CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-001

PHARMACOLOGY REVIEW

Fitzgerald

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DIVISION OF NEUROPHARMACOLOGICAL DRUG PRODUCTS
REVIEW AND EVALUATION OF PHARMACOLOGY & TOXICOLOGY DATA
Original Summary

[redacted]

Submission Date: 8/1/97
SR Date: 8/31/97
Review Date: 10/12/97

Drug: Almotriptan D,L Hydrogen Malate

Sponsor: [redacted] **US Agent:** Pharmaceutical Research Associates
16400 College Blvd.
Lenexa, KS 66219

Reviewer: T.D. Steele

Indication: Migraine

Pharmacological Classification: 5-$HT_{1B/1D}$ receptor agonist

Related INDs/NDAs/DMFs: [redacted]

Chemical Information:

Name(s): LAS 31416 D,L-hydrogen malate;
3-(2-dimethylaminoethyl)-5-(1-pyrrolidinyl-sulphonylmethyl)-1H-indole D,L-hydrogen malate

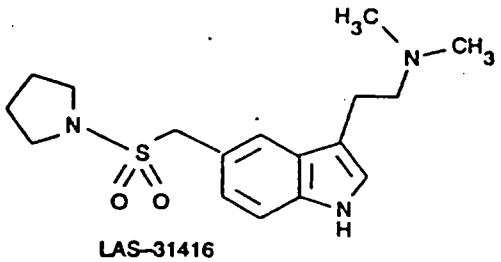
CAS numbers: none

Empirical Formula: $C_{17}H_{25}N_3O_2S - C_4H_6O_5$

Molecular Weight: 469.57 (base: 335.47)

Structure:

[redacted]



LAS-31416

Note: Portions of this review have been excerpted from the sponsor's submission

Proposed Clinical Study:

The proposed study is a randomized, single-dose, double-blind, parallel-group, placebo-controlled, phase II, multicenter, clinical trial in 300 evaluable migraine patients (male or female, 18-65 years of age) studied at home. There will be three parallel groups of 100 patients each (almotriptan or placebo). Patients should have experienced 1-6 migraine attacks per month during the two months prior to the screening visit. Subjects will be randomized to receive almotriptan (12.5 or 25 mg) or placebo. One or two doses of 12.5 or 25 mg of almotriptan or placebo will be administered by the oral route over a one to two day period. A patient's final visit must occur 1-2 days after the last dose of study medication. The primary efficacy parameter will be the number of attacks reduced from severe or moderate to mild or no pain within the two hour period following study medication, using a four-point headache intensity scale. A secondary efficacy parameter will be the reduction of headache. Safety will be studied by collecting adverse events throughout the study, performing physical examination, vital signs and 12-lead ECGs, and obtaining blood and urine samples at screening and at the end of the study (1 to 6 days after the last dose of study medication).

Previous Clinical Experience:

Around 750 patients or healthy volunteers have received almotriptan by either the subcutaneous, oral, sublingual, intranasal or intravenous routes. In nine Phase I studies in approximately 150 patients (300 drug administrations), almotriptan appeared safe and well tolerated, and no clinically significant changes in vital signs or laboratory parameters were recorded. Two Phase II studies were performed in inpatients, one by the subcutaneous and the other by the oral route of administration. In a Phase III study, the efficacy of oral almotriptan versus placebo and 100 mg of sumatriptan was evaluated. In the three completed Phase II and III studies, a total of 960 patients were evaluated, of which 604 received almotriptan, 194 received sumatriptan and 162 placebo. In a dose-finding Phase II study still under evaluation, doses of 2, 6.25, 12.5 and 25 mg were administered by the oral route to 742 patients, of which 662 have received almotriptan and 80 placebo, have participated this study.

Nonclinical Pharmacology/Toxicology Studies Reviewed:

A. PHARMACOLOGY

A.1 Mechanism of Action

A.1.a. Receptor Binding Studies

Summary: ALMO was characterized as a selective" 5-HT_{1D} ligand based on its high affinity for 5-HT1D sites in either brain membrane preparations or in cells transfected with genes for the different 5-HT receptor subtypes, and generally weak activity at other sites. Agonist activity was subsequently characterized in isolated tissue studies (see A.2). Activation of 5-HT1D receptors on cerebral vessels results in vasoconstriction with consequent relief of migraine.

A.1.a.1. 5-HT Receptors

5-HT Receptor Affinities (nM)

Receptor	Almotriptan	Sumatriptan
5-HT_{1D}	13	7.8
$5\text{-HT}_{1D\beta}$	12	3.6
$5\text{-HT}_{1D\alpha}$	13	7.8
5-HT_{1A}	850	460
5-HT_{1B}	<1000	-
5-HT_{2}	2500	8800
5-HT_{4}	14000	10000
5-HT_{6}	~3000	-
5-HT_{7}	<1000	-

A.1.a.2. Non 5-HT Receptors

Study 1 (FLMOL 7/94): adrenergic (α_1, α_2, β_1, β_2), adenosine (A_1, A_2), angiotensin (AT_1, AT_2)

Study 2 (1376 S 810 E): D_1, D_2, ET_A, ET_B (endothelin), H_2, M (non-specific), NK_1, NK_2, NK_3, opiate (non-specific), CGRP; adenosine, NE, and 5-HT uptake sites

Results: Weak affinity for non-specific opiate and muscarinic receptors (IC_{50} is slightly greater than 10 μM). No significant activity at other sites.

A.2 Efficacy in Models Predictive of Anti-Migraine Activity

A.2.a. Isolated Organ Studies

Summary: These studies provided quantitative information on the relative 5-HT receptor agonist activity of ALMO (eg. dog saphenous vein, meningeal artery, basilar artery), and also some prediction of efficacy by studying vessels thought to be important in migraine (eg. meningeal and temporal artery). Other vessels and tissues were studied to assess side effect potential. The general profile observed was that the activity of ALMO is ≥ SUMA in contracting vessels important in migraine (eg. meningeal, temporal), and ≤ SUMA in contracting vessels associated with potential side effects (eg. coronary artery, pulmonary vessels, bronchus).

Preparation	Receptor	Drug	Affinity (EC_{50}, μM)	Max. Effect (% 5-HT Resp)
Dog Saphenous Vein vasoconstriction	$5\text{-}HT_{1D}$	ALMO SUMA	0.39 0.57	105 105
Human Meningeal Artery vasoconstriction	$5\text{-}HT_{1D}$	ALMO SUMA	0.28 0.95	133 206
Human Basilar Artery vasoconstriction	$5\text{-}HT1_{D\beta}$	ALMO SUMA	0.31 0.17	57 83
Human Coronary Artery vasoconstriction		ALMO SUMA	0.93 0.58	63 77
Human Temporal Artery vasoconstriction		ALMO SUMA	0.32 0.24	65 67
Human Pulmonary Artery contraction		ALMO SUMA	1.2 0.2	4 34
Human Pulmonary Vein contraction		ALMO SUMA	1.0 0.4	33 40
Human Isolated Bronchus contraction		ALMO SUMA	0.5 0.6	180 93
Human Ophthalmic Artery vasoconstriction		ALMO SUMA	0.13 0.09	59 63
Human Internal Carotid Artery vasoconstriction		ALMO SUMA	0.82 0.52	83 83
Endothelium-denuded dog coronary artery constriction		ALMO SUMA	~10 ~10	

A.2.b. Effect on Carotid Circulation

Summary: Increases in carotid vascular resistance is a predictor of migraine efficacy. ALMO (1 mg/kg, i.v.), but not SUMA, increased blood pressure by 25% in anesthetized dogs without affecting heart rate (test doses: 0.001-3 mg/kg, i.v.). Neither compound had strong effects on blood pressure or heart rate in anesthetized cats (doses: 0.03-3 mg/kg, i.d.; 0.01 μg- 3 mg/kg, i.v.). In some studies, smaller effects of either compound on femoral or mesenteric beds were observed. A study of the effects of ALMO, SUMA and naratriptan on carotid arteriovenous anastomoses (AVAs) in anesthetized cats indicated that the increase in carotid resistance is due to an increased resistance of AVAs.

Model	Drug	ED_{50} (mg/kg,) (1 carotid R)
Anesthetized dog	ALMO SUMA	0.12 (i.v.) 0.04 "
Anesthetized cats	ALMO SUMA	0.34 (i.d.) 0.24 "
"	ALMO SUMA	0.01 (i.v.) 0.01 "

A.2.c. Additional Studies

1. Meningeal extravasation in guinea pigs: ALMO (0.3 mg/kg, i.v.) and SUMA (1 mg/kg, i.v.) caused similar inhibitory effects on plasma extravasation and vasodilatation in response to electrical stimulation of the trigeminal nerve. The ALMO metabolite LAS 31727 was 30-fold more potent than the parent compound.

2. Inhibition of forskolin-stimulated cAMP accumulation in transfected HeLa cells: ALMO and SUMA were equipotent 5-HT agonists in cells expressing 5-$HT1_{D\beta}$ receptors, but ALMO was less potent than SUMA in cells expressing 5-$HT1_{D\alpha}$ receptors (IC_{50}s, nM)

	5-$HT1_{D\alpha}$	5-$HT1_{D\beta}$
ALMO	6.5	1.6
SUMA	0.8	2.1

3. Effects on vertebral, mesenteric and renal blood flows in anesthetized dogs: ALMO increased mean blood pressure by 24% at 3 mg/kg, i.v. SUMA did not affect blood pressure. ALMO and SUMA caused a small decrease in vertebral blood flow, but not in the renal or mesenteric beds.

A.3 Safety Pharmacology

A.3.a. Cardiovascular Studies:

Model	Dose/Route	Effect
Isolated guinea pig heart	0.1-10 μM of ALMO or metab. (LAS 31727)	ALM: sl. ↓ HR, CO (9-10%); no effect on LVP, dP/dt, CF; Metab: ↓ HR, CO, dP/dt, CORF (15-32%)
Hemodynamics & ECG in conscious monkeys	1 & 3 mg/kg, s.c. ALMO or SUMA	HD: sl. ↑ BP (13%), HR (14%); sl. ↑ QT (30 ms) in 1 of 2 ALMO animals, and 2/2 SUMA animals
Hemodynamics & ECG in conscious dogs	0.003 - 1 mg/kg, i.v., ALMO, metab (LAS 31727), or SUMA	HD: ↑ BP(52%), HR (76%), CorF (121%); ↓ CorR (33%); ↑QTc (see below); Similar profile but smaller changes w/ metab. (>100 μg/kg) and SUMA (>10 μg/kg); (obs. at 1 hr post-Rx)
Agonist interactions in anesthetized cats	3 - 300 μg/kg, i.v. ALMO & SUMA	HD: ↑ hypertensive effects of EPI, NE, Tyr, AT, 5-HT_{1A} agonist
Hemodynamics in anesthetized dogs	0.001 -3 mg/kg, i.v. ALMO & SUMA	HD - ALMO: sl. ↓ CO, SV; ↑ TAR; no effect on BP, HR, contractility; HD - SUMA: sl. ↓ in most parameters
ECG effects in anesthetized dogs	10, 30 μg/min (intracoronary)	No toxicologically sig. effects on HR, BP, or ECG by ALMO or SUMA
Conscious rats	60 mg/kg, p.o.	No effect of either ALMO or SUMA

QTc effects: Data tables from the monkey and dog studies report are shown on the following pages. The potential importance of QTc lengthening in dogs was not addressed in the study report because the finding did not achieve statistical significance. Moreover, the sponsor cites the absence of QTc prolongation in anesthetized dogs to insinuate that the observed prolongations may be spurious. Obviously, the use of anesthetized versus conscious dogs and different routes of administration hinders comparisons between studies. However, the magnitude of the mean change in QTc with 1 mg/kg ALMO (> 100 ms) is large and may warrant monitoring in clinical studies.

An additional source of consternation with the dog findings is that they were omitted from the I.B. The sponsor has carefully tabulated the ECG findings up to the dose of 300 μg/kg, but not the values for the 1 mg/kg level. This is clearly a misrepresentation of these data, and the sponsor should be instructed to revise the I.B. to include these data.

TABLE 29. Value of the PR and QT intervals and HR in two *Macaca fascicularis* monkeys treated with 3 mg/kg s.c., sumatriptan, LAS 31416 and saline

TREATMENT	51759			51830		
	PR	QT	HR	PR	QT	HR
Saline	77	254	126	89	232	99
	±2	±4	±4	±3	±7	±6
Sumatriptan	80	287	115	83	264	99
	±2	±7	±4	±4	±9	±7
LAS 31426	81	281	120	88	243	103
	±2	±6	±5	±3	±7	±7

Values are expressed as mean ± s.e.m. of the readings each hour for 24 hours consecutively. PR and QT intervals are expressed in ms and the heart rate in beats/minute.

TABLE V. EFFECTS OF LAS 31416 ON PR, QRS, QT AND QTc INTERVALS OF ECG IN CONSCIOUS BEAGLE DOGS

TREATMENT	DOSE µg/kg i.v.	VALUES IN ms			
		PR	QRS	QT	QTc
LAS 31416	BASELINE	109 ±9	57 ±4	261 ±6	317 ±14
	3	107 ±5	55 ±3	250 ±1	334 ±13
	10	107 ±1	50 ±9	244 ±12	301 ±20
	30	-	55	239	333
	100	100 ±13	54 ±5	253 ±20	263 ±20
	300	102 ±5	57 ±4	250 ±7	326 ±12
	1000	89 ±8	128 ±35	286 ±49	441 ±87
	1 hour	98 ±8	68 ±9	232 ±7	302 ±27

N=3 animals. Statistical test: Student t for paired data compared to baseline. There were no statistically significant differences.

TABLE VIII. EFFECTS OF VEHICLE ON PR, QRS, QT AND QTc INTERVALS OF ECG IN CONSCIOUS BEAGLE DOGS

TREATMENT	DOSE mL/ ANIMAL	VALUES IN ms			
		PR	QRS	QT	QTc
VEHICLE	BASELINE	115 ±1	61 ±9	257 ±14	295 ±21
	0.25	116 ±6	58 ±11	268 ±13	310 ±45
	0.85	110 ±7	57 ±9	255 ±13	305 ±17
	2.55	156 -	82 ±11	328 ±38	338 ±52
	3.15	103 ±3	62 ±10	357 ±50	365 ±74
	4.85	120 -	50 ±7	255 ±18	258 ±13
	10.85	102 -	64 ±3	259 ±17	293 ±70
	1 hour	124 -	57 ±7	250 ±14	280 ±16

N=3 animals. Statistical test: Student t for paired data compared to baseline. There were no statistically significant differences.

TABLE VII. EFFECTS OF SUMATRIPTAN ON PR, QRS, QT AND QTC INTERVALS OF ECG IN CONSCIOUS BEAGLE DOGS

TREATMENT	DOSE µg/kg i.v.	VALUES IN ms			
		PR	QRS	QT	QTc
SUMATRIPTAN	BASELINE	109 ±2	55 ±6	259 ±4	294 ±9
	3	99 ±7	54 ±6	271 ±13	260 ±38
	10	113 ±11	52 ±4	239 ±4	298 ±32
	30	125 ±7	62 ±9	262 ±10	279 ±32
	100	117 ±13	56 ±5	239 ±7	301 ±16
	300	116 ±9	62 ±8	249 ±19	310 ±15
	1000	105 ±0	62 ±9	269 ±24	354 ±16
	1 hour	103 ±13	71 ±13	260 ±9	331 ±14

A.3.b. CNS/PNS Studies:

Model	Dose/Route	Effect
Behavioral tox (mice)	3 - 300 mg/kg, p.o.	No effect (72 hr. obs. period)
Motor activity (mice)	0.3-10 mg/kg, p.o.	No stimulation of LA (90 min. obs. period)
Hypothermia (g. pigs)	10 mg/kg, s.c.	No effect
Hex. sleep time (mice)	10 mg/kg, p.o.	"

Conclusion: ALMO appears to be devoid of CNS effects, and may not penetrate the Blood-Brain barrier.

A.3.c. Renal Studies:

Model	Dose/Route	Effect
Diuresis in rats	10 mg/kg, p.o.	No diuretic or antidiuretic effects over 4 hrs
Renal function in anesthetized dogs	1 mg/kg, i.v.	sl. ↑ RBF at 60-120 min; large ↑ urine and Na output at 40-60 min

Conclusion: ALMO has diuretic effect in anesthetized dogs, but not in conscious rats.

A.3.d. Anti-emetic/analgesic evaluations:

In a study to evaluate the potential anti-emetic effects of ALMO, a dose of 1 mg/kg, p.o., ALMO did not reduce the number of dogs that had an emetic response to apomorphine, but did reduce the total number of emesis episodes suggest a slight anti-emetic effect.

ALMO (10 mg/kg, p.o.) had significant analgesic activity in acetic acid- and phenylbenzoquinone-induced writhing models in mice.

A.3.e. Drug Interaction Studies:

Several studies were conducted to determine if ALMO enhanced or diminished the effects of other drugs.

Study	ALMO Dose	Results
Anti-emetic effect of domperidone and metoclopramide in APO-treated dogs	1 mg/kg, p.o.	ALMO potentiated effects of submaximal doses of DOM and METO
Caffeine-induced hyperactivity in mice	10 mg/kg, p.o.	ALMO did not increase CAF-hyperactivity
EtOH-induced sleep in mice	10 mg/kg, p.o.	ALMO did not prolong EtOH sleep times
ASA-analgesia in acetic acid-induced writhings in mice	10 mg/kg, p.o.	ALMO did not affect on ASA analgesia
DZP-induced sedation (Hex. sleep time) in mice	10 mg/kg, p.o.	ALMO did not potentiate sedative effects of DZP

B. TOXICOLOGY

B.1 Single Dose Toxicology

GLP single-dose toxicology studies were conducted in Sprague-Dawley rats and CD-1 mice by oral, intravenous, and subcutaneous routes. Several clinical signs including tremors, convulsions and early deaths occurred at the HD level. No major necropsy lesions were evident after the 14-day observation period.

Species (n)	**Route**	**Max. Non-Lethal**	**Min. Lethal**	**Observations**
Mouse (5M/5F) (14 day obs)	po			Ptosis, tremors, mydriasis, staggering gait, prostration. Clonic convulsions precede death.
	iv			
	sc			
Rat (5M/5F) (14 day obs)	po			similar to mouse
	iv			
	sc			

B.2 Multiple-Dose Toxicology

The sponsor has conducted short-term (1-4 weeks) studies by both the oral and subcutaneous routes, and long-term oral studies (26 weeks) in rats and dogs. Since only the oral route is proposed for the initial IND clinical study, and the doses in the 4-week studies were greater than those in the two-week studies, only the 4- and 26-week oral toxicology studies are reviewed. The only unique toxicity introduced by the subcutaneous route was injection site irritation.

B.2.a. 4-Week Subchronic Toxicity Study of LAS 31416 by the Oral Route in Sprague-Dawley Rats

Report #: T.31416.08 **GLP:** Yes
Conducted by: [redacted] **Start date:** 9/29/93

Dosing Information:

doses:	6, 60, 600 mg/kg/day (single administration/day) by gavage
N:	10/sex
lot:	H-002
vehicle:	[redacted]

Summary of Main Findings: The main findings are summarized in the following table. HD animals exhibited ptosis, salivation, and only slight and transient effects on food intake and body weight. The hematological findings are not considered toxicologically relevant because of the small magnitude of the mean changes; data were not obtained prior to treatment which precludes substantive conclusions. Several HD animals displayed elevations in ALP and AST (> 2 SD of mean), but there were no corollary liver histopathology findings. The statistically significant increase in organ weight was modest. Urinalysis revealed some altered electrolyte handling of minor significance, but several HD animals excreted high quantities of protein (300 mg/dl). The absence of renal histopathology may suggest a possible artefact of the test method. The "taller" thyroid epithelial structure observed in HD animals may represent an early stage of thyroid hypertrophy seen in the 26-week study. Thyroid hypertrophy has been observed with other sulfonamides, and may be related to an inhibition of thyroid hormone synthesis (P. Greaves, Histopathology of Preclinical Toxicity Studies). The few cases of bladder cystitis in HD animals are of uncertain significance since similar changes were not observed in the 26-week study. There were no striking urinalysis findings (eg. hematuria or crystalluria) that distinguished the animals with cystitis from unaffected animals.

