0.0301)	6 (0.0397)
Brain: Malignant glioma	0	0	2 (0.0958)	0

Female Rats

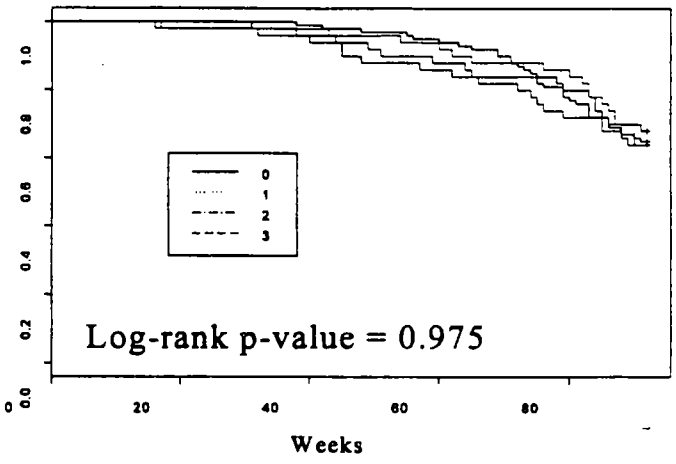
	Controls 1+2	50 mg	100 mg	200 mg
Total number of animals	100	50	50	50
Number of animals with tumor (*p*-value of pairwise comparison with control groups)				
Stomach: Non-glandular mucosa papilloma	0	0	0	2 (0.1401)
Uterus: Adenocarcinoma	0	1 (0.2667)	1 (0.3366)	2
Pancreas: Islet adenoma	1	1 (0.5340)	1 (0.5663)	2 (0.2885)
Brain: Malignant glioma	0	0	0	1 (0.3125)

Kaplan-Meier Plots

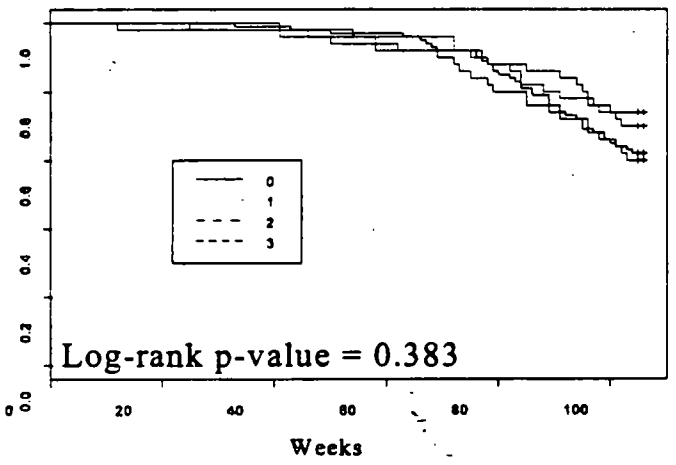
Female Mice



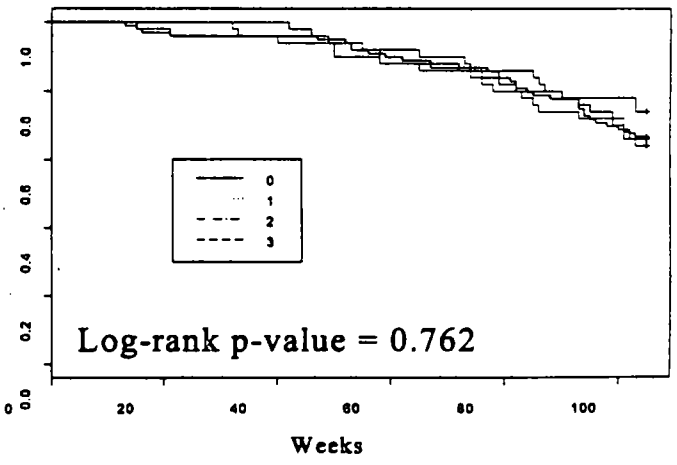
Male Mice



Female Rats



Male Rats



/S/ 8/12/96

Barbara A. Bono

concur: Dr. Lin /S/ 8/12/96
Dr. Nevius 8-12-96

cc:
Orig.
HFD-570 / Division File
HFD-570 / BKuzmik
HFD-570 / SWilliams
HFD-570 / JSun
HFD-715 / Division File, Chron
HFD-715 / ENevius, KLin, SWilson, BBono