TABLE 7

Inter group comparison of tumour incidence

KEY FOR TABLES

Tumours of similar histogenic origin were merged, as requested by the Pathologist.

Tumour type	Tissue/findings
Adrenal cortex adenoma	Adrenal cortex benign tumour adenoma
HLR leukaemia granulocytic	Haem/Lymph/Retic leukaemia granulocytic
Lungs bronchiolo-alveolar adenoma	Lungs bronchiolo-alveolar benign tumour adenoma
Lymph node mesenteric lymphangioma	Lymph node: mesenteric benign tumour lymphangioma
Nasal cavity/head squamous cell carcinoma	Nasal cavity/head malignant tumour squamous cell carcinoma
Pancreas islet cell adenoma	Pancreas islet cell benign tumour adenoma
Skin hair follicle tumours	Skin: lesion benign tumour hair follicle tumour
Skin sebaceous cell adenoma	Skin: lesion benign tumour sebaceous cell adenoma
Skin squamous cell papilloma	Skin: lesion benign tumour squamous cell papilloma
Subcutaneous tissue/dermis lipoma	Sub-cutaneous tissue/dermis benign tumour lipoma
Testes interstitial cell adenoma	Testes interstitial cell benign tumour adenoma
Thymus thymoma	Thymus benign tumour thymoma
Thyroid gland follicular adenoma	Thyroid gland follicular benign tumour adenoma
Adrenal medullary tumours	Adrenal medulla benign tumour phaeochromocytoma
	Adrenal medulla malignant tumour phaeochromocytoma
Glial cell tumours	Spinal cord malignant tumour astrocytoma
	Brain malignant tumour astrocytoma
Blood vessel tumours	Haemangioma – all sites
Histiocytic sarcoma	Histiocytic sarcoma – all sites
HLR lymphoid tumours	Haem/Lymph/Retic lymphoma – lymphoblastic
	Haem/Lymph/Retic lymphoma – large granular lymphocyte
	Haem/Lymph/Retic lymphoma – follicle centre cell
Mammary gland epithelial tumours	Mammary gland benign tumour adenoma
	Mammary gland benign tumour fibroadenoma
	Mammary gland malignant tumour adenocarcinoma
Pituitary tumours	Pituitary pars anterior benign tumour adenoma
	Pituitary pars anterior malignant tumour carcinoma
Skin/appendage fibroblastic tumours	Sub-cutaneous tissue/dermis benign tumour fibroma
	Sub-cutaneous tissue/dermis malignant tumour fibrosarcoma
Thyroid C-cell tumours	Thyroid gland C-cell benign tumour adenoma
	Thyroid gland C-cell malignant tumour carcinoma
Uterus & cervix stromal tumours	Uterus & cervix benign tumour stromal polyp
	Uterus & cervix malignant tumour stromal sarcoma

TABLE 7.1

Inter group comparison of tumour incidence

Tumour incidence in males: groups 1, 4 and 5 analysed
Results of tests for increasing and decreasing dose response

Tumour type		Number of tumour bearing animals in Group 1	4	5	Included in analysis	Groups 1v5	Groups 1>5	Tests
Adrenal cortex adenoma	NF	0	2	1	NF	NS	NS	P
HLR leukaemia granulocytic	NF	0	0	1				
	F	2	0	0				
	ALL	2	0	1	ALL	NS	NS	P
Lungs bronchiolo-alveolar adenoma	NF	0	1	2	NF	NS	NS	P
Lymph node mesenteric lymphangioma	NF	6	7	15	NF	P=.044*	NS	L
Pancreas islet cell adenoma	NF	6	6	4	NF	NS	NS	L
Skin hair follicle tumours	NF	2	0	2	NF	NS	NS	P
Skin sebaceous cell adenoma	NF	1	1	1	NF	NS	NS	P
Skin squamous cell papilloma	NF	3	3	1	NF	NS	NS	P
Subcutaneous tissue/dermis lipoma	NF	1	1	2				
	F	1	0	0				
	ALL	2	1	2	ALL	NS	NS	P
Testes interstitial cell adenoma	NF	3	5	7	NF	NS	NS	L
Thymus thymoma	NF	1	1	1	NF	NS	NS	P
Thyroid gland follicular adenoma	NF	2	0	1	NF	NS	NS	P
Adrenal medullary tumours	NF	9	8	13	NF	NS	NS	L
Glial cell tumours	NF	0	1	1				
	F	0	0	1				
	ALL	0	1	2	ALL	NS	NS	P
Blood vessel tumours	NF	3	3	1	NF	NS	NS	P
Histiocytic sarcoma	NF	1	1	2				
	F	1	0	0				
	ALL	2	1	2	ALL	NS	NS	P
Pituitary tumours	NF	21	30	32				
	F	14	12	5				
	ALL	35	42	37	ALL	NS	NS	L
Skin/appendage fibroblastic tumours	NF	10	7	10				
	F	3	4	2				
	ALL	13	11	12	ALL	NS	NS	L
Thyroid C-cell tumours	NF	11	7	6	NF	NS	NS	L