The NTE is considered to be 60 mg/kg.

Mean plasma Cmax and AUC values increases were greater than dose-proportional, and were higher on day 22 than on day 1 (data not shown). Levels of the metabolite decreased with time and dose suggesting that metabolic saturation contributes to lack of dose proportionality.

4-Week Oral Almotriptan Toxicity in Rats

		Dose Almotriptan (base)					
		6		60		600	
Observation (n = 10)		M	F	M	F	M	F
TK (d. 22)	Parent Cmax (µg/ml)	0.11	0.07	2.72	4.06	21.54	24.85
	AUC_{0-24}(µg.h/ml)			10.36	13.35	246.6	337.6
	Metab Cmax (µg/ml)	0.92	0.56	1.11	0.83	2.82	1.82
	AUC_{0-24}(µg.h/ml)			6.40	4.71	32.78	20.41
Clinical Observations							
body weight						↓d7-14	↓d7-14
food intake						↓d7	↓d7
ptosis						↑	↑
salivation						↑	↑
Ophthalmoscopy (wk 4)		no treatment-related effects					
Hematology (mean changes)							
rbc (slight)[a]				↓		↓	
Clin Chem							
↑ Alk P						4/10	8/10
↑ ALT							4/10
↓ Na, Cl						2/10	1/10
Urinalysis							
↑ protein (300 mg/dl)						7/10	3/10
Organ Weights							
liver (rel)						↑ 14%	↑ 12%
Histopathology							
thyroid[b]							
bladder, cystitis						2/10	1/10

[a] Group hematological variations occurred but only 1 LDF was anemic; there was a tendency for higher neutrophil counts in MDF,HDM, and HDF (uncertain significance)

[b] HD animals tended to have a "taller" epithelial structure (possibly an early stage of thyroid hypertrophy seen in the 26-week study

B.2.b. LAS 31416: 26-Week Oral (Gavage Administration) Toxicity Study in the Rat

Report #:	655/47-1050	**GLP:**	Yes
Conducted by:	[redacted]	**Start date:**	1/15/96

Dosing Information:

doses: 20, 100, 500 mg/kg/day (single administration/day) by gavage;
N: 20/sex;
lot: J-003 vehicle: [redacted]

Summary of Main Findings: The main findings are summarized in a table on the following page. None of the ten deaths that occurred during the study were considered treatment-related. MD and HD animals exhibited salivation from study day 4 for 0.5-2 hr after dosing. Transient paddling (<0.5 hr) and occasional squinting was observed in MD and HD animals. HDM experienced a slight decrease in weight gain for weeks 0-4, but were reduced only 5% relative to controls at termination. Group mean increases in neutrophil and decreases in lymphocyte count was observed in HDM at week 13, but not at week 26; thus, the significance of the finding is uncertain. Group mean increases in WBC in MDF and HDF were dose-dependent, and some individual variations were notable, possibly indicating an infection. Several statistically significant group mean changes in clinical chemistry parameters were identified (↑ Alk Phos in HDM and HDF at wks 13 and 25; ↑ ALT in HDF at wk 13; ↑ urea in HDF at wk 13, and in MDF at wk 25; ↑ inorganic P in HDM and HDF at wks 13 and 25, and in MDF at wk 13; ↑ Ca in HDM and HDF at wks 13 and 25). Only alkaline phosphatase levels displayed individual variations that were greater than 2 S.D. beyond control values in HD animals (see summary table), and were considered of toxicological importance. However, the elevated AlkP levels did not correlate with liver histopathology. The high levels of urinary protein are consistent with findings from the 4-week study, but were not associated with renal histopathology.

Centrilobular hypertrophy accompanied by a significant increase in liver weight in HDF raises the possibility of hepatic enzyme induction. The absence of a strong dose-relationship suggests that hepatic fibrosis and necrosis were incidental findings. Thyroid follicular cell hypertrophy with depletion of follicular colloid is likely a progression of drug-related effects on epithelial structure described in the 4-week study. Similar effects have been observed with other sulfonamides, and may be due to inhibition of thyroid hormone synthesis or an enhanced clearance. Either mechanism would reduce circulating T3 and T4 levels, resulting in excessive TSH secretion from the hypothalamo-pituitary axis. Clinical alterations in T_3 and T_4 levels are not a concern for the proposed limited dose regimen study, but may require monitoring in longer-term repeated dose studies.

The study report also documents a dose-related increased incidence of histopathological changes in the lungs (inflammatory cell foci, foamy histiocytes) in addition to the pneumonitis denoted in the table. These findings occurred in several controls and are of uncertain significance, but raise the possibility that the test compound increases the susceptibility of the lung to infection.

The NTE is considered to be 100 mg/kg/day, based on the clinical pathology and histopathology.

26-Week Oral Toxicity in Rat
Toxicology Results

	Dose Almotriptan (base)					
	20		100		500	
Observations (n = 20)	M	F	M	F	M	F
Clinical Observations						
salivation			↑	↑	↑	↑
paddling			↑	↑	↑	↑
ptosis			↑	↑	↑	↑
Ophthalmoscopy (13, 25 wks)	no treatment-related effects					
Hematology (13, 25 wks)						
↑ WBC, neuts, lymphos				1/10		4/10
Clinical Chemistry (13, 25 wks)						
↑ Alk Phos					9/9	1/10
Urinalysis						
↑ protein					9/10	1/10
Organ Weights						
adrenals (rel)					↑ 18%	↑ 27%
liver (rel)					↑ 13%	↑ 25%
kidney (rel)					↑ 9%	↑ 9%
heart (rel)						↑ 10%
Histopathology						
liver,						
centrilobular hypertrophy						3/20
fibrosis			1/20		1/20	
necrosis			1/20	1/20	2/20[a]	
thyroid,						
follicular cell hypertrophy					6/20	8/20
(assocd w/ ↓ colloid)						
lung,						
pneumonitis	1/20				3/20	7/20
(also in 1 ConM, 1 ConF)						

Mortality: 10 animals died, but none were considered treatment-related. However, liver necrosis was seen in 2 HDM that died on study, and was listed in Path report as C.O.D. No CC changes predicted lesion.

Clin. path. samples were collected for 10 of 20 animals per group

Mean plasma Cmax values increased approximately proportional to dose, but AUC increases were greater than dose proportional, possibly indicating metabolic saturation. Plasma levels were much greater at week 13 than at day 1, but only slightly greater at week 26 compared to week 13. No sex differences were apparent.

Toxicokinetic Results

		20		100		500	
Observations (n = 20)		M	F	M	F	M	F
Cmax (µg/ml)	day 1	0.20	0.16	2.46	2.90	9.13	9.05
	wk 13	0.86	1.08	4.84	4.90	39.20	26.12
	wk 26	1.00	1.43	5.92	6.87	21.46	32.72
AUC_{0-24} (µg.hr/ml)	day 1	0.84	0.59	14.87	19.81	130.36	140.12
	wk 13	2.66	2.83	25.82	34.90	406.18	322.06
	wk 26	2.86	3.02	38.26	41.53	260.61	300.03

B.2.c. LAS 31416: 28-Day Oral (Capsule) Sub-Chronic Toxicity Study in the Beagle Dog

Report #:	655/34-1050	**GLP:**	Yes
Conducted by:	[redacted]	**Start date:**	3/9/94

Dosing Information:

Doses:	0, 2, 5, 12.5 mg/kg/day
N:	3/sex
lot:	H-002
vehicle:	[redacted]

Summary of Main Findings: The main findings are summarized in a table on the following page. A number of clinical signs were evident post-dose and persisted for the duration of the working day. ALMO did not affect body weight or food consumption. The most noteworthy toxicological finding was QTc prolongation at the MD and HD; these effects were most pronounced on day 1, but a dose-related trend for prolongation was evident at week 4. These findings are consistent with the findings from the safety pharmacology study, but smaller in magnitude. Tachycardia was also prominent. There were no clearly treatment-related effects on ophthalmalogy or clinical pathology parameters. The histopathology findings may be incidental in view of the lack of a dose-response relationship or occurrence in controls (cystitis found in 1 Control F), but are noted as consistent with rat findings.

The NT is considered to be 2 mg/kg/day, based on the ECG changes and tremors and 5 mg/kg.

Toxicokinetic analyses revealed that plasma concentrations of the parent compound and metabolite LAS 31911 increased approximately proportional to dose. There was a slight shift (increase) in the parent:metabolite ratio over time (wk 4 vs. wk 1), perhaps due to saturation. Gender differences were not marked.

4-Week Oral Toxicity in Beagle Dogs

Dose Almotriptan (base)

Observation (n = 3)		2		5		12.5	
		M	F	M	F	M	F
314161 Cmax (µg/ml)	wk 1	0.39	0.37	1.22	0.82	1.63	1.91
	wk 4	0.33	0.42	1.29	1.18	3.19	2.84
314161 AUC (µg.h/ml)	wk 1	1.26	0.93	3.91	3.08	9.23	8.09
	wk 4	1.16	1.16	4.35	3.40	10.05	10.07
319111 Cmax (µg/ml)	wk 1	0.18	0.15	0.33	0.35	0.61	0.57
	wk 4	0.15	0.14	0.29	0.37	0.40	0.57
319111 AUC (µg.h/ml)	wk 1	1.03	0.63	2.14	2.06	4.68	4.81
	wk 4	0.62	0.59	1.79	1.79	3.38	4.02
Clinical Observations							
tremors				↑ (few)	↑ (few)	↑	↑
mydriasis		↑	↑	↑	↑	↑	↑
vocalization				↑	↑	↑	↑
splayed hindlimbs				↑	↑	↑	↑
ECG (d1 or 2, & wk 4)[a]							
↑ heart rate (bpm)				74		114	76
↑ QTc (ms)						36 (ns)	45
(no effect on bp)							
Ophthalmoscopy (wk 4) Hematology Clinical Chemistry Urinalysis		no treatment-related effects					
Histopathology							
bladder, cystitis							1/3
thyroid, foll. cell hypertrophy			1/3				
lung, pneumonitis				1/3			

[a] ECG - day 1 recordings (slightly lower @ wk 4)

B.2.d. LAS 31416: 26-Week Oral (Capsule Administration) Toxicity Study in the Beagle Dog

Report #: 655/49-1050
Conducted by: [redacted]
GLP: Yes
Start date: 1/17/96

Dosing Information:

Doses:	0, 2, 5, 12.5 mg/kg/day
N:	4/sex
lot:	J-003
vehicle:	[redacted]

Summary of Main Findings: The main findings are summarized in a table on the following page. A number of clinical signs including mydriasis, vocalization and splayed hindlimbs were evident post-dose; their intensity and frequency dissipated with repeated dosing. One death (HDF) occurred, but this was not considered treatment-related by the study pathologist (hemorrhagic and necrotic enteritis). ALMO did not affect body weight or food consumption. As in the 28-day study, the most noteworthy toxicological finding was QTc prolongation and tachycardia in MD and HD animals; these effects were most pronounced on day 1. There were no clearly treatment-related effects on ophthalmalogy, hematology or urinalysis. The serum chemistry changes were not accompanied by histopathological changes.

The NOAEL is considered to be 2 mg/kg/day, based on the ECG changes at 5 mg/kg.

Toxicokinetic analyses revealed that plasma concentrations of ALMO increased approximately proportional to dose. There were no marked differences with respect to day of sampling or gender.

[redacted]

26-Week Oral Toxicity in Beagle Dogs

		Dose Almotriptan					
		2		5		12.5	
Obsn (n = 4)		M	F	M	F	M	F
Cmax (µg/ml)	day 1	0.28	0.39	0.80	0.82	2.06	1.73
	wk 4	0.31	0.36	0.78	1.04	2.49	1.99
AUC_{0-24} (µg.h/ml)	day 1	1.34	1.18	3.30	3.45	9.88	10.20
	wk 4	1.35	1.23	3.18	3.45	9.48	8.92
Clinical Observations mortality							1
mydriasis		all animals - all doses					
vocalization splayed hindlimbs		d.r.- increase in frequency					
Ophthalmoscopy (wk 25)		no treatment-related effects					
EKG [a] (day 2/3, wk 25 @ 1&24hrs) ↑ heart rate (bpm) PR, QT QTc (ms) QRS PVC				↑	70 ↓ ↑24 (ns)	76 ↓ ↑	82 ↓ ↑33 ↑ 1
Hematology (wk 13, 26) Urinalysis (wk 13, 26)		no treatment-related effects					
Clinical Chemistry (wk 13,26) ↑ Alk Phos (wk 26) ↑ urea, creatinine		1	1	1	1 1 (w26)	3 1 (w13)	1
Organ Weights liver ~~(rel)~~			↑23%		↑12%	↑ 21%	↑ 8%
Histopathology		no treatment-related findings					

[a] EKG results from wk 1 (effects diminished at wk 26); PVC (wk 26 at 24 hrs post-dose) was considered artefact (no additional episodes on follow-up of animal)

B.3. Reproductive Toxicology

The reproductive studies received a cursory review that was adequate to ensure that appropriate clinical precautions are in place. A more thorough review should be conducted for the NDA.

Summary: The major findings are presented in the following Table. The most notable finding in the Segment I study was a high rate of infertility in HDF rats due to an extended estrus cycle (persistent vaginal cornification). The possibility that drug treatment prevented the LH surge necessary for ovulation was raised, but studies involving potential alterations of hormonal mechanisms by ALMO were not been conducted. The rate of pre- and post-implantation losses in MD and HD animals was higher than in controls, but the absence of a dose relationship and lack of statistical significance led to the conclusion that these were not treatment-related. Modest developmental impairments were evident in the offspring.

ALMO was not teratogenic in the rat Segment II studies at doses that caused maternal toxicity (decreased food intake and body weight during the treatment period). HD animals had an increased number of resorptions (mostly early), and an increased number of post-implantation losses. Impaired fetal weight development and delays in ossification, most notably in the sternebrae and vertebrae, were evident in offspring of HD animals.

ALMO did not produce clear teratogenicity in rabbits in either a dose-ranging study, or in the definitive study. Two HD (60 mg/kg/day) fetuses had spina bifida, but this was considered within the historical range (supporting tables were provided). The most notable finding in the main rabbit study was a significant increase in post-implantation losses at the HD. The number of pre-implantation losses appeared to increase with dose; statistical significance was not achieved, but the effects may be toxicologically important (incidence rate - control: 7.2%; HD: 13.4%). An increase in post-implantation losses was also observed in the dose-ranging study at 100 and 150 mg/kg/day.

It is noted that the reproductive toxicities associated with ALMO are less dramatic than those reported in the sumatriptan labeling (embryolethality, vascular and skeletal abnormalities and pup deaths). It would be of interest to compare the reproductive toxicities among this relatively new class of compounds to determine if any consistent toxicities are associated with these agents.

Toxicokinetic analyses in the Seg. II studies were conducted on treatment day 13 in pregnant rats (gestation day 7).Dose-related increases in parent compound and metabolite (LAS31911) were evident. The amounts of metabolite formed by rats was small relative to the parent compound. By contrast, the in the rabbit dose-ranging study, generally higher levels of metabolite than parent compound were observed (see Sponsor's Table XXVII).

Reproductive Toxicology Major Findings

Study	Doses (mg/kg, p.o.)	Design	Findings
Segment I, Rat	25, 100, 400; n = 48 M & F lot J-003	Rx males: 28 days Rx females: day -14 to day 13 of gestation or through weaning	Clin: ↓ b.w.g. & f.i. at MD in HD; Mating: extended estrous in MD & HD; 10/47 HDF were infertile; Preg. Params: extended gestation at HD; possible ↑ pre-implant loss at MD & HD; F1: slight impairment of b.w. development at HD (day 7 - weaning)
Segment II, Rat	250, 500, 1000; n = 10/dose lot I-002	Rx days 6-15 p.c.; C-section (day 20)	Clin: d.r.↓ maternal b.w.g. & f.i.; clonic contractions of hindlimbs at HD Preg. Params: ↑ resorptions & post-implant losses at HD; F1: ↓ fetal wt. at HD; delayed ossification at all doses
Segment II, Rabbit	5, 20, 60; n=20 lot I-001	Rx days 6-18 p.c.; sac. day 28	Clin: transient↓ maternal b.w.& f.i. at HD; Preg. Params: ↑ pre- and post-implant losses at HD (led to ↓ live fetuses); Litter Params: no effect on fetal development;

Toxicokinetic Data

			250	500	1000
Rat, Seg II (day 13)	31416	Cmax (µg/ml)	9.0	16.4	28.9
		AUC_{0-24} (µg.hr/ml)	113.8	253.9	479.0
	31911	Cmax (µg/ml)	0.9	1.4	2.2
		AUC_{0-24} (µg.hr/ml)	13.7	24.3	36.5

XXVII.- Main pharmacokinetic parameters obtained in New Zealand female rabbits on days 1 and 13 of treatment (days 6 and 18 of pregnancy) with oral doses of 25, 50, 100 and 150 mg/kg/day.

DOSE (mg/kg/day)	PARAMETER	LAS 31416		LAS 31911	
		DAY 1	DAY 13	DAY 1	DAY 13
25	C_{max} (µg/ml)	0.174 ± 0.082	0.753 ± 0.251	7.382 ± 1.345	10.271 ± 2.017
	t_{max} (h)	1.3 ± 0.6	1.0 ± 0.0	1.3 ± 0.6	1.0 ± 0.0
	C_{min} (µg/ml)	ND	ND	ND	ND
	$t_{1/2}$ (h)	1.9 ± 0.6	1.4 ± 0.1	1.5 ± 0.2	0.8 ± 0.2
	AUC(0-8) (µg.h/ml)	0.537 ± 0.084	1.532 ± 0.962	22.054 ± 3.922	19.277 ± 3.513
	AUC(0-24) (µg.h/ml)	0.537 ± 0.084	1.532 ± 0.962	24.265 ± 5.055	19.277 ± 3.513
50	C_{max} (µg/ml)	0.668 ± 0.245	2.308 ± 0.445	16.813 ± 2.525	14.815 ± 3.494
	t_{max} (h)	1.3 ± 0.6	1.7 ± 0.6	1.0 ± 0.0	1.0 ± 0.0
	C_{min} (µg/ml)	ND	ND	ND	ND
	$t_{1/2}$ (h)	2.2 ± 0.5	1.6 ± 0.2	1.2 ± 0.2	1.4 ± 0.4
	AUC(0-8) (µg.h/ml)	2.758 ± 0.415	9.334 ± 2.849	52.963 ± 4.068	38.452 ± 7.387
	AUC(0-24) (µg.h/ml)	3.390 ± 0.311	10.697 ± 3.346	55.643 ± 4.626	42.661 ± 6.300
100	C_{max} (µg/ml)	7.241 ± 5.539	10.794 ± 3.493	18.879 ± 3.253	16.166 ± 0.657
	t_{max} (h)	2.0 ± 1.7	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
	C_{min} (µg/ml)	ND	ND	ND	ND
	$t_{1/2}$ (h)	2.1 ± 1.4	2.1 ± 0.6	1.9 ± 1.0	2.4 ± 0.4
	AUC(0-8) (µg.h/ml)	16.455 ± 10.387	40.378 ± 5.791	65.712 ± 8.366	52.775 ± 7.420
	AUC(0-24) (µg.h/ml)	18.898 ± 9.759	48.637 ± 2.429	78.888 ± 15.683	68.893 ± 13.627
150	C_{max} (µg/ml)	8.377 ± 3.211	17.314 ± 5.165	26.009 ± 8.305	12.188 ± 0.862
	t_{max} (h)	1.7 ± 0.6	3.0 ± 1.4	1.0 ± 0.0	1.5 ± 0.7
	C_{min} (µg/ml)	ND	0.091 ± 0.019	0.283 ± 0.021	0.987 ± 0.183
	$t_{1/2}$ (h)	2.2 ± 0.5	2.7 ± 0.1	3.8 ± 0.3	6.5 ± 0.4
	AUC(0-8) (µg.h/ml)	37.972 ± 15.280	96.657 ± 20.794	82.359 ± 20.164	59.115 ± 5.629
	AUC(0-24) (µg.h/ml)	47.553 ± 19.131	157.20 ± 13.760	102.97 ± 19.751	101.81 ± 15.911

ND not detected (<0.025 µg/ml LAS 31416; <0.050 LAS 31911)

B.4. Mutagenicity

Almotriptan did not display any genotoxic activity in an adequately conducted standard battery of testing.

Assay	Doses/Concs	Observation
Ames Test	8-5000 µg/plate	**Negative** in all strains tested + or - S9 (TA 1535, 1537, 98, 100, 102)
In vitro cytogenetics (human lymphocytes)	- S9: 276-563 µg/ml +S9: 1643-3352 µg/ml	**Negative**
Mouse Micronucleus	2000 mg/kg, p.o. (2X); 5M, 5F per time point (24, 48 hr)	**Negative**
Mouse Lymphoma/ Thymidine Kinase	312.5-5000 µg/ml	**Negative**

C. PHARMACOKINETICS/ADME

C.1. Single Dose Pharmacokinetics

The single-dose pharmacokinetic profile of ALMO has been extensively studied in several species by the oral, intravenous, and subcutaneous routes. Only the oral and iv study results are presented in the following summary table. The studies included the use of both radiolabeled and non-radiolabeled methods.

Species variations were evident with respect to bioavailability of the parent compound , metabolism to LA 31911. The elimination $t_{1/2}$ of the parent compound was relatively short in all species (< 2.4 hr); the elimination $t_{1/2}$ of radioactivity was between 1.6-91 hr in rats and 3.8-4.6 hrs in dogs.

Species	Dose (mg/kg)	Rte	Cmp.	Cmax (µg/ml)	AUC (µg.hr/ml)	Tmax (hr)	t½ (hr)	Vz (L/kg)	Cl (l/hr/kg)	F (%)
Monkey	10	po	31416	0.2	0.8	3.5	1.7			18.7
			31911	1.1	3.9	3.0	1.1			
		iv	31416	5.2	4.3	-	1.3	4.4	2.4	
			31911	1.8	3.3	0.8	1.2			
Dog	5	po	31416	0.9	3.6	0.8	2.4			81.8
			31911	0.5	2.9	3.3	1.6			
		iv	31416	1.9	4.4	-	2.3	3.7	1.2	
			31911	0.4	2.5	2.5	1.7			
Rat	10	po	31416	0.5	0.6	0.25	1.6			36.0
			31911	1.3	3.5	0.5-1	1.3			
		iv	31416	3.5	1.7	-	0.8	5.9	5.7	
			31911	1.7	3.6	0.5	1.2			
Human	12.5 mg	po	31416	0.04	0.28	2.7	3.4			
	25 mg			0.075	0.53	2.5	3.5			

C.2. Distribution

The distribution, excretion and milk transfer of a single intravenous or oral dose of 5 mg/kg [^{14}C]-ALMO was evaluated in rats. The main uptake after p.o. treatment was in organs of absorption (g.i. tract), elimination (kidney, liver), and skin. Retention of radiolabel was evident in the skin and eyes of pigmented rats. Blood-brain barrier and blood-placental transfer was low. Excretion into milk was high (milk:plasma ratio = 6.7 after 6 hrs).

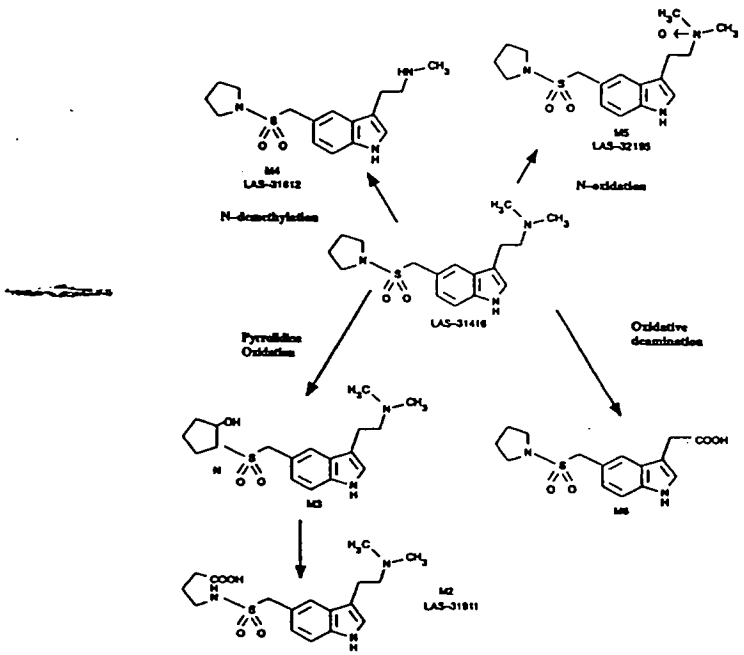
C.3. Metabolism

In preliminary *in vitro* metabolism studies with liver subcellular fractions from rat, guinea pig, dog, monkey and humans, the percent biotransformation of ALMO was determined:

human (12%) < dog = rat (33-37%) < monkey (47%) < guinea pig (87%)

The main product of ALMO *in vitro* metabolism in rat differed depending on the sub-cellular fraction. Rat microsomes hydroxylated the pyrrolidine moiety, producing M3, while the liver S9 fraction produced M2 as a major metabolite, probably due to further M3 oxidation at the pyrrolidine moiety and ring opening. The main metabolic reactions in Guinea pig and dog were N-dimethylamino N-oxidation (M5) and N-demethylation (M4). In monkeys, the pyrrolidine oxidation (M2), N-dimethylamino group N-oxidation (M5) and N-demethylation (M4) were equally important *in vitro* metabolic reactions. Human microsomes displayed only a low extent of microsomal metabolism. Pyrrolidine oxidation (M2), tertiary amine N-demethylation (M4) and oxidative deamination (M6) were the main metabolic pathways (see sponsor Fig. 1):

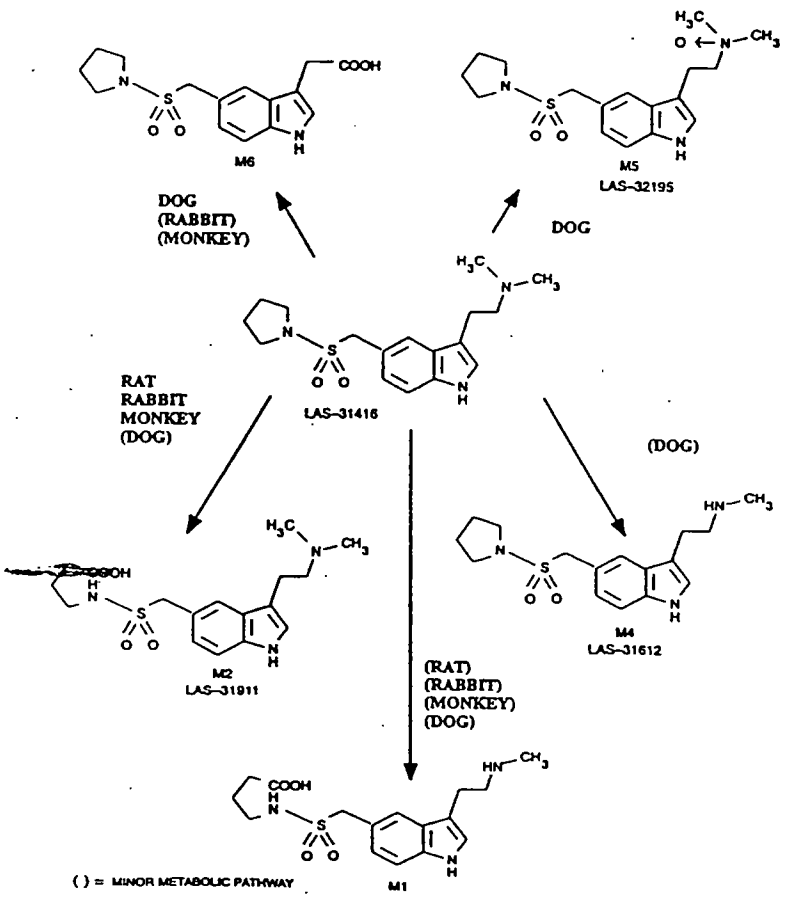
Figure 1 Main *in Vitro* metabolic pathways of LAS 31416



The *in vivo* metabolic profile of ALMO was assessed in plasma and urines of rat, rabbit, dog and monkey (see sponsor Fig 2). In rat and rabbit plasma, metabolite M2 was present at concentrations five times higher than those of the parent compound. A second minor metabolite was identified as M5 and/or M6. M2 and M5/M6 were also detected in dog plasma at concentrations similar to those of unchanged ALMO. Up to eight different metabolites were detected in the urine of animals treated with ALMO. The quantitative distribution of urinary ALMO metabolites among the different species can be summarized as:

human:	40-50% parent 20-25% M6 (31911 was a minor metabolite)
dog:	30-50% parent 15-20% 31911, M5, M6 (each)
rat, rabbit, monkeys:	40-45% 31911 10-15% parent

Figure 2 LAS 31416 main metabolic pathways in laboratory animals

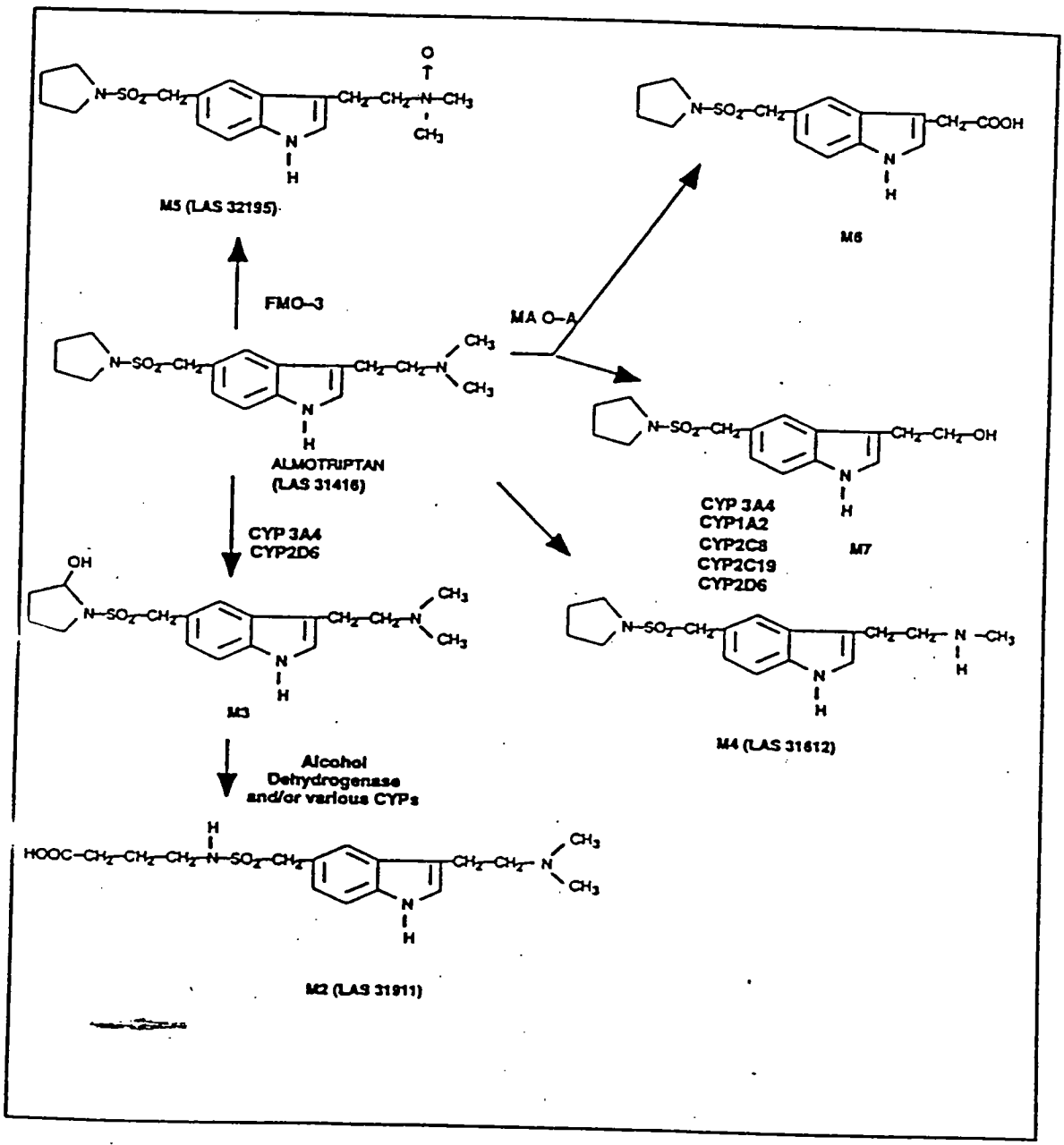


Consistent with the results of the *in vitro* microsome studies, urinary excretion studies of almotriptan in humans indicated that oxidative metabolism was a minor clearance pathway (~20% of biotransformation) compared to other animal species. Two Phase I metabolites were detected in human urine, corresponding to the γ-aminobutyric acid (M2) and indoleacetic acid (M6) derivatives (see Sponsor's Figure). Additional *in vitro* studies identified the human enzymes involved in these reactions.

Five different metabolites were detected during these studies, including the *in vivo* metabolites M2 and M6. The formation of the indoleacetic acid (M6) and alcohol (M7) metabolites of almotriptan was inhibited by MAO inhibitors clorgyline (MAO-A) and deprenyl (MAO-B) (clorgyline > deprenyl). The formation of the other metabolites was dependent on the presence of a NADPH generating system suggesting P_{450} involvement. Almotriptan hydroxylation at the pyrrolidine ring to generate M3 was mediated by CYP3A4/5 and to a lesser extent by CYP2D6. Further oxidation of the pyrrolidine ring to the γ-aminobutyric acid derivative (M2) and N-demethylation (M4) also appeared to be mediated by CYP3A4 and CYP3A4/5. However, the use of recombinant human P450 enzymes show that CYP1A2, CYP2C8, CYP2C19 and CYP2D6 also catalyzed this reaction. As a minor *in vitro* metabolic route, ALMO was N-oxidized at the tertiary amine group (M5), a reaction that could not be correlated to the different CYP activities possibly indicating that a microsomal flavin monoxygenase (FMO-3) is responsible for this pathway. The hydroxylated derivative (M3) is not detected *in vivo,* because the pyrrolidine moiety is further oxidized to the γ-aminobutyric acid metabolite (M2). This reaction could be catalyzed by various CYP and/or by alcohol/dehydrogenase.

The following diagram (Figure 1) shows the proposed metabolic pathway of almotriptan in man. This pathway has been confirmed after oral administration of ^{14}C-almotriptan to six healthy male subjects at a nominal dose level of 25 mg (see Excretion).

Figure 1. Metabolic Pathway of Almotriptan (LAS 31416) In Man



C.4. Excretion

Excretion studies were conducted in rats, dogs and monkeys (not shown) using both radiolabelled and unlabeled materials. Urinary excretion was the primary route in rats, dogs and humans. Most of the material was eliminated within 24 hrs. The total excretion of unchanged drug and metabolite suggests a significant first pass effect in all animals species studied. There were no notable gender differences in excretion in rats or dogs:

% Dose of Drug-related Material Excreted

		interval	urine	feces
Rat	5 mg/kg [^{14}C]-ALMO, p.o.	72 hr	54	42
	" , i.v.	72 hr	64	31
	10 mg/kg ALMO, p.o.	24 hr	38	
Dog	1 mg/kg [^{14}C]-ALMO, p.o.	8 days	80	24
	" , i.v.	8 days	80	23
	5 mg/kg ALMO, p.o.	24 hr	42	
Human	25 mg [^{14}C]-ALMO, p.o.	24 hr 7days	71 76	 13

In animal studies with the unlabeled material and in the human radiolabelled studies, the relative amounts of parent compound and the major urinary metabolites were determined:

Urinary Excretion Profile of ALMO and Metabolites (% dose)

Species	Dose/Route	ALMO	LAS 31911 (M2)	LAS 32195 (M5)	(M6)
Rat	10 mg/kg, p.o. " , i.v.	7.2 14.2	30.2 21.5		
Dog	5 mg/kg, p.o. , i.v.	20.8 20.8	10.5 7.0	10.5 7.9	
Human	25 mg [^{14}C]-ALMO, p.o.	28-36	minor		14-18

Thus, the relative amounts of drug-related material excreted by urinary and fecal routes are relatively similar among species, but the urinary metabolic profile suggests quantitative differences in metabolism of ALMO. A large fraction, designated M6 in human urine, was not quantitated in the dog study noted above, but was detected in dog urine following subcutaneous administration at levels that were higher than LAS 31911 (Report B-31416-DMO2).

C.5. Plasma protein binding

The relative amounts of plasma protein binding among species were:

[redacted]

Rat	-	59%
Dog	-	55%

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ON ORIGINAL

D. SUMMARY AND EVALUATION

Almotriptan (ALMO) as an NCE of the "triptan" class that is under development for migraine relief. The pharmacological mechanism of ALMO, like other "triptans", is proposed to be due to specific activation of 5-HT_{1D} receptors of the cerebral vasculature. This action results in vasoconstriction of vessels that are putatively excessively dilated during migraine. Although ALMO appears strikingly similar to sumatriptan in its chemical structure and pharmacological actions, the sponsor posits, and presents some preliminary evidence to support, that ALMO may be clinically superior to sumatriptan by virtue of its diminished effects on tissues associated with side effects (eg. coronary artery).

The proposed study is a randomized, single-dose, double-blind, placebo-controlled Phase II study in approximately 300 patients with migraine. Extensive clinical experience with ALMO has already been generated in 750 patients or healthy volunteers in foreign studies. ALMO has been administered by several different routes; the oral route is proposed in the initial U.S. study. No serious safety concerns have arisen thus far in clinical studies.

The toxicology package presented in the submission is adequate to support the proposed single-dose trial with a caveat related to the drug batch that was used in the 26-week study (see below). Single dose studies in rat and mouse determined a high minimum oral lethal dose (2000 mg/kg). Multiple-dose studies of up top 26-weeks duration were conducted in rats and dogs. Reproductive toxicology studies (Segment I in rat, and II in rat and rabbit) were adequate to allow inclusion of woman with appropriate precautions against pregnancy. An adequate mutagenicity battery was negative. The critical studies conformed to GLP.

The drug batch (J-003) that was used in the 26-week toxicology studies had levels of "related substances" of 0.07%. The certificates of analysis of the drug used in the clinical supplies report higher levels of "related substances" (0.2 and 0.3%). Thus, the animals were not exposed to the same level of impurities as humans will be exposed. Further, the sponsor has not completely identified degradants in the clinical supply (according to chemist). As the levels of impurities/degradants are relatively low, and humans have already been exposed to the drug product, this is not a reason for a "HOLD" of the initial study; however, they should informed of this potential problem in their development program so that appropriate actions can be taken to correct it (i.e., development cleaner clinical batches or rerun their tox studies).