F = fatal
NF = non-fatal
L = large sample tests
P = permutational tests

NS = not significant for increasing or decreasing dose response (P>0.05)
* = p<.05
** = p<.01
*** = p<.001

TABLE 7.2

Inter group comparison of tumour incidence

Tumour incidence in females: groups 1, 4 and 5 analysed
Results of tests for increasing and decreasing dose response

Tumour type		Number of tumour bearing Animals in Group 1	4	5	Included in analysis	Groups 1v5	Groups 1+5v4	Tests
Nasal cavity/head squamous cell carcinoma	NF	1	0	0				
	F	0	2	0				
	ALL	1	2	0	ALL	NS	NS	P
Pancreas islet cell adenoma	NF	4	2	3	NF	NS	NS	P
Thymus thymoma	NF	3	2	2	NF	NS	NS	P
Thyroid gland follicular adenoma	NF	1	1	1	NF	NS	NS	P
Adrenal medullary tumours	NF	0	3	1	NF	NS	NS	P
Histiocytic sarcoma	NF	1	0	0				
	F	1	0	1				
	ALL	2	0	1	ALL	NS	NS	P
HLR lymphoid tumours	F	1	2	1	F	NS	NS	P
Mammary gland epithelial tumours	NF	23	26	22				
	F	17	8	20				
	ALL	40	34	42	ALL	NS	NS	L
Pituitary tumours	NF	36	22	28				
	F	15	18	17				
	ALL	51	40	45	ALL	NS	NS	L
Skin/appendage fibroblastic tumours	NF	2	3	0				
	F	4	1	0				
	ALL	6	4	0	ALL	P=.015 *	NS	L
Thyroid C-cell tumours	NF	9	8	14	NF	NS	NS	L
Uterus & cervix stromal tumours	NF	6	9	6				
	F	0	0	1				
	ALL	6	9	7	ALL	NS	NS	L

F = fatal
NF = non-fatal
L = large sample tests
P = permutational tests

NS = not significant for increasing or decreasing dose response (P>0.05)
* = P<.05
** = p<.01
*** = P<.001

2. 96/103 Week Oral Gavage Mouse Carcinogenicity Study (Vol 1.37-1.40)

CLE Report No: 655/67-D6154

Performing Laboratory: [redacted]

Dates Performed: Treatment initiated 2/5/97, necropsies completed 2/2/99.

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

Doses: 0 (control), 40, 100 and 250 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 at least 25-times that achieved at human therapeutic dose level and AUC was expected to be $\geq$25-times that achieved at human therapeutic dose level (12.5 mg/day). The low dose was expected to achieve an AUC of 1 to 2-times that achieved in humans.

Procedure: Crl:CD-1(ICR)BR strain rats (Charles River, UK), 51/sex/group in the main study groups and 15/sex in the satellite groups (consisting of the 3 drug treated and group 1 controls for toxicokinetics), 6 weeks of age (mean body weights of 22.2-38.4 g for males and 16.5-28.5 g for females) at the start of dosing), received LAS 31416 (DL hydrogen malate) (Batch no. K001; purity specified as 71.0% base and 28.8% acid) by once daily oral gavage. The animals were housed 3 per cage. All animals were examined daily for mortality and clinical signs and each week they received a detailed clinical examination, including palpation. Animals showing poor health were isolated and moribund animals were killed and necropsied. Body 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 before death if possible; from the abdominal aorta blood samples. Blood films for differential counts were prepared but not examined. 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.