The clinical signs of toxicity in the rat were rather minor, including ptosis and salivation. The liver (centrilobular hypertrophy) and thyroid (follicular cell hypertrophy associated with depletion of colloid) were identified as potential target organs. Similar thyroid changes have been induced by other sulfonamides; this effect may be related to inhibition of thyroid hormone synthesis which results in overstimulation of TSH release from the pituitary to due loss of feedback inhibition. No specific endocrine studies to address the mechanism were conducted. The finding is not a major clinical concern for a single dose study, but the medical officer will be advised on the implications with longer-term studies.

In the dog studies, the major adverse or toxic events were cardiovascular changes (QTc prolongation, tachycardia). The QTc prolongation was observed in a safety pharmacology study in conscious dogs; the sponsor diminished the significance of the finding since similar effects were not observed in anesthetized dog CV safety study. The occurrence of the QTc prolongation in the dog toxicology study, and other findings from the CV safety studies in conscious dogs and, to a lesser extent, monkeys provided additional evidence that the drug produces marked profound cardiovascular effects at high doses (↑ blood pressure and heart rate). Thus, cardiovascular parameters including EKG changes warrant monitoring in clinical studies.

The pharmacokinetic and metabolism data suggest that the dog is more closely related to humans with respect to drug disposition. In humans, the primary drug-related species detected in urine were parent compound (major fraction), and a metabolite M6. This metabolite was detected as a major component in dog urines from a subcutaneous study, and in dog plasma following oral administration. In contrast, the metabolite LAS 31911 is the major drug-related component in rat and rabbit plasma and urine; it is present in quantities greater than the parent compound. The elimination half-life for the parent compound was not markedly different among species, but was longest in humans (3.5 hrs). Bioavailability was high in dogs, and low in monkeys and rats.

Comparison of the animal toxicokinetic data with human plasma levels at the proposed doses reveals that a wide margin exists between expected human exposures and toxic levels in the rat. This was not the case in the dog (2.3-fold based on AUC) where cardiovascular toxicity was manifested, which emphasizes the need for adequate CV/EKG monitoring in the clinical studies.

Species	Parameter	Dose	Cmax (µg/ml)	AUC (µg.hr/ml)
[redacted]				
Rat	NT (26 wk study)	100 mg/kg/day	5.9	38
Dog	NT (4 & 26 wk study)	2 mg/kg/day	0.3	1.2

E. RECOMMENDATIONS

To the Medical Officer:

1. Tachycardia, hypertension and QTc prolongation were notable findings in the dog safety pharmacology and/or chronic toxicology studies. The protocol does not specify that these parameters will be monitored after drug ingestion (while the drug is present in the patient's system). Consider requesting these measures if plausible.

2. Changes in thyroid epithelial structure with depletion of colloid in the rat suggest a possible inhibition of thyroid hormone synthesis or increased thyroxine clearance. This may be of minimal concern in a single dose study, but thyroid monitoring should be considered in future protocols that call for repeated dose administrations.

To the Sponsor:

1. A comparison of the impurity/degradant levels in drug batches used in the 26-week animal toxicology studies and in the clinical drug supply suggests that the trial subjects will be exposed to higher levels of unknown materials than were animals in the toxicology studies. Because the total level of impurities in the clinical supply is relatively low (0.2-0.3%), the imminent risk to patients is not great enough to warrant a clinical hold. However, it is recommended that you conduct chronic toxicology studies for an NDA submission with drug batches that contain impurity/degradant levels that are at least equivalent to the levels in clinical batches.

2. Infertility occurred in 10/47 female rats treated with 400 mg/kg/day of almotriptan. A warning to this effect should be included in the Informed Consent.

3. [redacted]

[redacted]

/S/

Thomas D. Steele, Ph.D.

cc:

/HFD-120, Div. File
/GFitzgerald, Ph.D.
/AOliva, M.D.
/LChen, R.Ph.
/TSteele, Ph.D.
/S. Stolzenberg, PhD

NDA 21-001

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
Original Summary

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ORIGINAL NDA DATED: 12/17/99
CENTER RECEIPT DATE: 12/20/99
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SPONSOR: Pharmacia & Upjohn
Kalamazoo, MI 49001-0199
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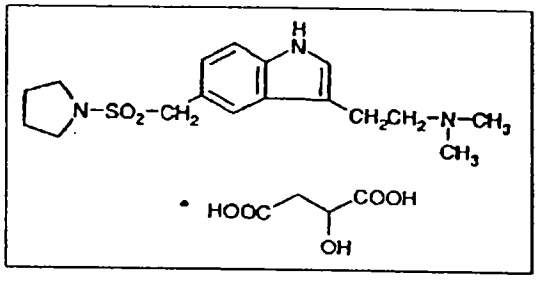
DRUG: AXERT™ (Almotriptan malate) tablets (code name: LAS 31416)



MW: 469.56

FORMULATION: 6.25 and 12.5 mg (as base) tablets for oral administration. [redacted]

Excipients per tablet include mannitol, cellulose, povidone, sodium starch glycolate, sodium stearyl fumerate, titanium dioxide, and carnuba wax.

PHARMACOLOGICAL CLASS: Serotonin receptor subtype 5-$HT_{1B/1D}$ partial agonist

PROPOSED INDICATION: Acute treatment of migraine attacks

DOSAGE REGIMEN: One 6.25 or 12.5 mg tablet, not exceeding 25 mg within 24 hours, and not more than 3 times in a month. (The sponsor recommended 50 mg as the highest dose within 24 hours but the clinical data was not sufficient to support this dose.)

RELATED APPLICATION: [redacted]

RELATED COMPOUNDS: Imitrex® (sumatriptan succinate)

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I. BACKGROUND

AXERT™ (almotriptan) is being developed for use in acute treatment of migraine headaches. Like other triptans of its class, almotriptan is considered to be a selective agonist at the 5-$HT_{1B/1D}$ receptor, and it also binds selectively to 5-HT_{1F} receptors. It is believed to act selectively on extracerebral, intracranial arteries to inhibit excessive dilation of these vessels in migraine, and on the nerve terminals in an activated trigeminal system. Sponsor claims that almotriptan has demonstrated *in vivo* efficacy by inhibiting plasma protein extravasation in a guinea pig model and it caused increased vascular resistance in the cat and dog carotid vasculature. The action of almotriptan on headache is hypothesized to involve activation of an inhibitory receptor on perivascular fibers, which either inhibits neuropeptide release and impulse conduction in trigeminovascular neurons or simply constricts the affected vessels. It is claimed that almotriptan has a pharmacologic efficacy and safety profile comparable to or more favorable than that of sumatriptan.

II. PHARMACOLOGY

A partial review of pharmacology appears in the Initial Pharmacology Review of Thomas D. Steele, Ph.D. (See Appendix III). The following is predominantly from the sponsor's summary with several modifications by the reviewer.

A. In Vitro Studies Related to Indication

Sponsor's Table 2 that follows indicates high affinities for 5-HT_{1B}, 5- HT_{1D} and 5-HT_{1F} receptors and relatively low affinities for other 5-HT receptor sites.

Table 2. Binding Affinity of Almotriptan and Sumatriptan for Serotonin Subtypes, Expressed as the IC_{50} (M) or K_i(M).

Receptor	Almotriptan	Sumatriptan	Tissue Source
5-HT_{1A}	$8.5 \pm 0.7 \times 10^{-7}$	$4.6 \pm 0.7 \times 10^{-7}$	Rat hippocampus
5-HT_{1B}*	$1.2 \pm 0.7 \times 10^{-8}$	$3.6 \pm 0.8 \times 10^{-9}$	HeLa 5A14 cells – Human
5-HT_{1D}*	$1.3 \pm 0.1 \times 10^{-8}$	$7.8 \pm 3 \times 10^{-9}$	HeLa MA6A cells – Human
5-HT_{1D}	$1.3 \pm 0.1 \times 10^{-8}$	$7.8 \pm 1.6 \times 10^{-9}$	Caudate nucleus – Bovine
5-HT_2	$2.5 \pm 1.1 \times 10^{-5}$	$8.8 \pm 2.5 \times 10^{-5}$	Cerebral cortex – Human
5-HT_4	$1.4 \pm 1.1 \times 10^{-4}$	1×10^{-5} (n=1)	Caudate nucleus – Human
5-HT_3	$> 1 \times 10^{-4}$	Not tested	N1E-115 cells
5-HT_6	$< 1 \times 10^{-5}$	Not tested	HEK 293 cells – Rat
5-HT_7	$< 1 \times 10^{-6}$	Not tested	HEK 293 cells – Rat
5-HT_{1F}	2.7×10^{-8}	2.94×10^{-8}	HeLa cells – Human 5-HT_{1F}

*The serotonin subtype receptor nomenclature adopted here differs from that used in the original study report. The human 5-HT_{1B} receptor was identified as the 5-$HT_{1D\beta}$ originally. Present nomenclature conforms to that proposed by the Serotonin Club Nomenclature Committee [131]. The 5-HT_{1D} receptor was identified as the 5-$HT_{1D\alpha}$ originally.

The contractile response of the isolated saphenous vein preparation of the dog, which results from activation of 5-HT receptors, has been used as an initial screening model for migraine treatment. Binding affinities for almotriptan and sumatriptan were generally similar and both drugs produced maximal contractile effects in the dog saphenous vein.

With human isolated tissue preparations, almotriptan and sumatriptan produced qualitatively similar constriction in vascular beds supplying the cranium, and for human meningeal artery tissue the affinity for almotriptan was 3-times greater than for sumatriptan. The M4 metabolite of almotriptan, LAS 31612 has an affinity for meningeal receptors that was equivalent to parent compound.

In human superficial temporal artery, sumatriptan showed slightly greater affinity than almotriptan. Almotriptan and sumatriptan caused a maximal contraction of 65 to 67% in temporal arteries, indicating that both are partial agonists on this tissue.

In human vascular tissue preparations not generally considered to be involved in migraine expression, almotriptan generally produced constriction that was less than or equal to sumatriptan, such as human basilar rings and carotid artery.

Studies were conducted in human ophthalmic artery preparations in order to assess the possibility that almotriptan might cause an ischemic optic neuropathy similar to that described for sumatriptan. The EC50 of almotriptan was moderately higher than sumatriptan but the maximal vasoconstriction was nearly identical. Sponsor suggests this indicates that the likelihood for ischemic optic neuropathy is lower with almotriptan than with sumatriptan.

The EC50 of for induction of coronary vasospasm was higher for almotriptan (EC50 = 0.93 uM) than for sumatriptan (EC50 = 0.58 uM) or serotonin (EC50 = 0.09 uM). The 3 substances were also compared for spasmogenic efficacy and potency in isolated human pulmonary muscle preparations of arterial, venous and bronchial origin. Almotriptan produced no contraction in pulmonary rings, whereas sumariptan resulted in concentration dependent contractions about half that of 5-HT. Sumatriptan contracted the pulmonary vein tissue with approximately 33% of the amount observed for 5-HT, while contractions from sumatriptan were 40% of that with 5-HT. Neither sumatriptan nor almotriptan caused substantial contraction in isolated bronchus.

In appropriate tissue, receptor activation by 5-HT_1 agonists produces a G protein-mediated inhibition of adenylate cyclase. Almotriptan and sumatriptan similarly inhibited cAMP accumulation in HeLa cells expressing 5-HT_{1B} (IC_{50} = 1.5 and 2.1 nM, respectively), but almotriptan was about 8-fold less potent than sumatriptan in expressing 5-HT1D (IC_{50} = 6.5 and 0.78 nM, respectively).

B. In Vivo Studies Related to Indication

Electrical stimulation of the trigeminal ganglion results in leakage of plasma proteins from meningeal vessels, an event resulting in neurogenic inflammation and hypothesized

to be involved in the etiology of migraine pain. Almotriptan and sumatriptan were compared for their abilities to inhibit plasma protein extravasation in anesthetized guinea pigs following electrical stimulation of the trigeminal ganglion. Almotriptan exhibited slightly greater potency than sumatriptan in inhibiting the extravasation of dye following ganglionic stimulation. The lowest effective dose of almotriptan found to inhibit protein extravasation was 0.3 mg/kg, compared with the lowest effective dose of sumatriptan of 1 mg/kg.

The effects of intravenous almotriptan and sumatriptan on blood flow in the carotid, femoral and mesenteric vascular beds were compared in the anesthetized cat. No consistent changes in mean blood pressure or heart rate were found following sumatriptan or almotriptan administration. Almotriptan exhibited its greatest effect on carotid vascular resistance, increasing resistance by 276% over control and decreasing blood flow by 72%. The extent of these effects was nearly identical to that following sumatriptan treatment. Maximal femoral blood flow reductions were equivalent for almotriptan and sumatriptan (56% reduction for both compounds), though maximal femoral resistance tended to be higher with almotriptan than with sumatriptan (207% and 101%, respectively). Effects of almotriptan and sumatriptan were fully reversible by treatment with the 5-HT antagonist, metithepine. Similar measures of cardiovascular function were observed in cats following intraduodenal administration. Mean arterial blood pressure was modestly reduced from baseline by both almotriptan and sumatriptan (decreasing by 10% and 8%, respectively), and the decrease by almotriptan reached statistical significance at 1 and 3 mg/kg. The reductions of carotid blood flow induced by almotriptan and sumatriptan were essentially identical at 1 mg/kg (decreasing by 49% and 55%, respectively), as were the increases in carotid resistance (82% and 117%, respectively). Femoral blood flow was reduced by similar amounts from baseline by almotriptan and sumatriptan (52% and 47%, respectively), while femoral resistance increased 103% and 105%, respectively).

The increase in carotid vascular resistance from serotonin agonists is thought to be due to a selective increase in the resistance of carotid arteriovenous anastomoses. Colored microspheres of 15 gm in diameter were injected into the lingual artery of cats 15 minutes after intravenous injection of either almotriptan, naratriptan or sumatriptan. The distribution of carotid flow was obtained from postmortem measurement of color arising from cerebral and extracerebral tissue, as well as that found in the lungs after passing through the arteriovenous anastomoses. The vasoconstrictor effects of all three migraine palliatives were attributable to their selective increase of vascular resistance in the arteriovenous anastomoses. Cerebral blood flow increased in both absolute and relative terms, while extracerebral circulation decreased slightly.

Sumatriptan and almotriptan were also compared for effects upon carotid, femoral and coronary vascular resistances in anesthetized beagle dogs. In contrast to similar studies in cats, sumatriptan was three times more potent than almotriptan in increasing resistance in the carotid vasculature. Maximum increases in carotid resistance were similar following almotriptan or sumatriptan, and while neither compound affected coronary flow, almotriptan moderately increased coronary resistance at 0.3 mg/kg and higher.

Almotriptan displayed a selectivity for carotid vasculature compared to femoral or coronary vasculature. A comparison was made of intravenous almotriptan and sumatriptan upon vertebral, mesenteric and renal blood flow in the dog. No blood pressure changes were observed with sumatriptan, but almotriptan treatment resulted in a 24% increase in mean blood pressure at the highest dose (3 mg/kg). Sumatriptan caused a significant decrease in vertebral blood flow of 38% at a dose of 1 mg/kg, whereas the maximal decrease in blood flow after almotriptan treatment was a nonsignificant 20% at a dose of 1 mg/kg. These effects on the vertebral vascular bed were smaller than those previously observed on the common carotid artery. No important or significant effects were observed in the mesenteric or renal vasculature.

C. General Pharmacodynamic Effects

The behavioral effects of a maximum single oral dose of 300 mg/kg of almotriptan were studied in mice. No effects were attributable to almotriptan over the 3 days following treatment. In another study, the effects of almotriptan and other 5-HTIB/D agonists on motor activity were studied in mice. Almotriptan, sumatriptan, naratriptan, and CP93129 did not alter motor activity levels, but anpirtoline, a 5-HT_{1B} agonist with CNS activity, induced a significant increase in motor activity at doses of 1 mg/kg and greater. This provides indirect evidence that almotriptan has limited penetration into brain, since centrally active 5-HT agonists generally produce activity increases.

Other indirect evidence of the lack of CNS activity with almotriptan was provided by a study of the hypothermic effects of 5-HT_{1D} agonists in guinea pigs. Almotriptan, sumatriptan and a centrally active 5-HT_{1D} agonist, 5-carboxamido-tryptamine (5-CT), were administered to guinea pigs implanted with temperature transducers. Neither sumatriptan nor almotriptan (10 mg/kg subcutaneous) affected body temperature, while animals treated with 5-CT experienced a mean temperature reduction of 2.24°C. Almotriptan was evaluated in two experiments for possible analgesic or antinociceptive activity in chemically induced writhing tests in mice. Almotriptan had a moderate analgesic effect in both assays. Sumatriptan exhibited a similar level of analgesia against acetic acid writhing, but was inactive in phenylbenzoquinone writhing. In addition, no synergism was noted with combination treatment of aspirin with almotriptan.

No diuretic or antidiuretic effect was detected with almotriptan in fasted rats. In anesthetized dogs, however, almotriptan caused a slight increase in renal blood flow with a transient diuresis (20 minutes) after administration of 1 mg/kg intravenous, without any change in glomerular filtration rate or filtration factor.

In mice, almotriptan demonstrated no significant drug interaction with hexobarbital, diazepam plus hexobarbital, ethanol or caffeine. The effects of combining almotriptan with various stimulants upon cardiac parameters were investigated in the anesthetized cat. Almotriptan alone provoked a slight increase in blood pressure at the highest dose (300 ug/kg intravenous), whereas sumatriptan evidenced no consistent effects on blood pressure or heart rate. Both almotriptan and sumatriptan potentiated the pressor effects of adrenaline, noradrenaline, tyramine, and angiotensin II in a dose-dependent manner. In

addition, almotriptan increased the effects of dimethylphenylpiperazinium, whereas sumatriptan did not.

Nausea and vomiting are common accompanying symptoms to migraine attacks. Antiemetic effects are desirable in migraine treatment. Almotriptan was evaluated for effectiveness against apomorphine-induced emesis in the dog. Almotriptan diminished the frequency of emesis episodes, but did not abolish their occurrence. In another study, almotriptan was administered in combination with marginal antiemetic doses of domperidone or metoclopramide in apomorphine-treated dogs. The antiemetic effects of domperidone and metoclopramide were significantly potentiated by the addition of almotriptan.

Sumatriptan is known to delay gastric motility in therapeutic use, an action thought to be related to stimulation of 5-HT_{1A} receptors in the gastrointestinal tract. In view of this, a study was conducted in rats measuring the rate of removal of glass microspheres from the stomach. Sumatriptan and almotriptan caused similar reductions in the rate of stomach emptying, but only at a high dose of 100 mg/kg.

D. Safety Pharmacology Studies

Almotriptan caused an increase in vascular resistance in the carotid circulation in anesthetized cats and dogs at 0.3 to 3 mg/kg, i.v., but was not found to cause significant changes in systemic blood pressure or hemodynamic parameters in the rat or dog. In cynomolgus monkeys, s.c. injection of almotriptan produced only slight and transitory cardiovascular effects, which required a higher dose for expression than that required by sumatriptan. No change in heart rate or blood pressure was observed in freely moving telemetered rats following oral administration of 60 mg/kg of almotriptan or sumatriptan. Intravenous infusion of 1 mg/kg of almotriptan to conscious dogs caused a modest increase in blood pressure and heart rate, which was accompanied by an increase in coronary artery flow and a fall in coronary vascular resistance. Similar effects were found following administration of a 0.3 mg/kg dose of sumatriptan. No significant effects upon ECG intervals were observed with either sumatriptan or almotriptan in this study. When infused directly into the coronary artery of anesthetized dogs, almotriptan did not cause consistent increases on the QTc interval and had only minor effects on hemodynamic parameters. In open chest assessment of cardiac function in dogs with i.v. administration of either almotriptan or sumatriptan, no consistent effects on blood pressure, heart rate or cardiac contractility were observed with any dose of almotriptan (high dose of 3 mg/kg). The only abnormal observations were a slight increase in total resistance, as well as a decrease in cardiac output and stroke volume. Some cardiac depression was observed with sumatriptan, while the remaining effects were similar to those of almotriptan, though generally more intense.