Group number	Group description	Dose level (mg/kg/day)	Survival	
			Male	Female
1	control I	0	18 (35)	25 (49)
2	Low	40	15 (29)	21 (41)
3	Intermediate	100	16 (31)	21 (41)
4	High	250	18 (35)	16 (31)
5	control II	0	20 (39)	26 (51)

Percentage survival presented in parentheses

In males, there was no significant difference in mortality between Group 1 and Group 5 ($P>0.05$). There was no significant dose-response in mortality across groups ($P>0.05$). There was some evidence that the mortality in Group 2 males was higher than that in the combined control groups (unadjusted $P=0.038$; $P>0.05$ after Bonferroni adjustment); however, the nature and incidence of the causes of morbidity and mortality in controls and treated animals were similar, and consistent with the expected profile in this strain of mouse. The mortality in Groups 3 and 4 were not significantly different from that in the combined control groups ($P>0.05$ for both comparisons).

In females, there was no difference in mortality between control groups 1 and 5. There was evidence of an increasing dose-response in mortality across female groups ($P<0.001$). The mortality in groups 2 and 3 was not significantly different from that in the combined control groups ($P>0.05$ for both comparisons). The mortality in group 4 was significantly higher than that in the combined control groups ($P<0.001$). The nature and incidence of causes of morbidity and mortality in control and treated animals were similar, and consistent with expected profile in this strain of mouse.

Sponsor concluded that there was no adverse effect of administration of LAS 31416 on the incidence or nature of the causes of morbidity and mortality. The executive-CAC that met on 7/18/00 concluded that the MTD for female mice was 250 mg/kg/day, based on mortality.

Body weight: See sponsor's Figures 3 & 4, Table 4.2 which follows. There was no evidence of an effect of treatment on mean body weight or body weight gain. There was a slight increase in the group mean body weight gain of treated male animals ($P<0.05$ using the dose response test; table 4.2) which was not considered to be of toxicological significance.

FIGURE 1

Group survival – males

Test Article	Control I		LAS 31416		Control II
Group	1	2	3	4	5
Level (mg/kg/day)	0	40	100	250	0

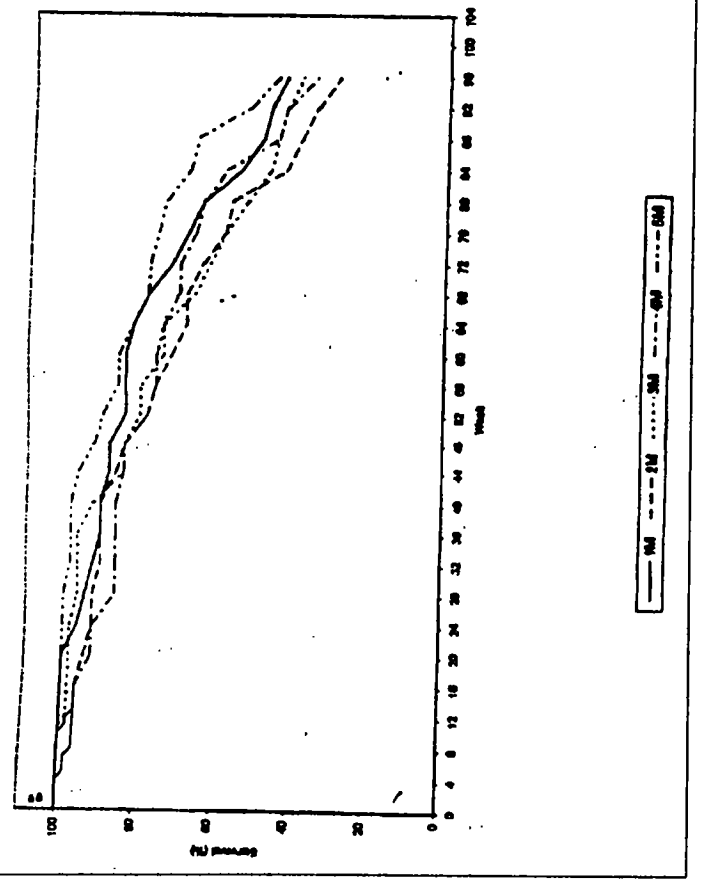

FIGURE 2

Group survival – females

Test Article	Control I		LAS 31416		Control II
Group	1	2	3	4	5
Level (mg/kg/day)	0	40	100	250	0