When studied in conscious telemetered cynomolgus monkeys, almotriptan caused only slight and transitory increases in blood pressure and heart rate and a decrease in the QA interval. The QA interval was defined as the interval between the R wave of the ECG and the beginning of the ascending line of the blood pressure curve. Emesis was produced in

both monkeys with 3 mg/kg sumatriptan, and one monkey following 3 mg/kg almotriptan. Neither almotriptan nor sumatriptan caused significant changes in the shape of the ECG, but sumatriptan lengthened the QT interval in both monkeys at a dose of 3 mg/kg subcutaneous, whereas this effect was seen in only one animal treated with almotriptan at this dose.

Almotriptan and sumatriptan were tested for effects upon action potentials of guinea pig isolated papillary muscles in order to further assess cardiotoxicity. Neither the resting membrane potential nor the action potential of right ventricular tissue were significantly altered by the addition of concentrations of 0.1 to 3 uM of either almotriptan or sumatriptan.

Sumatriptan and almotriptan were compared for vasoconstrictive effects in human coronary artery preparations. The 50% effective concentration for coronary artery constriction was 0.93 uM for almotriptan and 0.58 uM for sumatriptan. The maximal contraction produced by almotriptan was also slightly lower than that caused by sumatriptan (63% vs 77% of initial contraction by 10 uM 5-HT, respectively).

The contractile effects of almotriptan and sumatriptan were also evaluated in isolated, endothelium-denuded coronary arteries from dogs. Almotriptan and sumatriptan produced similar concentration-dependent increases in muscle contraction, with maximal effects of 55% and 53%, respectively, at the highest concentration tested (10 uM).

E. Absorption, Distribution, Metabolism, Excretion (ADME)

1. Pharmacokinetics after Single Dosing

The single-dose pharmacokinetics of almotriptan was characterized in rats, dogs and monkeys (See sponsor's Table 9 which follows). Almotriptan was rapidly eliminated with a short half-life of 0.8, 2.0 to 2.3 and 1.3 hours after intravenous administration in rats, dogs, and monkeys, respectively. The volume of distribution of almotriptan at steady state in the three animal species (2.6 to 3.6 L/kg) was greater than total body water (ca. 0.5 to 0.8 L/kg), indicating that the compound was distributed into tissues. Almotriptan was eliminated by both renal and metabolic routes, with CLr accounting for 13% to 30% of total systemic clearance across species. In rat, the remaining clearance was greater than the hepatic blood flow, indicating possible extrahepatic metabolism. The remaining clearance was about half, and 77% of the hepatic blood flow in dogs and monkeys, respectively. Oral bioavailabilities were 20% to 37% in rats, 82% in dogs, and 19% in monkeys, suggesting incomplete absorption and/or first pass metabolism in rats.

2. Pharmacokinetics (or Toxicokinetics) After Multiple Dosing: Covered on pages 81 to 86, after sections on Toxicology.

Table 9. Summary of Selected Single Dose Almotriptan Pharmacokinetic Data.

Species (Strain)	Route	Dose Level mg/kg (FBE) males/ females	Mean Values of Parameters							Almotriptan Urinary Elimination % of dose (0-24 h)	Ref
			tmax (h)	Cmax (µg/mL)	AUC (µg·h/mL)	t½ (h)	Vz (L/kg)	CL (L/h/kg)	F (%)		
Rats (Sprague-Dawley)	IV	10	0.1	3.43†	1.69	0.8	6.4	5.9		15.1	50
		10	0.1	3.60†	1.82	0.7	5.3	5.5		13.4	
	PO	10	0.25	0.57	0.62	2.0			36.5	7.0	51
		10	0.25	0.45	0.64	1.2			35.3	7.4	
	IV	5	0.1	2.45†	1.41	0.7	3.4	3.35			55
		5	0.1	1.82†	1.02	0.78	5.2	4.62			
	PO	5	0.1	0.095	0.276	1.48			19.2		
		5	0.25	0.210	0.311	0.83			29.2		
Dogs (beagle)	IV	5	0.1†	1.95†	4.39	2.3	3.7	1.2		20.8	53
	PO	5	0.8	0.92	3.60	2.4			81.8	20.8	
	IV	1	0.1†	0.32	0.59	1.96	4.78	1.68			56
	PO	1	0.44	0.18	0.45	2.15			79.6		
Monkeys (cynomolgus)	IV	10	0.08†	5.18	4.32	1.3	4.4	2.4		29.6	54
	PO	10	3.5	0.20	0.79	1.7			18.7	6.5	
Humans	[redacted]										60

† at first sampling time

Abbreviations: AUC = area under the plasma concentration-time curve; CL = total plasma clearance; Cmax = maximum plasma concentration; F = absolute oral bioavailability; FBE = free equivalents; h = hour(s); IV = intravenous; PO = oral; SD = standard deviation; tmax = time of maximum plasma concentration; t½ = terminal half-life; Vz = volume of distribution determined from terminal phase

3. Distribution

Radioactivity was widely distributed in albino rats after oral administration of ^{14}C-almotriptan. At all time points, the highest concentrations were found in the gastrointestinal tract, liver and kidney. By 168 hours, detectable concentrations of radioactivity were found only in the large and small intestines, kidney, liver, and skin. At 24 hours, radioactivity concentrations were below or near the limit of detection in half of the tissues examined. Tissues with concentrations generally less than plasma included blood cells, bone, bone marrow, brain, eye, fat, muscle, ovary, skin, spinal cord, testis, and uterus. Radioactivity concentrations in the thyroid were similar to plasma through 6 hours and below the limit of detection at 24 hours. No notable concentrations of radioactivity in other tissues were found by whole-body autoradiography. In pigmented rats, concentrations of radioactivity in eyes were higher than in the eyes of albino animals and declined slowly (half-life of 22 days), consistent with melanin binding commonly observed for basic drugs. There was limited exposure to fetuses; concentrations of radioactivity in amniotic fluid and fetuses were about 10-fold lower than in maternal plasma at corresponding time points. Drug-related materials were excreted in milk in rats where highest concentrations of radioactivity were observed at 3 hours after dosing but was still high at 6 hours after dosing when the concentration in milk was around 6.7-fold higher in milk than in plasma.

4. Protein Binding

Protein binding for almotriptan in serum was determined by ultrafiltation and by equilibrium dialysis at concentrations ranging between 0.05 and 1000 uM.. By the ultrafiltration method, mean unbound fraction was 41.1, 45.3 and 17% in rats, dogs and humans, and was relatively constant between 0.05 and 10 uM in all 3 species.. By equilibrium dialysis, unbound fractions at 10 uM were 67.5, 70.8, 73.1, 70.5 and 59.1 for the mouse, rat, dog, rabbit and human, respectively. In human serum albumin, with 0.05 to 10 uM almotriptan, mean unbound fractions were between 82.1 and 86.6%, whereas in alpha-1 glycoprotein at physiological concentrations, unbound almotriptan was high; $\geq$91.4%. Almotriptan was not highly bound to serum proteins.

5. Metabolism

Almotriptan was extensively metabolized in animals and in humans (See sponsor's Tables 6 and 7 on pages 12 and 13, Figure 1 on page 14). The major routes of metabolism characterized across species were oxidation of the pyrrolidine ring and oxidative deamination, leading to the formation of a gamma-aminobutyric acid derivative (M2), and to an indole carboxylic acid (M6), respectively. Other pathways of metabolism included N-dealkylation, N-oxidation (with formation of M4 and M5, respectively) and various hydroxylations of almotriptan. Based on comparison of the AUC for total radioactivity in plasma with AUC data obtained for almotriptan, unchanged drug accounted for approximately 10%, 15% and 36% of the total circulating drug-related material in rats, dogs and humans, respectively. Thus, metabolic products, particularly M2 in the mouse and rat, M1, M2, M5 and M6 in the dog, are considerably higher in urine and plasma of these animals than in humans.

Metabolite profiles were compared across species in plasma, urine and feces (Tables 7 and 8 on pages 12 and 13, Figure 1 on page 14). Qualitative similarity in metabolite profiles was observed, with no evidence of differences based on gender or route of drug administration. All metabolites observed in human plasma were also observed in animals. M2 and M6 were observed in urine and plasma of all species studied, including humans. M2 was the major circulating metabolite in rats with an AUC 5-fold higher than parent drug after oral dosing. Formation of M2 was saturated at higher doses in rats and it represented approximately 30% of the dose recovered in urine. In dogs, the major circulating metabolite of almotriptan after oral administration was M2, with an AUC ratio of M2 to almotriptan of 0.75. Urinary recovery in dogs was approximately 12% each for M2 and M5. M4 was not observed in plasma in rats (5 mg/kg), dogs (1 mg/kg) or humans (25 mg dose) (Table 7, page 13), but was a minor metabolite detected in urine in all three species. Metabolite profiles in feces were qualitatively similar to those observed in urine in these studies. M4 was observed as a minor metabolite in plasma of rats that received a 100 mg dose (Table at bottom on page 12).

The metabolism of almotriptan was studied in vitro using both hepatic microsomes and S9 fractions prepared from a number of nonclinical species and from humans. In general, the relative extent of metabolism observed in vitro was predictive of that seen in vivo. M6 was not detected following incubation of almotriptan with microsomes or S9 fractions from nonclinical species, but was produced during incubation with human microsomes as were M2, M4 and M5. Thus, the use of human hepatic systems in vitro was appropriate to study the enzymology of almotriptan metabolism.

The integrated summary table of metabolites found in plasma with comparisons in different species (including rats that received a dose of 5 mg/kg and humans that received a dose of 25 mg) is shown on page 13, but this table does not include mice. The table at the bottom of page 12 summarizes plasma metabolic products found in 3 different species after receiving 100 mg/kg. This includes mice and rats. Metabolites M2 and M6 and parent compound are the main drug products in the plasma of mice, rats and humans. Although other metabolic products are found in urine of humans, those products were not quantifiable in human plasma at the 25 mg dose.

At 2 hours post-dosing in mice at 100 mg/kg, parent compound was a minor drug-related component (244 ng/mL) relative to M2 (3477 ng/mL) and M6 (3257 ug/mL). In humans, at around 1.5 hours post-dosing (25 mg or 0.5 mg/kg based on a 50 kg man), the concentration of parent compound was about 3.6- or 1.8-fold higher than M2 or M6. At these dose levels, the metabolism of almotriptan is considerably greater in mice than in humans. Nevertheless, the circulating levels of parent compound were 244 ng/mL in the mouse, compared to 65.1 ng/mL in humans.

After an oral dose of 5 mg/kg in the rat at 2 hours post dosing, the concentration of M2 and M6 were about 6 fold higher and 0.5 fold than parent compound, respectively, indicating that metabolism in rats is generally higher than in humans. However, at 100 mg/kg at 2 hours post-dosing in rats, almotriptan comprised the highest amount of drug

related material in the plasma, which suggests enzyme saturation with the higher dose. M2 and M6 levels were considerably higher at the higher dose in rats.

Table 6. Composition of Drug-Related Material Identified in Urine from Male Rats, Male Dogs, and Humans Following Oral Administration of Almotriptan at Doses of 5 mg/kg, 1 mg/kg, and 25 mg, Respectively.

Species	Rats [82]	Dogs [83]	Humans [61]
Dose	5 mg/kg PO	1 mg/kg PO	25 mg PO
Component*	Urine 0 to 72 hours (% dose †)	Urine 0 to 72 hours (% dose †)	Urine 0 to 24 hours (% dose †)
Almotriptan	5.55	15.44	
M1	nq	6.83	
M2	32.61	12.95	
M4	0.12	2.77	
M5	nq	11.82	
M6	0.47	13.16	
M8	nq	nq	
M10	7.64	nq	
M11	nq	1.20	
Total % dose	57.37	76.34	

*Metabolites are quoted as M numbers as used in the text. Equivalent P numbers quoted in certain Study Reports and LAS numbers (where these exist) are shown in Table 5.

†Figures quoted are mean values derived from data from individual animals/subjects.

Abbreviations: nq = not detected or not measured; PO = oral

The mean plasma levels of LAS 31416 and metabolites in mouse, rat and rabbit plasma, 2 hours after a single oral dose administration of 100 mg/kg of LAS 31416 are given in the table below:

	Plasma Concentration (µg/ml)						
Species	**M1**	**M2**	**M4**	**M5**	**M6**	**M10**	**LAS31416**
Mouse	1.611 ± 0.529	3.477 ± 1.596	0.030 ± 0.028	0.045 ± 0.061	3.257 ± 1.798	ND	0.244 ± 0.195
Rat	[redacted]18 ± 0.043	1.426 ± 0.146	0.111 ± 0.015	0.121 ± 0.013	1.365 ± 0.233	0.099 ± 0.006	2.293 ± 0.467
Rabbit	2.372 ± 0.272	8.520 ± 1.596	0.043 ± 0.000	0.066 ± 0.059	1.746 ± 0.420	ND	1.507 ± 0.028

Mean ± standard deviation ND: not detected

In summary, this study demonstrates that mice, rats and rabbits, the species used in toxicology studies are exposed to the major human metabolites of LAS 31416, M2 and M6, as well as to several other minor metabolites including M1, M4, M5, M10 (rat) and M15 (mouse).

Table 7. Composition of Drug-Related Material Identified in Plasma from Male Rats, Male Dogs, and Humans Following Oral Administration of Almotriptan at Doses of 5 mg/kg, 1 mg/kg, and 25 mg, Respectively.

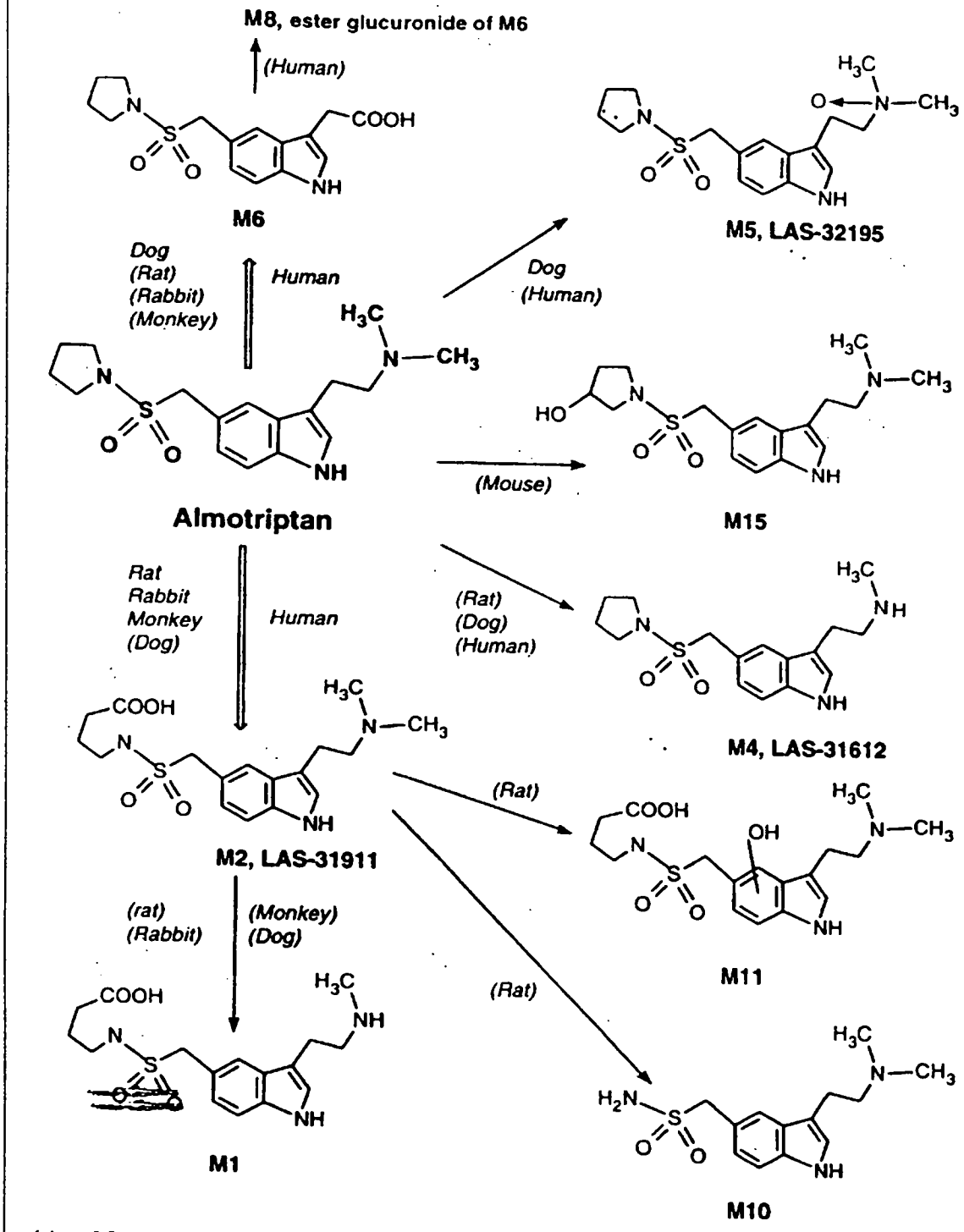
Species	Rats [82]					Dogs [83]					Humans [61]
Dose	5 mg/kg PO					1 mg/kg PO					25 mg PO
Component	Plasma 0 to 6 hours (ng.eq/mL†)					Plasma 0 to 6 hours (ng.eq/mL†)					Plasma 0 to 6 hours (ng.eq/mL†)
Sample time (hours)	0.25	1.0	2.0	4.0	6.0	0.25	1.0	2.0	4.0	6.0	
Almotriptan	149.2	62.2	65.5	31.7	nq	198.3	155.7	134.6	Nq	nq	
M1	nq	nq	nq	26.6	nq	nq	57.7	114.3	Nq	nq	
M2	169.0	337.9	385.8	231.4	182.4	nq	125.3	145.6	227.8	nq	
M4	nq	nq	nq	nq	nq	nq	nq	nq	nq	nq	
M5	nq	nq	nq	nq	nq	nq-	93.4	68.6	nq	nq-	
M6	nq	nq	31.4	25.8	34.2	nq	nq	71.0	141.7	nq	
M8	nq	nq	nq	nq	nq	nq	nq	nq	nq	nq	
M10	nq	nq	nq	nq	nq	nq	nq	nq	nq	nq	
M11	nq	nq	nq	nq	nq	nq	nq	nq	nq	nq	
Total	398.9	424.8	510.1	353.7	216.6	198.3	432.1	534.2	369.5	175.4	

*Metabolites are quoted as M numbers as used in the text. Equivalent P numbers quoted in certain Study Reports and LAS numbers (where these exist) are shown in Table 5.

†Figures quoted are mean values derived from data from individual animals/subjects.

Abbreviations: nq = not detected or not measured; PO = oral

Figure 1. Major Metabolic Pathways of Almotriptan Identified In Vivo in Nonclinical Species and in Human.



( ) = Minor Metabolic Pathway

6. Enzymology

Studies were performed in vitro using microsomes and recombinant enzymes to elucidate the enzymatic basis of human hepatic metabolism of almotriptan. Information was obtained on the identities of four enzymes responsible for the formation of six metabolites, including M2 and M6, the major metabolites of almotriptan in vivo (See Figure 1 on page 14). CYP isoforms CYP2D6 and CYP3A4 were indicated in the formation of M4 via N-demethylation, and formation of M3, a carbanolamine intermediate not observed in vivo, produced via pyrrolidine ring oxidation. However, the high substrate concentrations used in these studies prevented unambiguous assignment of the isoforms involved. The N-oxide metabolite, M5, was formed via the action of FMO-3. The major metabolites M2 and M6 were the products of nonmicrosomal enzymes. Oxidation of carbinolamine, M3, by the cytosolic enzyme aldehyde dehydrogenase resulted in the formation of gamma-aminobutyric acid, M2, a route previously described for phencyclidine. M6 was formed as a result of oxidative deamination of almotriptan catalyzed by MAO-A, with further oxidation of the aldehyde intermediate by aldehyde dehydrogenase. The presence of the latter two enzymes indicated contamination of the human hepatic microsomal preparation used in this work with cytosolic and mitochondrial enzymes, but did not affect the conclusions of the study. Similar contamination of human microsomes with mitochondrial enzymes has been reported in the literature.