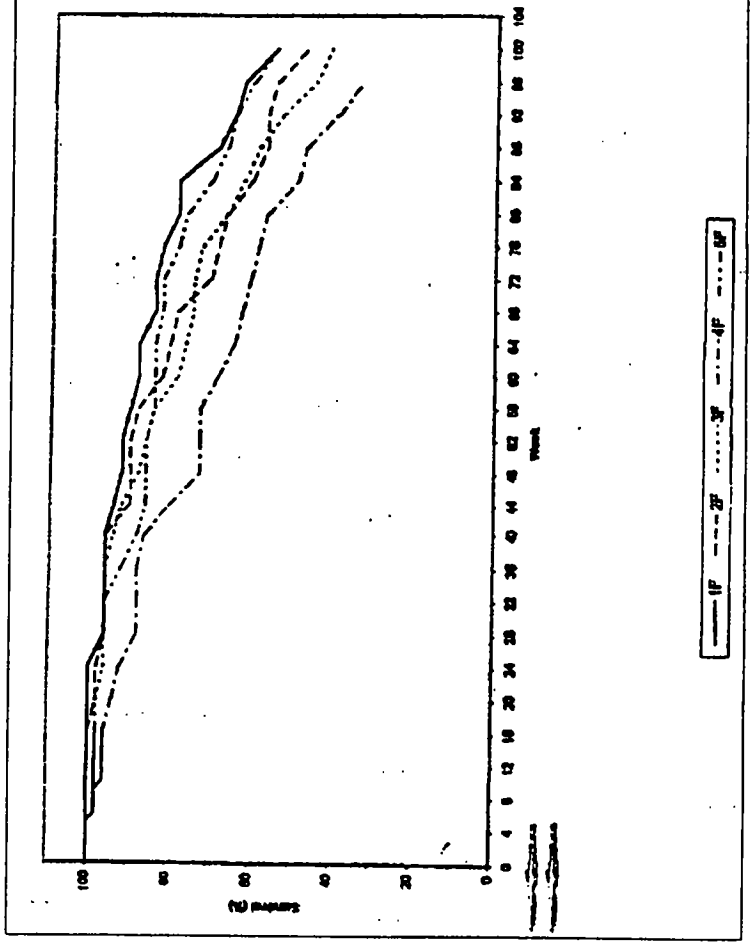

FIGURE 3

Group mean body weight - males

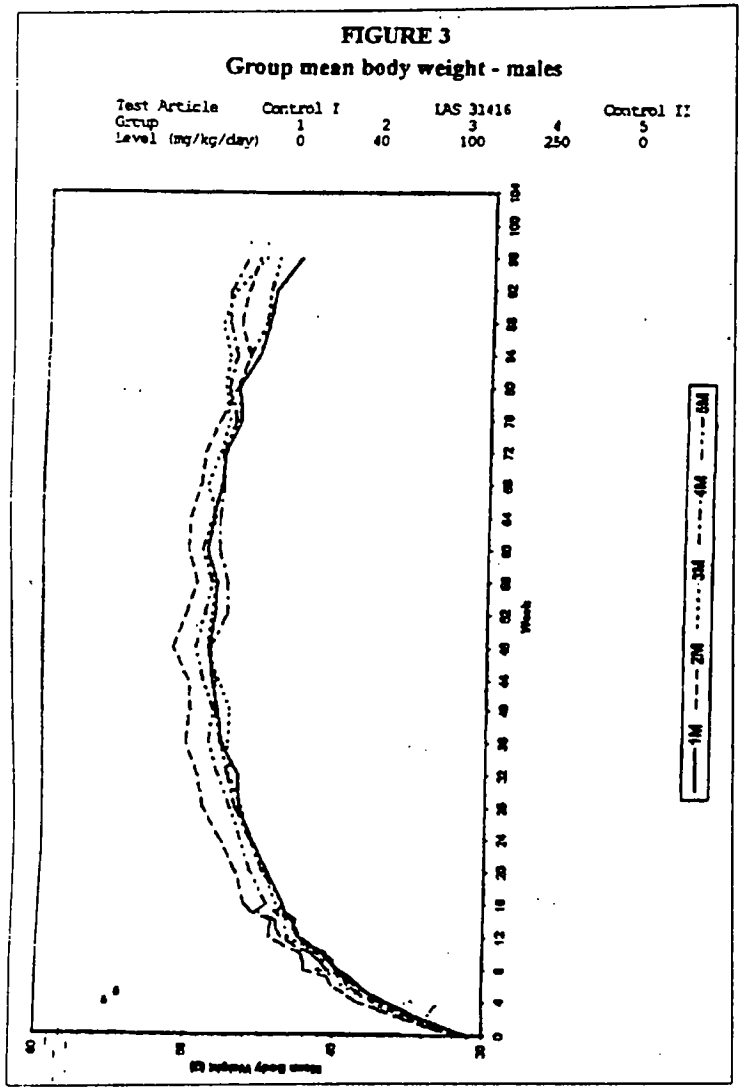

FIGURE 4

Group mean body weight - females

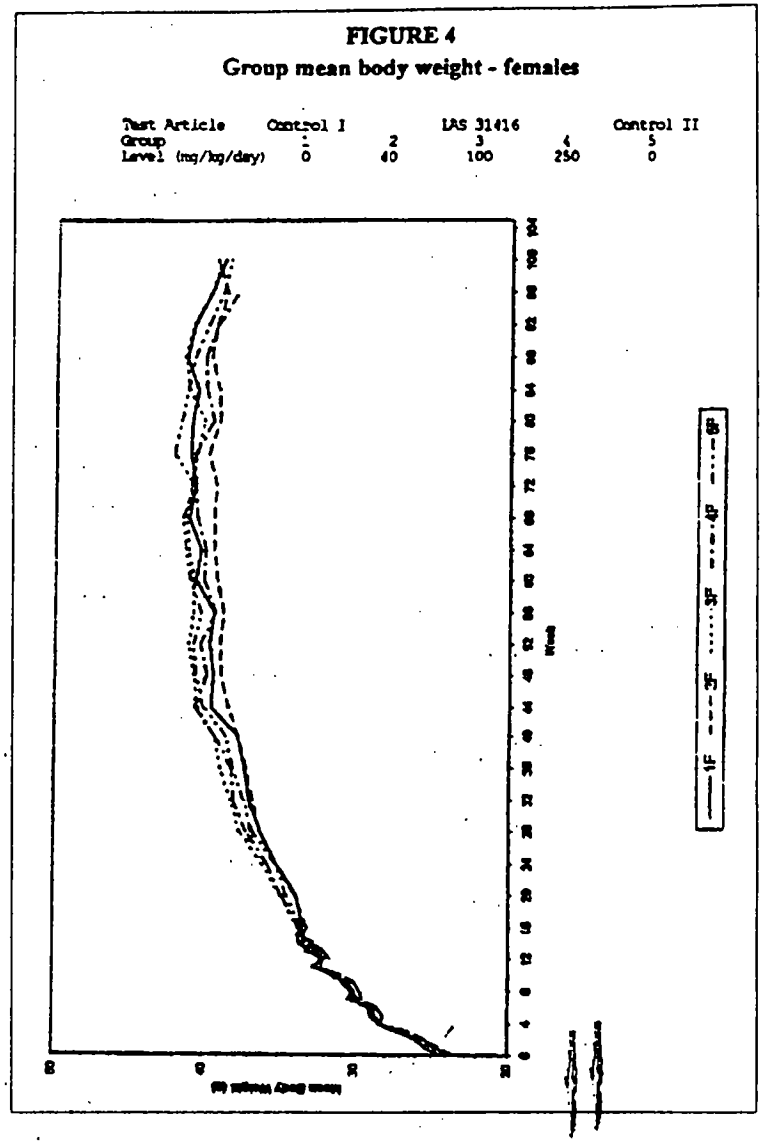

TABLE 4.2

Group mean body weight gains

Test article	Control I		LAS 31416		Control II
Group	1	2	3	4	5
Level (mg/kg/day)	0	40	100	250	0

Week to Week of study		Mean body weight gains (g) for Group: 1M	2M	3M	4M	5M	Statistics
0 to 13	Mean	11.7	12.7	11.9	11.6	12.9	A
	SD	2.50	3.17	2.48	2.54	3.67	
0 to 96	Mean	12.3	14.2	14.5	15.0	13.4	DR* A
	SD	3.98	3.51	4.20	3.76	3.54	
13 to 24	Mean	2.8	3.2	2.5	3.2	2.6	A
	SD	2.20	1.86	1.98	2.08	2.03	
24 to 52	Mean	2.5	2.9	2.9	2.1	2.5	A
	SD	2.72	3.48	2.41	3.56	4.29	
52 to 76	Mean	-1.8	-2.7	-1.7	-1.1	-2.3	A
	SD	3.12	3.19	2.72	2.06	2.69	
76 to 96	Mean	-3.2	-2.3	-1.9	-1.7	-3.2	A
	SD	4.38	2.71	1.42	3.25	3.83	