The potential inhibition of human hepatic drug-metabolizing enzymes by almotriptan was investigated in human microsomal and mitochondrial preparations. Almotriptan was a weak competitive inhibitor of CYP2D6 with a Ki value of 87 uM. Based on the reported plasma C_{max} in humans of [redacted] following single oral administration of 50 mg almotriptan, the predicted fractional inhibition constant (i) for inhibition of CYP2D6 by almotriptan in vivo was calculated to be 0.73%. This suggests that at clinically relevant doses, almotriptan would not be expected to alter the metabolism of drugs that are substrates of CYP2D6. Almotriptan showed little or no capacity to inhibit or to function as a metabolism-dependent (reversible or irreversible) inhibitor of CYPI A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2E1, CYP3A4/5, or CYP4A9/11 activity. Almotriptan has little or no capacity to be a reversible inhibitor of MAO-A or MAO-B or to function as a metabolism-dependent irreversible inhibitor of these enzymes. Collectively, these data indicate that almotriptan would not be expected to alter the metabolism of other drugs that are metabolized by either the CYP isozymes listed (including CYP2D6) or MAO-A or MAO-B.

The potential for enzyme induction was examined following 14-day oral administration of almotriptan to male and female rats. None of the hepatic changes normally associated with induction of drug-metabolizing enzymes were observed. Minimal effects were seen on activities of CYP subfamilies, but none for uridine-diphosphateglucuronyltransferase. These observations are consistent with the lack of increase in clearance in animals from single-dose to repeated-dose administration, and sponsor believes that it is unlikely that they hold any significance for humans.

7. Cardiovascular System (Dogs)

Cardiovascular findings in both male and females dogs were increases in heart rate within 1 hour after dosing by oral, subcutaneous or intravenous routes. Increases in heart rate were noted at oral doses of 2 mg/kg/day and greater. The data were highly variable and comparisons were not statistically significant. Nevertheless, these data were viewed as a signal of effects on ventricular repolarization in dogs. The ECG and heart rate changes had recovered to baseline or control values by 24 hours after dosing in all repeated dose studies.

Oral escalating doses of almotriptan, ranging from 3 to 12 mg/kg/day, produced increases in heart rate and blood pressure 1 hour after dosing. Blood pressure was not affected in the 4-, 26- or 52-week repeated-dose dog studies. On weeks 1 and 4, a dose-related increase in group mean heart rate with shortened PR and QT intervals was seen 1 hour after dosing in the 4-week study. These changes were statistically significant at 5 mg/kg/day males and in both sexes at 12.5 mg/kg/day. On week 1, similar changes in heart rate and PR and QT intervals were seen in the 26-week dog study 1 hour after dosing in the 5 and 12.5 mg/kg/day groups. Increases in QRS intervals were also seen at 5 mg/kg/day males and in both sexes at 12.5 mg/kg/day, which could have been attributed to the trend toward QTc prolongation. On week 25, the ECG changes had lessened and consisted of increases in heart rate in all groups and decreases in QT interval in the 5 and 12.5 mg/kg/day females. Because of the ECG changes at 5 and 12.5 mg/kg/day, the 2 mg/kg/day dose was considered the NOAEL for both the 4-week and 26-week dog studies.

Because of great variability in the ECG data, it was difficult to discern definitive changes in the 52-week study. On week 1, heart rates were increased in males and females in the 5 and 12.5 mg/kg/day groups. Decreases in QT intervals in treated females and a trend toward prolongation of the QTc interval were observed in the 12.5 mg/kg/day males. Changes in heart rate and ECG intervals were noted in the 5 and 12.5 mg/kg/day females on weeks 25 and 51. Dr. Oliva, the medical reviewer, confirmed the sponsor used the Bazett formula (QTc = QT / square root of RR) to correct the raw QT for heart rate. The Bazett correction OVER-corrects the QT in drugs that cause tachycardia, i.e., making it appear that the drug prolongs the QT, when in fact, it does not, or at least not to the extent that the Bazett correct QTc would suggest.

One female dog in the 12.5 mg/kg/day group was found dead on week 39. Histologic findings for this dog, including hemorrhage in the myocardium and coronary groove, coronary arteritis and vascular engorgement in other tissues, suggested a terminal cardiovascular event. Clinical signs, body weights, food consumption, drug plasma levels and ECG data collected from this female were comparable to other females in the group with the exception of increases in heart rate and a trend toward QTc interval prolongation on week 25. The cause of death in this animal was considered to be treatment-related. The NOAEL for the 52-week study was determined to be 5 mg/kg/day.

III. TOXICOLOGY

A. Acute (Vol 1.20)

Reviewed and summarized in the Original Review for [redacted] The following is sponsor's summary of single dose studies

Table 5. Summary of Single-Dose Toxicity Studies with Almotriptan.

Species	Route	Minimum Lethal Dose (mg/kg)	Maximum Non-lethal Dose (mg/kg)	Reference
Mouse	Oral	2000	1000	[98]
	Intravenous	125	50	[99]
	Subcutaneous	600	300	[100]
Rat	Oral	2000	1000	[101]
	Intravenous	100	50	[102]
	Subcutaneous	--	600	[103]

Abbreviations: – = no lethality

Mortalities occurred within a few hours after parenteral administration and within 48 hours after oral administration. Clinical signs included palpebral ptosis, tremors, abnormal gait, mydriasis (rats) and clonic convulsions which generally preceded death. Injection site irritation was noted after subcutaneous administration in both species.

B. Subchronic and Chronic

1. Mouse

13-Week Oral Gavage Dose-Finding Mouse Study (Vol 1.21)

Report No: B.31416.29/4.8
CHE Study No. 655/66

Performing Laboratory: [redacted]

Dates Performed: Initiated 10/2/96, necropsies performed 1/6/97.

Quality Assurance: A statement of compliance with GLP is included.

Doses: 0, 20, 160 and 320 mg/kg/day (dose level expressed in terms of base)

Procedure: Crl:CD-1 (ICR) BR mice, 12/sex/group, around 42 days of age, body weights of 24.9-34.5 g for males and 21.4-27.6 g for females, received almotriptan D,L-hydrogen malate (Batch K001; >99% purity) daily, by oral gavage. The animals were observed daily for clinical signs, morbidity and mortality, body weights and food measurements

were obtained weekly. Organ weights were obtained for brain, heart, kidneys, liver, prostate, spleen and testes with epididymides combined. All the tissues listed for the mouse carcinogenicity test (page 42) were collected at necropsy but only the following organs from control and high dose groups and decedent animals were examined; adrenals, brain, cecum, colon, duodenum, gall bladder, heart, ileum, jejunum, kidneys, liver, lungs ovaries, pancreas, pituitary, prostate, seminal vesicles, spleen, stomach, testes and epididymis, thyroids with parathyroids and uterus were examined by a pathologist.

Orbital sinus blood samples were collected in Week 13 at 1, 3, 6 and 24 hours after dosing for plasma level measurements and toxicokinetics. Cmax and AUCs derived from this study are shown on page 82.

Mortality: 1 male at high dose at week 2 with cause of death unknown, 2 female controls at weeks 2 due to eye injury and at week13 due to misintubation, 1 female at low dose and 1 female mid dose due to misintubation.; no compound related deaths.

Results: There were no compound related effects on clinical signs, body weight (see figures 1 and 2 that follow), food intake, organ weights, gross or microscopic pathology.

Toxicokinetic findings for this study are reviewed on page 82.

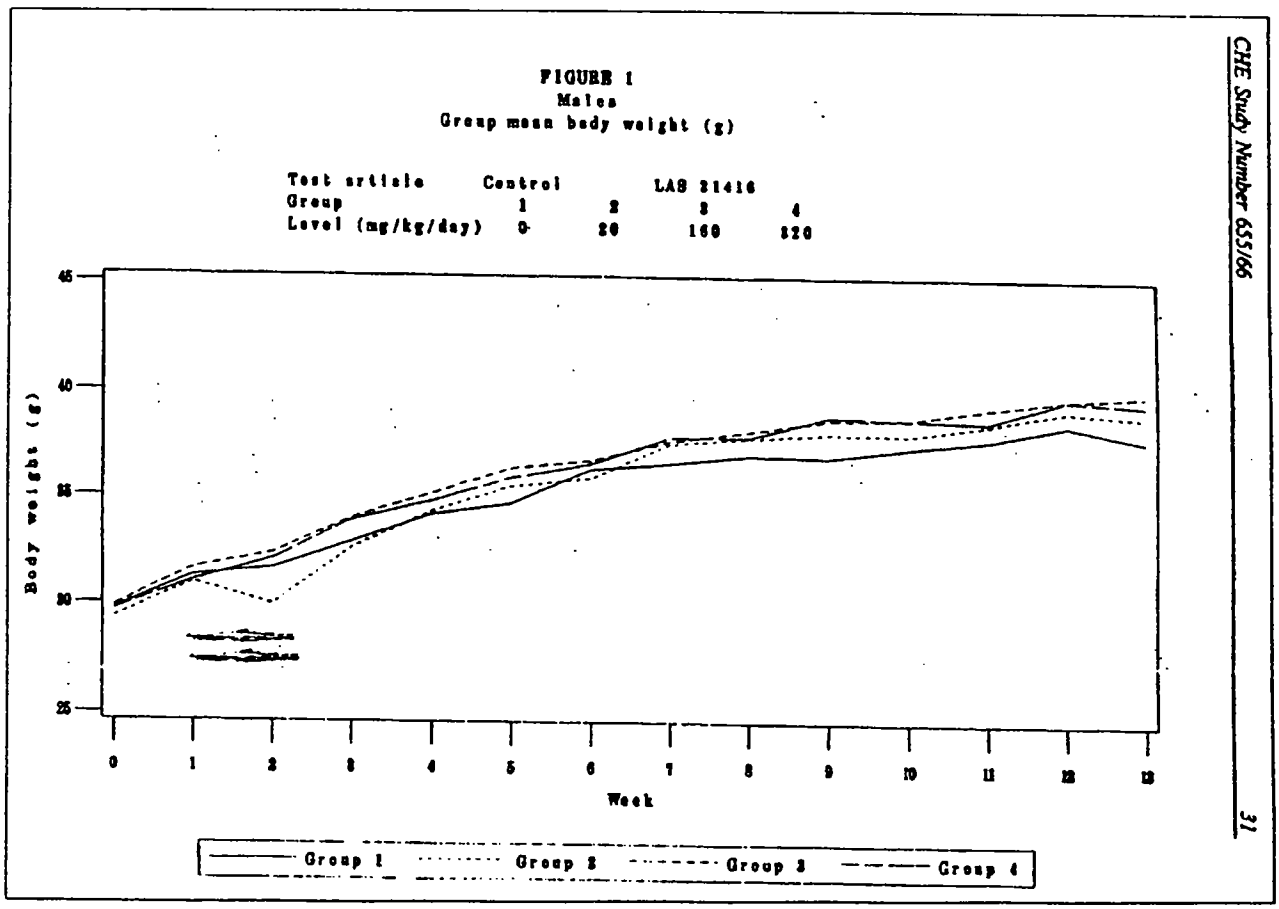
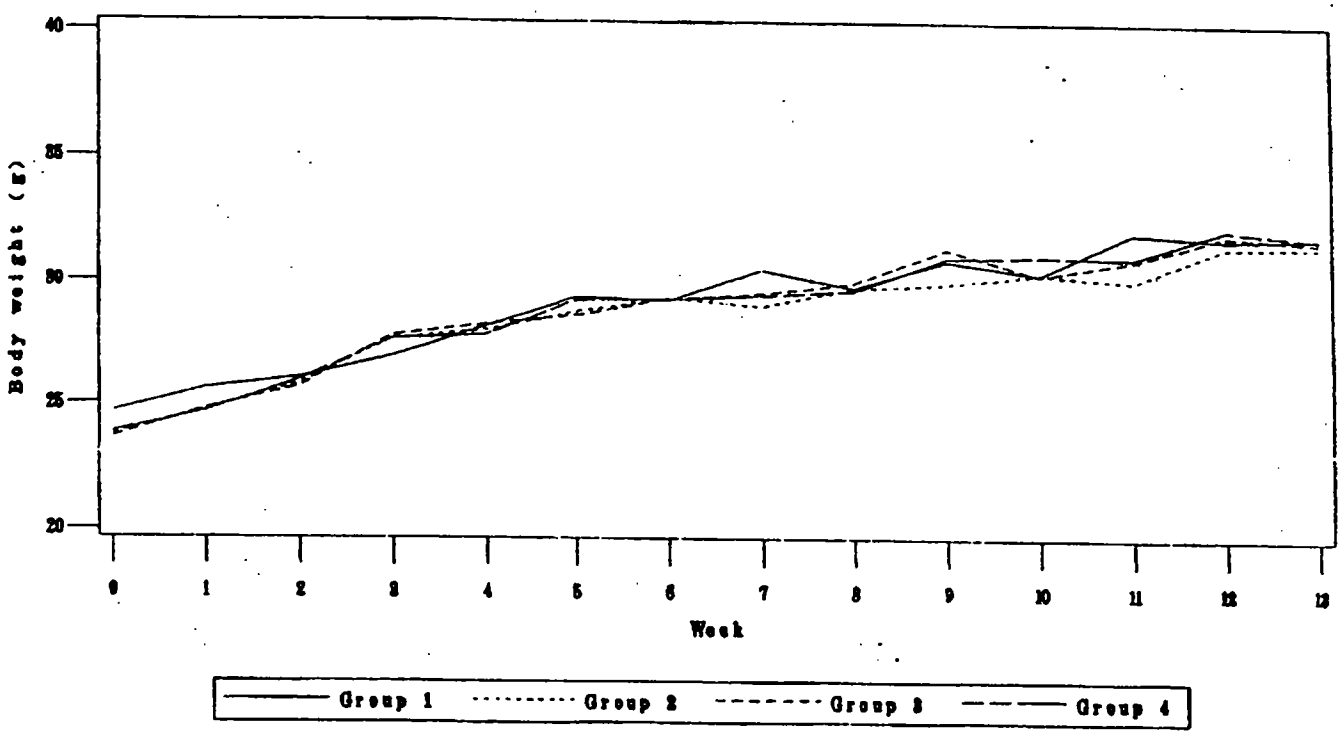


FIGURE 2
Females
Group mean body weight (g)

Test article	Control	LAB 31416		
Group	1	2	3	4
Level (mg/kg/day)	0	20	160	820



2. Rat (Vol 1.22)

In a 4-week study, doses were 0, 6, 60 and 600 mg/kg/day and in a 26-week study doses were 0, 20, 100 and 500 mg/kg/day. Both studies have been previously reviewed in the Initial Pharmacology Review [redacted] The main findings only at the highest doses in both sexes of both studies were increased alkaline phosphatase and increased liver weights; in the 26 week study, increased adrenal and kidney weights in both sexes and increased heart weight in females were also observed. At the 500 mg/kg/day dose of the 26 week study, histopathology changes included centrolobular hypertrophy or fibrosis and necrosis of the liver, thyroid cell follicular cell hypertrophy and lung pneumonitis. The NOAEL in the 4-week study was 60 mg/kg/day. The NOEL for the 26-week study was considered to be 100mg/kg/day; however at 100 mg/kg/day, liver fibrosis was seen in 1 female, liver necrosis was seen in 1 male and 1 female, none in controls or low dose. There were no effects on mortality, morbidity or weight gain in the 4 or 26-week studies. (See figures 1 and 2 that follow for body weights in the 26-week study.)

Toxicokinetic findings for all rat studies are summaarizedon pages 81-4.

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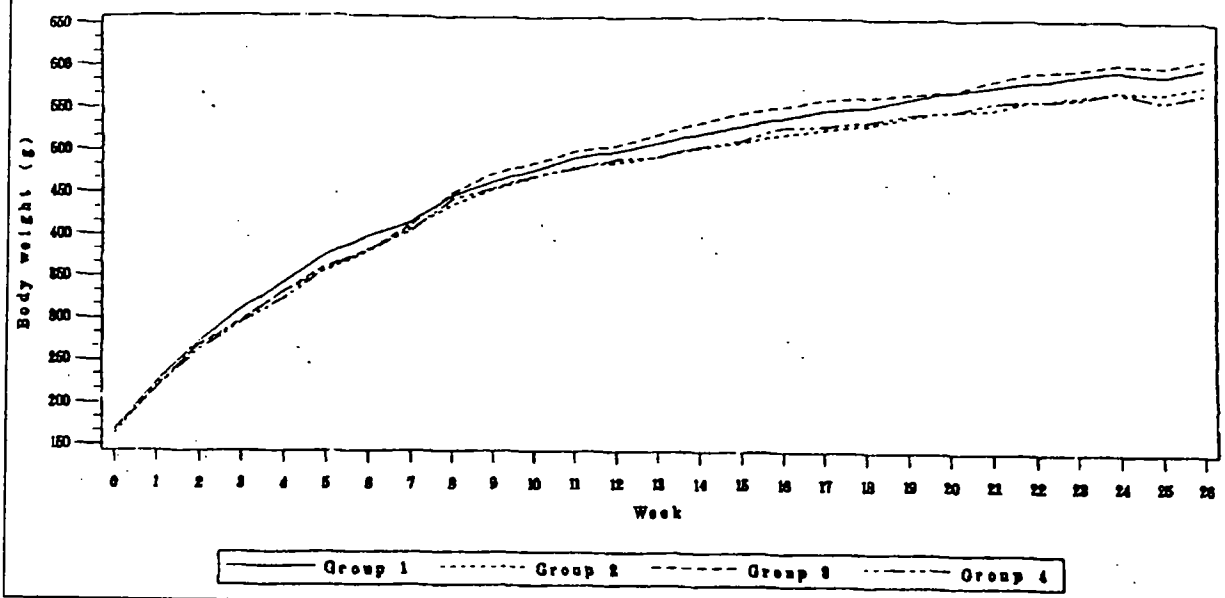
FIGURE 1
Males
Group mean body weight (g)

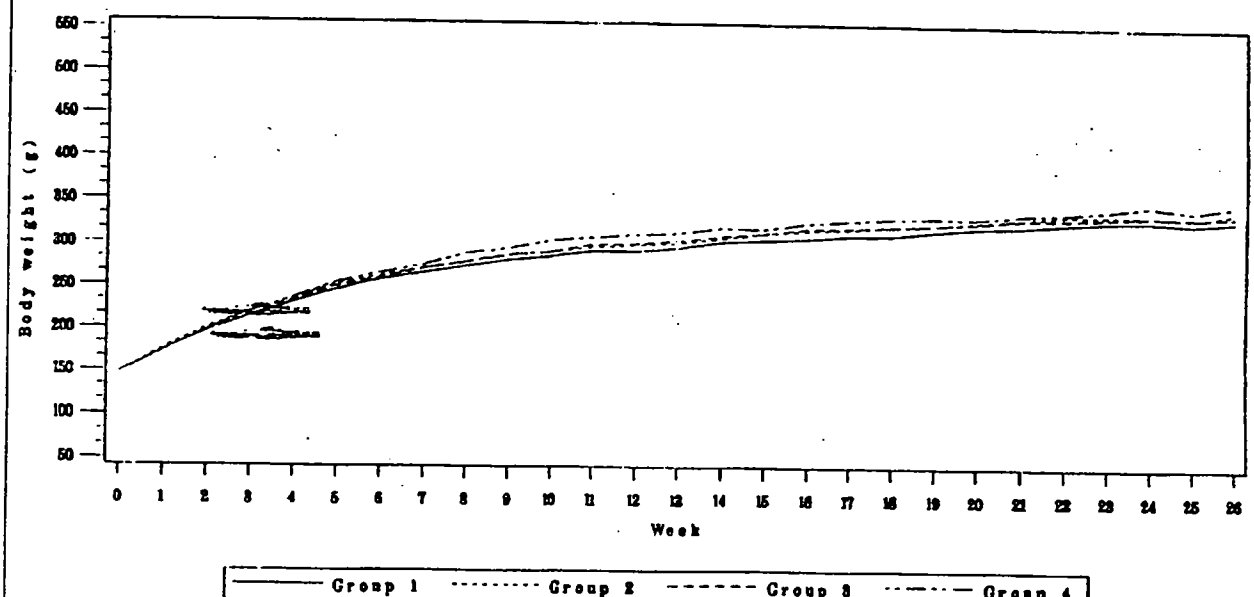
Test article	Control	LAS 31416		
Group	1	2	3	4
Level (mg/kg/day)	0	20	100	500



CHE Study Number 655147

FIGURE 2
Females
Group mean body weight (g)

Test article	Control	LAS 31416		
Group	1	2	3	4
Level (mg/kg/day)	0	20	100	500



CHE Study Number 655147

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3. Dog

Results of 4- and 26-week dog studies were reviewed in the Initial Review for IND [redacted]. The following 52-week study was previously reviewed for IND [redacted]

52-Week Oral Capsule Dog Study with 4 Week Recovery (Vol 1.28)

CLE Report No: 655/69-D6154

Performing Laboratory: [redacted]

Dates Performed: Initiated 11/13/96, completed 12/10/97.

Quality Assurance: A statement of compliance with GLP is included.

Doses: 0, 2, 5 and 12.5 mg/kg/day (dose level expressed in terms of base)

Rationale for Dose Selection: Based on previous 28 day and 26 week studies with dogs (CLE Study Nos. 655/33, 655/34 and 655/49). The high dose was expected to "produce target organ toxicity or non-specific toxicity". However, we find that the highest dose, 12.5 mg/kg/day in the 28 day and 26 week dog studies, elicited only a number of transient clinical signs, such as tachycardia, dilated pupils, splayed/stiff legs, abnormal gait, tremors, dry and warm nose, but no drug related effect on mortality, body weight or body weight gain, organ weights, gross or microscopic pathology.

Procedure: Beagles, 4/sex/group in the main study and an additional 2/sex in control and high dose group for the 5 week recovery study, 8-9 months of age, weighing 6.25-11.15 kg for males and 6.05-8.35 kg for females at the start of dosing, received almotriptan D,L-hydrogen malate (Lot no. K001) in gelatin capsules. The animals were examined daily for mortality and clinical signs, and were given detailed physical exams weekly; body weight was recorded weekly. Food consumption was measured daily. Each animals in all 4 groups was offered 400 g food per day, but from week 32 to termination, high dose males were given 500 g of food per day because 1 of the high dose males was losing weight. Ophthalmologic exams were performed on all animals at pre-treatment, weeks 25 and 51 of treatment and week 56 (after the treatment-free period). Electrocardiography was performed on all animals at pre-treatment and at approximately 1 and 24 hours after dosing on Day 2 (males) or Day 3 (females), and in Weeks 25 and 51; also in Week 55 after the treatment-free period. Blood pressure measurements from the ear artery were taken 1 hour after dosing on the same days as ECGs. Blood samples for toxicokinetics were taken from the jugular vein before dosing, 0.5, 1, 2, 4, 8, and 24 hours after dosing, on Day 1, Weeks 26 and 52. Blood samples for clinical pathology were obtained from all dogs after an 18 hour fast at pre-dosing, then before treatment at Weeks 13, 26, 31, 39, 52 and 56 for evaluation of effects on hematology (RBC, HB concentration, PCV, MCV,

MCHC, total and differential WBC and platelet count, PT, and APTT) and clinical chemistry (AST, ALT, alkaline phosphatase, GGT, Na, K, Ca, inorganic P, Cl, total protein, albumin, globulin, A/G, total cholesterol, glucose, urea, total bilirubin and creatinine). Urinalyses was conducted at pre-treatment, weeks 12 and 25 of treatment. Post-mortem included gross pathology, organ weights, and histopathology of all animals. The table that follows lists all organs that were fixed and examined for histopathology and specifies organs that were weighed before fixing to obtain mean absolute and relative (to final body weight) organ weights.

Adrenals		†	Oesophagus		
Aorta			Ovaries (x2)		†
Bone marrow smear (femur)	a,c		Pancreas		
Brain (x5)		†	Pituitary		†
Caecum			Prostate		†
Colon			Rectum		
Duodenum			Salivary glands		
Eyes and optic nerves	b		Sciatic nerves (x2)		
Femur with bone marrow and articular surface			Skin		
Gall bladder			Spinal cord cervical		
Gross lesions			Spinal cord lumbar		
Heart (x3)		†	Spinal cord thoracic		
Ileum			Spleen		†
Jejunum			Sternum with bone marrow		
Kidneys		†	Stomach (x3)		
Lacrimal glands			Testes + epididymides		†
Liver (x 2)		†	Thymus		
Lungs (x2) including mainstem bronchi			Thyroids + parathyroids		†
Mammary			Tongue		
Mandibular lymph nodes			Trachea		
Mesenteric lymph nodes			Urinary bladder		
Muscle			Uterus (x2)		†
			Vagina		

Fixative = 10% neutral buffered formalin except where indicated by:
a - methanol
b - Davidson's fluid
c - Examined by a hematologist for myeloid/erythroid ratios
A dagger denotes organs that were weighed before fixing

Results

Mortality: One female at high dose was unexpectedly found dead in Week 39. The animal had consumed all of its 400 g of food on the previous day but had not consumed any food on the day of its death (animal fed in morning and death occurred at 2:30 p.m.). A blood sample collected in the morning of the animal's death showed liver markers that were within normal range. Microscopic findings included hemorrhage in the myocardium and coronary groove, coronary arteritis and prominent vascular engorgement in other tissues, suggesting a pre-terminal cardiovascular event. The investigators claimed that there was no evidence of cardiovascular compromise in other

treated animals. Sponsor claimed, "The cause of death for this animal was not determined", but its relationship to treatment cannot be ruled out.

Clinical Signs: Dilated pupils were seen after dosing in treated animals of all 3 groups throughout the treatment period. Duration of this effect on each day was not indicated. Vocalization was frequently seen after dosing in all mid and high dose dogs throughout the treatment period, but the incidence tended to decrease after the first few weeks in most animals. Some low dose animals also vocalized after dosing. Splayed legs were seen in all high dose animals after dosing on some days during the first week, and in 1 male and 1 female at mid dose during one occasion at weeks 1 or 2. A high dose male also showed lack of coordination on all legs after dosing on one day in Week 1, and 2. Females were found to be "nervous" after dosing on Day 1.

Body Weight and Food Consumption: No discernable treatment related effect on body weight or body weight gains were evident, but mean food consumption of males and females tended to be higher in treated animals (dose related and statistically significant dose response in males at Weeks 1-4, 27-52 and 1-52).

Ophthalmoscopy: At Week 51, three males had "unilateral, slight opacities in the center of the cornea"; one dog at each of the 3 dose levels. The eyes of these dogs had been clear when examined at pre-treatment and at Week 25. The high dose treated dog with the opacity was in the recovery group, and at the end of the 4 week treatment free period, the opacity was no longer evident.

Cardiovascular: At Week 1 after dosing, rapid heart rate at 1 hour after dosing was observed in all mid and high dose animals throughout the treatment period; 1 male and 1 female at low dose was also occasionally observed with rapid heart rate ($P<0.05$ for dose response in females). The mean blood pressure of mid dose males was increased at Week 1 compared to control ($P<0.05$), but this was not seen at low and high doses. The group mean PR interval of low and high dose males and intermediate dose females were decreased compared to controls but these differences did not show a dose-related response and sponsor suggests that their significance to treatment is equivocal. The group mean QT interval of treated females was decreased compared to controls ($P<0.05$ to $P<0.01$ for all 3 treated groups). All of these intervals returned to baseline values by 24 hours after dosing. One male at mid dose showed the occurrence of ventricular premature beats/tachycardia 24 hours after dosing on Day 3, but this was not apparent in repeat traces of this animal in Week 4. Since this was not found in other animals and did show recurrence in the same animal, sponsor considered this to be of unlikely toxicological significance.

At Week 25, there were no clear differences in ECG intervals of treated males at 1 hour after dosing compared to pre-dose or controls. Heart rate of mid and high dose females were increased and PR and QT intervals decreased at 1 hour after dosing, compared to control values. However, they were not different compared to pre-dose values and sponsor considers their significance as equivocal. At 24 hours post dosing, heart rate was below pre-dose and control values for high dose males and females.

At Week 51, the group mean heart rate of treated females was increased and the group mean PR interval of mid and high dose females was decreased ($P<0.01$ for mid dose) at 1 hour after dosing compared to control, but were not different compared to pre-dose values; therefore sponsor considered the significance of these differences as equivocal. At 24 hours post-dosing, heart rate of mid and high dose females was decreased, compared to pre-dose and control values; however, heart rate of control females was also decreased compared to pre-dose values.

At the end of the treatment free period (Week 55), heart rate of high dose animals remained low compared to control and pre-dose values (Week –9). It should also be noted that in each of the time intervals when ECG measurements were taken, at 24 hours post dosing, there was usually a compensatory decrease in heart rate compared to controls, generally at mid and high doses and dose related, which reached statistical significance in males and females at high dose during Week 25

Results of toxicokinetics for all dog studies, including the present one, are found under the section on Toxicokinetics (See pages 81 and 83.

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TABLE 3

Group mean electrocardiography
Occasion: Week -9

Test article	Control	LAS 31416		
Group	1	2	3	4
Level (mg/kg/day)	0	2	5	12.5

Group and sex		Heart rate beats/min	Interval (ms) PR	QRS	QT	QTc
1M	Mean	125	99	44	208	303
	SD	11	9	5	20	37
2M	Mean	139	98	46	191	289
	SD	28	23	4	21	11
3M	Mean	140	94	40	181*	277
	SD	19	15	6	8	9
4M	Mean	137	98	39	191	286
	SD	35	10	4	18	18
				DR*		
Statistics		A2	A2	A2	A2	A2

Group and sex		Heart rate beats/min	Interval (ms) PR	QRS	QT	QTc
1F	Mean	142	95	38	188	290
	SD	23	8	5	13	13
2F	Mean	140	107	43	185	284
	SD	27	11	4	3	27
3F	Mean	152	76*	38	179	286
	SD	15	7	6	10	13
4F	Mean	132	91	36	186	275
	SD	29	8	4	18	16
Statistics		A2	A2	A2	A2	A2

* P<0.05
** P<0.01
*** P<0.001
DR = significant dose response test

A2 = two-way ANOVA, regression and Dunnett's

Note: P values and other statistical symbols noted above are the same for all tables pertaining to cardiovascular parameters that follow.

Group mean electrocardiography
Occasion: Week 1

Test article	Control	LAS 31416		
Group	1	2	3	4
Level (mg/kg/day)	0	2	5	12.5

1 hour after dosing

Group and sex		Heart rate beats/min	Interval (ms) PR	QRS	QT	QTc
1M	Mean	140	101	45	193	293
	SD	27	13	5	11	20
2M	Mean	176	86	46	178	305
	SD	33	12	4	10	24
3M	Mean	179	93	46	176	304
	SD	49	14	4	4	42
4M	Mean	185	84	49	191	336
	SD	21	9	14	18	20
			DR*			
Statistics		A2	A2	A2	A2	A2

24 hours after dosing

Group and sex		Heart rate beats/min	Interval (ms) PR	QRS	QT	QTc
1M	Mean	135	105	41	190	286
	SD	17	13	5	11	18
2M	Mean	141	91	45	185	280
	SD	42	11	4	6	32
3M	Mean	121	98	37	188	266
	SD	27	7	6	4	29
4M	Mean	121	98	41	211	295
	SD	45	14	7	21	49
Statistics		A2	A2	A2	A2	A2

Group mean electrocardiography
Occasion: Week 1

Test article	Control	LAS 31416			
Group	1	2	3	4	
Level (mg/kg/day)	0	2	5	12.5	**

1 hour after dosing

Group and sex		Heart rate beats/min	Interval (ms) PR	QRS	QT	QTc
1F	Mean	126	98	44	220	320
	SD	25	10	11	26	52
2F	Mean	141	106	43	192*	294
	SD	36	11	6	10	25
3F	Mean	183	73**	46	186**	325
	SD	42	16	4	16	23
4F	Mean	177	86	45	190**	322
	SD	60	13	10	14	48
		DR*				
Statistics		A2	A2	A2	A2	A2

24 hours after dosing

Group and sex		Heart rate beats/min	Interval (ms) PR	QRS	QT	QTc
1F	Mean	118	96	42	241	337
	SD	29	14	6	33	57
2F	Mean	100	95	44	203	263*
	SD	17	7	4	13	14
3F	Mean	110	86	46	208	281
	SD	13	5	4	11	15
4F	Mean	89	95	42	235	279
	SD	24	10	3	66	67
Statistics		A2	A2	A2	A2	A2

Group mean electrocardiography
Occasion: Week 25

Test article	Control	LAS 31416		
Group	1	2	3	4
Level (mg/kg/day)	0	2	5	12.5

1 hour after dosing

Group and sex		Heart rate beats/min	Interval (ms) PR	QRS	QT	QTc
1M	Mean	120	106	41	205	290
	SD	24	13	3	23	46
2M	Mean	137	97	44	190	283
	SD	43	10	7	15	24
3M	Mean	137	99	40	198	300
	SD	14	16	5	35	50
4M	Mean	144	99	40	193	297
	SD	36	13	4	19	33
Statistics		A2T	A2	A2	A2	A2

24 hours after dosing

Group and sex		Heart rate beats/min	Interval (ms) PR	QRS	QT	QTc
1M	Mean	130	102	43	195	283
	SD	31	13	6	15	33
2M	Mean	122	106	44	207	288
	SD	38	16	5	53	54
3M	Mean	113	96	39	190	260
	SD	31	6	7	8	43
4M	Mean	92*	104	40	228	274
	SD	32	18	3	37	30
Statistics		A2	A2	A2	A2	A2

Group mean electrocardiography
Occasion: Week 25

Test article	Control	LAS 31416		
Group	1	2	3	4
Level (mg/kg/day)	0	2	5	12.5

1 hour after dosing

Group and sex		Heart rate beats/min	Interval (ms) PR	QRS	QT	QTc
1F	Mean	111	105	41	224	304
	SD	14	11	2	17	28
2F	Mean	107	108	42	205	272
	SD	36	16	7	7	37
3F	Mean	149	85*	42	190*	299
	SD	20	5	4	13	17
4F	Mean	146	90	42	188*	285
	SD	56	6	6	20	26
Statistics		A2T	A2	A2	A2	A2

24 hours after dosing

Group and sex		Heart rate beats/min	Interval (ms) PR	QRS	QT	QTc
1F	Mean	125	101	43	192	276
	SD	19	7	2	8	18
2F	Mean	92	112	46	214	265
	SD	9	18	5	8	22
3F	Mean	90	92	51	230	279
	SD	21	9	13	33	42
4F	Mean	86*	92	40	224	264
	SD	16	13	2	57	45
Statistics		A2	A2	A2	A2	A2

Group mean electrocardiography
Occasion: Week 51

Test article	Control	LAS 31416		
Group	1	2	3	4
Level (mg/kg/day)	0	2	5	12.5

1 hour after dosing

Group and sex		Heart rate beats/min	Interval (ms) PR	QRS	QT	QTc
1M	Mean	121	106	49	231	327
	SD	23	9	4	35	41
2M	Mean	129	93	44	189*	273
	SD	46	13	4	11	34
3M	Mean	157	93	46	186*	300
	SD	33	12	6	7	35
4M	Mean	138	97	42*	203	306
	SD	25	14	4	40	46
Statistics		A2	A2	A2	A2	A2

24 hours after dosing

Group and sex		Heart rate beats/min	Interval (ms) PR	QRS	QT	QTc
1M	Mean	120	107	44	210	292
	SD	37	13	3	16	43
2M	Mean	121	97	45	236	334
	SD	37	10	6	35	61
3M	Mean	101	103	43	211	281
	SD	33	12	7	20	65
4M	Mean	102	109	42	245	317
	SD	26	17	3	43	58
Statistics		A2	A2	A2	A2	A2

Group mean electrocardiography
Occasion: Week 51

Test article	Control	LAS 31416		
Group	1	2	3	4
Level (mg/kg/day)	0	2	5	12.5

1 hour after dosing

Group and sex		Heart rate beats/min	Interval (ms) PR	QRS	QT	QTc
1F	Mean	119	110	43	216	304
	SD	26	10	3	18	36
2F	Mean	141	109	46	203	312
	SD	33	21	5	20	59
3F	Mean	161	79**	45	194	318
	SD	26	11	4	28	44
4F	Mean	142	94	45	195	301
	SD	26	6	5	16	38
Statistics		A2	A2	A2	A2	A2

24 hours after dosing

Group and sex		Heart rate beats/min	Interval (ms) PR	QRS	QT	QTc
1F	Mean	106	106	44	264	347
	SD	22	13	5	58	57
2F	Mean	94	111	47	201*	252*
	SD	8	15	7	8	15
3F	Mean	75	95	43	220	251*
	SD	14	10	2	2	22
4F	Mean	81	98	44	237	274
	SD	11	10	5	39	39
Statistics		A2	A2	A2	A2	A2

Group mean electrocardiography
Occasion: Week 55

Test article	Control	LAS 31416		
Group	1	2	3	4
Level (mg/kg/day)	0	2	5	12.5

Group and sex		Heart rate beats/min	Interval (ms) PR	QRS	QT	QTc
1M	Mean	138	99	41	222	335
4M	Mean	66	113	46	218	227

Group and sex		Heart rate beats/min	Interval (ms) PR	QRS	QT	QTc
1F	Mean	137	109	40	227	346
4F	Mean	99	101	42	202	258

Ophthalmology: Dilated pupils were noted, as described above under clinical signs. At week 51, 3 males (1 low dose, 1 mid dose and 1 high dose) had corneal opacities in the center of the cornea. The eyes of all 3 animals had been found to be clear at pre-dose and Week 25. The high dose dog with corneal opacity was in the reversibility group and the opacity seen at Week 51 was no longer apparent 4 weeks after the treatment-free period.

Clinical Pathology: There were no consistent compound related effects on hematology or clinical chemistry that could be attributed to compound treatment.

Postmortem

Organ Weights: At high dose, mean uterine weight was increased around 2-fold higher than control, but this was considered due to animals in estrus and not compound related. An increase in mean thyroid weight at mid dose was considered to be incidental, not accompanied by histopathology.

Pathology: There were no gross or microscopic pathology findings that could be attributed ~~to treatment~~.

Toxicokinetics: Summarized on page 83.

C. Carcinogenicity Studies

1. 104/95 Week Oral Gavage Rat Carcinogenicity Study (Vol 1.43-1.46)

CLE Report No: 655/68-D6154

Performing Laboratory: [redacted]

Dates Performed: Treatment initiated 9/27/96, necropsies completed 7/30/98 (females) and 10/2/98 (males).

Quality Assurance: A statement of compliance with GLP is included.

Doses: 0 (control), 10, 27 and 75 mg/kg/day with 2 control groups that received vehicle (dose level expressed in terms of base). The dose volumes were 10 mL/kg, based on individual body weight. Vehicle used was purified water.

Rationale for Dose Selection: The doses selected were based on the AUC option. For high dose, C_{max} was expected to be 50-times that achieved at human therapeutic dose level and AUC was expected to be ≥25-times that achieved at human therapeutic dose level. The low dose was expected to achieve an AUC >2-times that achieved in humans.

Procedure: Crl:CDBR strain rats (Charles River, UK), 70/sex/group in the main study groups and 10/sex in the satellite groups (satellite study had 1 vehicle control and 3 drug treated groups for toxicokinetics), were used. Rats were 6 weeks of age, mean weights of 191.6-263.2 g males and 137.4-208.5 g females) at the start of dosing. They received LAS 31416 (DL hydrogen malate) (Lot no. K001; purity not specified) once daily by oral gavage. The animals were housed 5 per cage. All animals were examined daily for mortality and clinical signs and each week they received a detailed clinical examination, including palpation. Moribund animals were killed and necropsied. Weight and food consumption for all animals were recorded weekly during the first 16 weeks, then every 4 weeks thereafter. Hematology (red and white blood counts) was performed on surviving animals at termination and on decedent animals prior to death, if possible; from the abdominal aorta blood samples. Postmortem of main study animals included gross pathology examination of all decedent and surviving animals, histopathology of tissues listed below of all control and high dose animals and decedent animals of all 4 groups. The females were terminated at week 96 because of high mortality in all 5 groups, including the 2 control groups.

Tail vein blood samples from satellite group animals were collected from 3/sex at week 26, 52 and 97 (females) or 104 (males) at 1, 3, 6 and 24 hours after dosing, for determination of plasma drug concentrations. After collection of blood samples, satellite animals were discarded without examination. Results of toxicokinetics for all rodent animal studies are found under the section on Toxicokinetics on page 81.

Adrenals		Š
Animal identification		
Aorta		
Brain		Š
Caecum		Š
Colon		Š
Duodenum		Š
Eyes		Š
Femur with bone marrow and articular surface		
Gross lesions		Š
Harderian glands	d	
Head		
Heart		Š
Ileum		Š
Jejunum		Š
Kidneys		Š
Lacrimal glands	d	
Larynx		Š
Liver		Š
Lungs with mainstem bronchi		Š
Mammary	f	Š
Mandibular lymph nodes		Š
Mesenteric lymph nodes		Š
Muscle (quadriceps)		Š
Nasal Turbinates	d	
Nasopharynx	d	
Oesophagus		Š

Optic nerves		Š
Ovaries		Š
Pancreas		Š
Pituitary		Š
Prostate		Š
Rectum		Š
Salivary glands		Š
Sciatic nerves		Š
Seminal vesicles		Š
Skin		Š
Spinal cord cervical		Š
Spinal cord lumbar		Š
Spinal cord thoracic		Š
Spleen		Š
Sternum with bone marrow		Š
Stomach		Š
Testes + epididymides		Š
Tissue masses		Š
Thymus		Š
Thyroids + parathyroids		Š
Tongue		
Trachea		Š
Urinary bladder		Š
Uterus		Š
Vagina		Š
Zymbal glands	d	

d - preserved with the head in situ
f - female only

Histopathology was performed on all tissues denoted by Š from:

-control and high dose animals

-decedents

-all gross lesions and tissue masses from all animals

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Survival/Mortality: See table below and sponsor's Figures, 1 and 2 which follow. The following is sponsor's summary of survival during the 104-week study period.

Group number	Group description	Dose level (mg/kg/day)	Survival	
			Male	Female
1	control I	0	34 (49)	30 (43)
2	Low	10	40 (57)	21 (30)
3	Intermediate	27	35 (50)	25 (36)
4	High	75	32 (46)	30 (43)
5	control II	0	46 (66)	25 (36)

Percentage survival presented in parentheses

Survival among the high dose males was lower than the combined control groups ($P<0.05$). This difference is considered to have arisen due to high survival in male control group II. There was no difference in survival between control group I and high dose males; therefore sponsor considers this finding to be of no toxicological significance. Among females, there were no statistically significant differences in survival between groups. In summary, there was no obvious adverse effect of administration of LAS 31416 on mortality. Additionally, there was no effect on the incidence or nature of the masses observed by palpation nor on morbidity.

Body Weight: See sponsor's Figures 3 and 4 and Table 4.2 which follows. An initial increase in mean body weight gain (around 10%; $P<0.001$) was seen in high dose females, limited to the first 13 weeks of treatment and was not seen as dosing continued. In males, there was a dose-related decrease in mean body weight gain (13.6% for high dose vs. control), between Weeks 24 and 52. Overall, there were little or no differences in mean body weights between control and treated groups during the course of the study.

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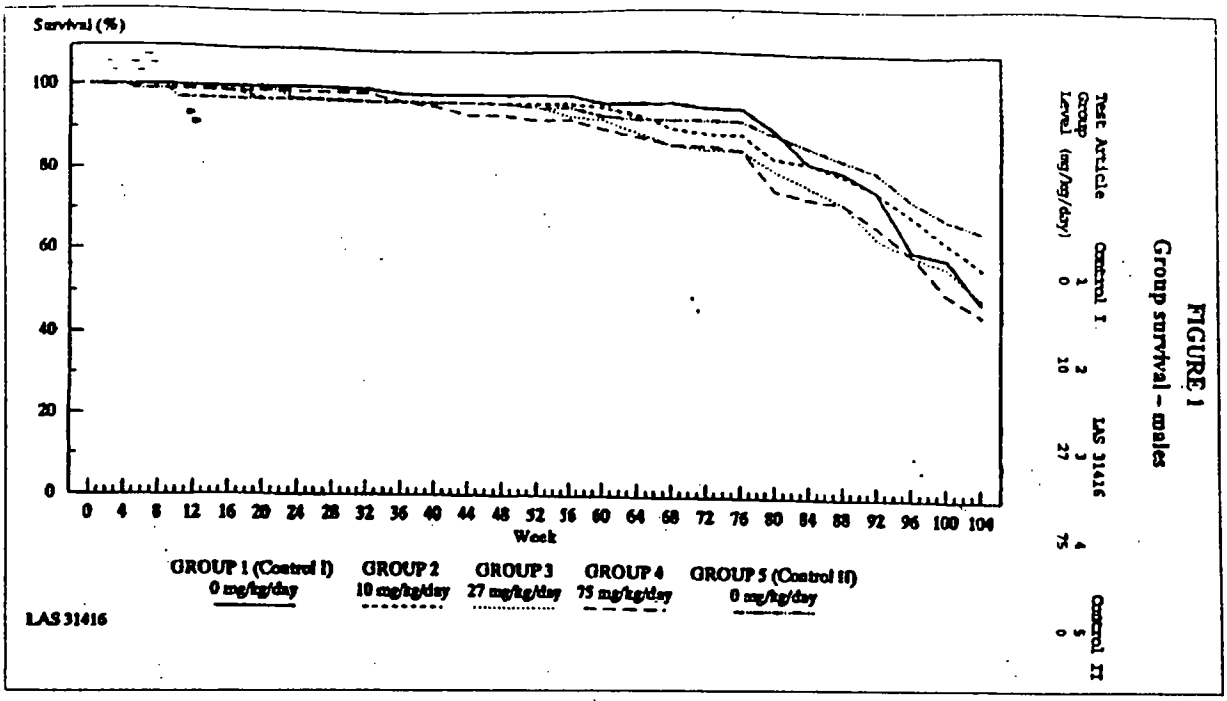

FIGURE 1
Group survival – males

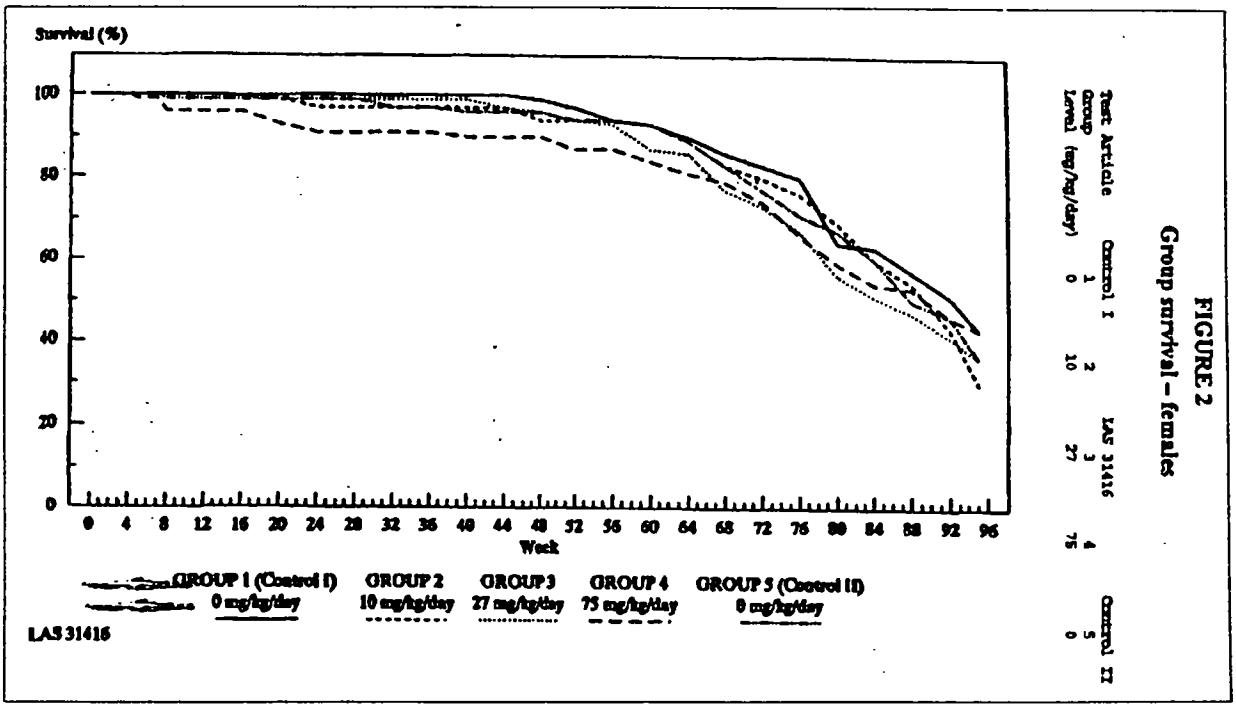

FIGURE 2
Group survival – females

TABLE 4.2

Group mean body weight gains

Test Article	Control I		LAS 31416		Control II
Group	1	2	3	4	5
Level (mg/kg/day)	0	10	27	75	0

Week of study		Group mean body weight gain (g) for : 1M	2M	3M	4M	5M	Statistics
0 to 13	Mean	281.5	292.4	292.7	292.9	283.1	A
	SD	43.64	44.64	46.17	43.44	41.09	
0 to 104	Mean	543.4	516.0	524.9	506.9	517.8	A
	SD	91.96	119.18	95.33	100.78	100.98	
13 to 24	Mean	80.9	84.8	85.8	85.2	85.2	A
	SD	21.98	23.46	21.39	20.48	19.88	
24 to 52	Mean	114.9	98.8	99.2	96.0	99.3	DR* A
	SD	31.34	43.31	34.29	34.10	34.37	
52 to 76	Mean	37.6	39.0	32.2	37.1	39.3	A
	SD	49.39	37.83	42.24	29.85	38.50	
76 to 104	Mean	31.7	5.5	20.4	1.3	5.1	A
	SD	66.30	64.66	46.55	66.00	74.80	

TABLE 4.2

Group mean body weight gains

Test Article	Control I		LAS 31416		Control II
Group	1	2	3	4	5
Level (mg/kg/day)	0	10	27	75	0

Week of study		Group mean body weight gain (g) for: 1F	2F	3F	4F	5F	Statistics
0 to 13	Mean	115.9	127.1	125.2	132.8***	124.9	A
	SD	19.52	22.15	19.79	19.02	20.31	
0 to 92	Mean	331.1	316.7	313.0	358.1	348.4	A
	SD	73.35	97.14	79.16	70.49	86.42	
13 to 24	Mean	31.1	31.3	28.2	29.0	30.1	A
	SD	11.33	14.80	12.86	13.30	15.27	
24 to 52	Mean	90.1	89.9	84.9	89.8	98.1	A
	SD	41.29	43.14	35.51	35.84	42.02	
52 to 76	Mean	75.9	68.0	67.9	84.3	68.1	A
	SD	38.15	39.25	31.12	25.82	38.87	
76 to 92	Mean	29.9	-2.9	27.4	45.1	28.3	J
	SD	37.90	83.53	37.93	28.23	41.06	

* P<0.05
** P<0.01
*** P<0.001

A = ANOVA, regression and Dunnett's
J = Kruskal-Wallis, Terpstra-Jonckheere, Wilcoxon

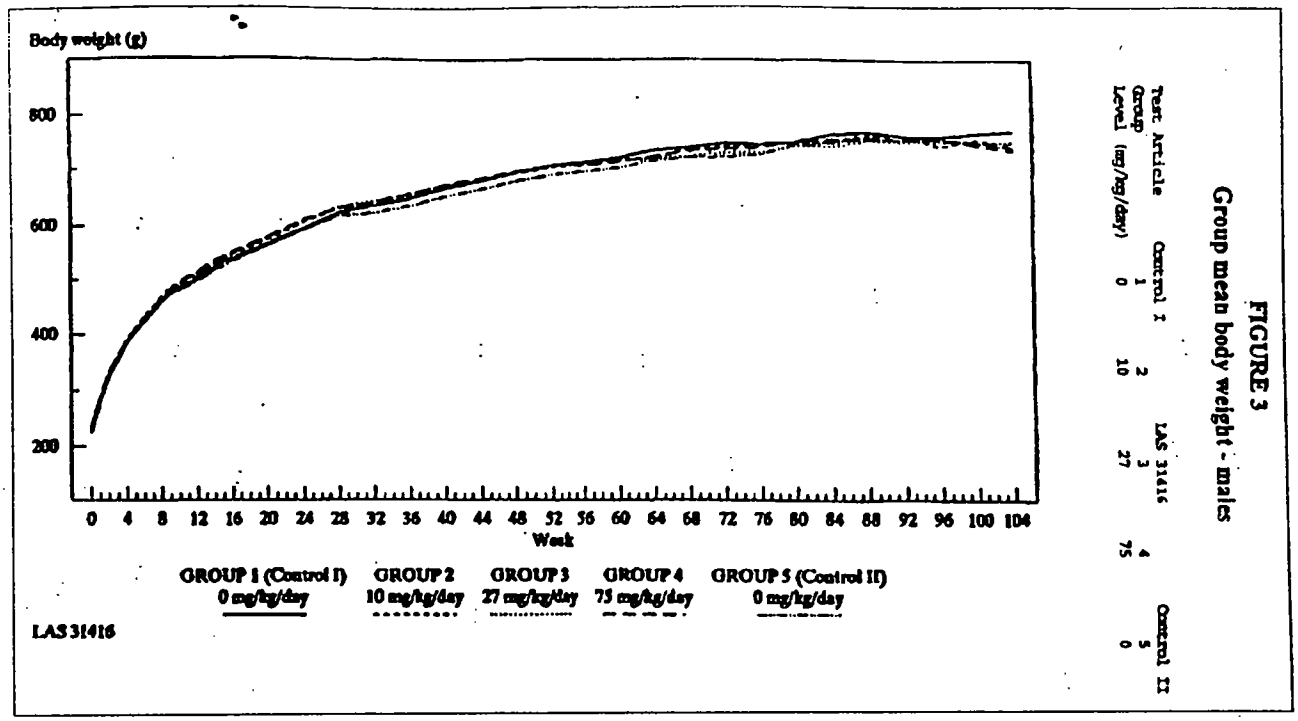

FIGURE 3

Group mean body weight - males

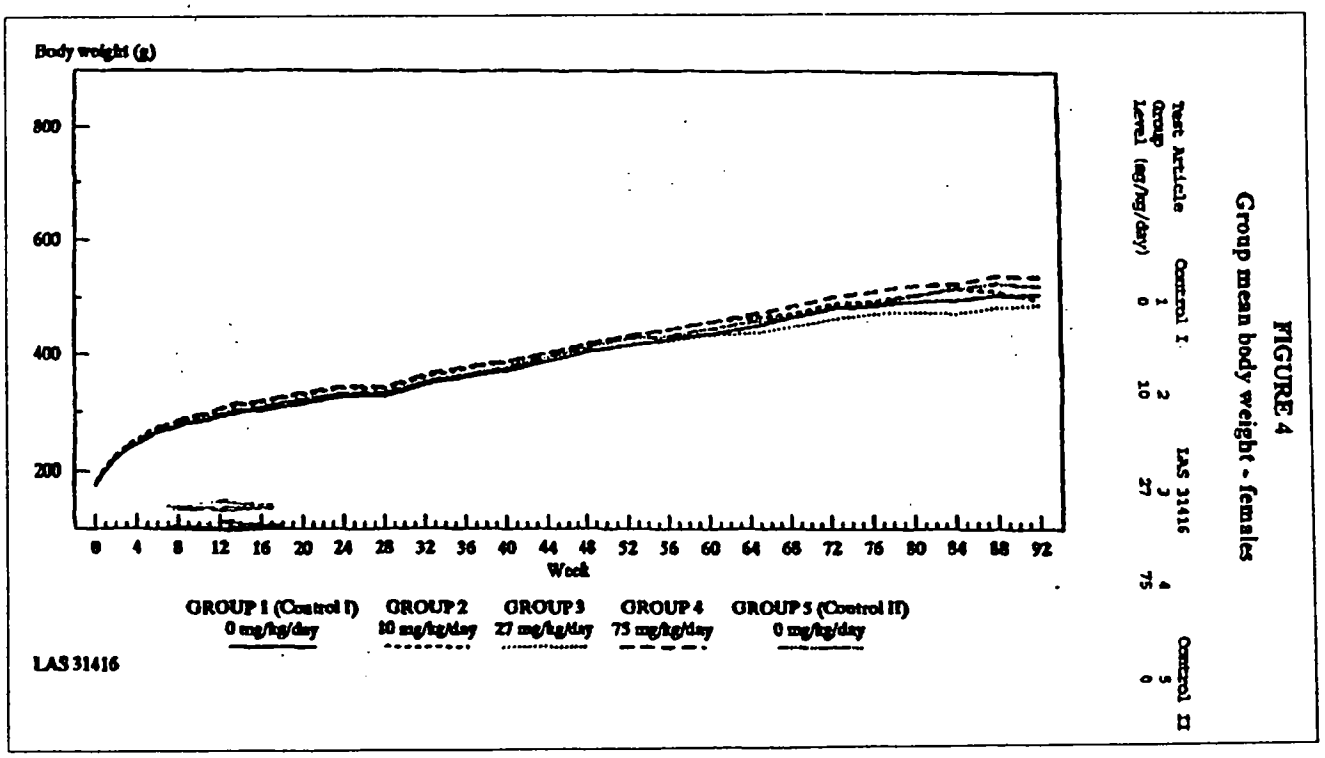

FIGURE 4

Group mean body weight - females

Pathology: There were no treatment-related effects on gross pathology. An inter-group comparison of tumor incidence in male and female rats is found in table 7 that follows. A complete summary of tumor incidence in decedents and terminal kill combined is found in Appendix II. There were no statistically significant differences between the incidences of tumors in high dose males or females compared to the 2 controls combined for each sex. There were some differences in tumor incidence between the 2 control groups; lymph node mesenteric lymphangioma in males ($P<0.044$) and skin/appendage fibroblastic tumors in females ($P<0.015$). For fatal and non-fatal skin/appendage fibroblastic tumors combined, sponsor indicated there was no significant difference in incidence between high dose and control groups 1 ($P>0.05$), but there was some evidence of an increase in high dose compared with control group 5 ($P<0.014$). See page 41. However, FDA guidelines recommends a significance level of $P<0.01$ for differences in common tumors.

Non-Tumor Histopathology: At the doses tested, there were no indications of any specific organ histopathology related to compound treatment.

The investigators concluded that there was no evidence of a change in incidence of any tumor nor the occurrence of an unusual tumor type to indicate that the drug was carcinogenic.

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