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

A = ANOVA, regression and Dunnett's

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	40	100	250	0

Week to Week of study		Mean body weight gains (g) for Group: 1F	2F	3F	4F	5F	Statistics
0 to 13	Mean	8.4	8.9	8.1	9.4	8.6	A
	SD	2.54	2.54	2.37	2.63	2.04	
0 to 96	Mean	15.2	14.9	15.7	14.6	15.4	A
	SD	5.45	6.58	4.22	3.07	4.85	
13 to 24	Mean	2.3	2.6	3.8*	2.6	3.1	A
	SD	2.23	2.13	2.91	2.82	2.49	
24 to 52	Mean	4.1	4.0	4.9	4.3	5.3	A
	SD	3.51	2.57	3.17	2.58	3.97	
52 to 76	Mean	1.5	0.6	0.3	0.9	1.0	A
	SD	2.69	2.35	3.57	3.34	2.56	
76 to 96	Mean	-2.0	-1.2	-1.6	-2.3	-3.0	A
	SD	5.13	3.43	2.29	3.61	3.12	

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

A = ANOVA, regression and Dunnett's

Pathology: Sponsor's Table 9 which follows is inter-group comparison of tumor incidence. A complete summary of tumor incidence in decedents and terminal kill combined is found in Appendix I. There were no findings for statistically significant differences between the incidences of tumors in high dose males or females compared to both controls combined.

Non-Tumor Pathology: There were no effects on gross pathology. 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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TABLE 9

Inter group comparison of tumour incidence

KEY FOR TABLES

Tumours of similar histogenic origin were merged, as requested by the Pathologist.

Tumour type	Tissue	Finding(s)
Pituitary adenoma	Pituitary	B-adenoma
HLR lymphoid tumours	Haem/lymph/retic	M-lymphocytic leukaemia
	Haem/lymph/retic	M-lymphoma
	Haem/lymph/retic	M-lymphocytic lymphoma
	Haem/lymph/retic	M-pleomorphic lymphoma
Liver hepatocellular tumours	Liver	B-hepatocellular adenoma
	Liver	M-hepatocellular carcinoma
Lung alveolar epithelial tumours	Lung	B-bronchiolo alveolar adenoma
	Lung	M-bronchiolo alveolar carcinoma
Mammary gland epithelial tumours	Mammary gland	B-adenoma
	Mammary gland	M-carcinoma
Uterus stromal tumours	Uterus	B-polyp
	Uterus	M-stromal sarcoma
Uterus smooth muscle tumours	Uterus	B-leiomyoma
	Uterus	M-leiomyosarcoma
Blood vessel tumours	Foot/leg	B-haemangioma
	Liver	B-haemangioma
	Spleen	B-haemangioma
	Uterus	B-haemangioma
	Blood vessel	M-haemangiosarcoma
	Uterus	M-haemangiosarcoma
	Bone	M-haemangiosarcoma
Histiocytic sarcoma	Skin+subcutis	M-histiocytic sarcoma
	Uterus	M-histiocytic sarcoma
	Connective tissue	M-histiocytic sarcoma
	Rectum	M-histiocytic sarcoma
Osteogenic tumours	Bone	B-osteoma
	Bone	M-osteosarcoma
Skin/appendage fibroblastic tumours	Skin+subcutis	B-fibroma
	Skin+subcutis	M-fibrosarcoma
	Skin+subcutis	M-sarcoma

TABLE 9.1

Inter group comparison of tumour incidence

Tumour incidence in males: Groups 1, 4 and 5 analysed
Results of tests for increasing and decreasing dose response

Tumour type		Number of tumour bearing animals in Group 1	4	5	Included in analysis	Groups 1v5	Groups 1+5v4	Tests
HLR lymphoid tumours	NF	1	1	1				
	F	6	3	4				
	ALL	7	4	5	ALL	NS	NS	L
Liver hepatocellular tumours	NF	8	9	14				
	F	2	0	0				
	ALL	10	9	14	ALL	NS	NS	L
Lung alveolar epithelial tumours	NF	6	12	9				
	F	1	1	2				
	ALL	7	13	11	ALL	NS	NS	L
Blood vessel tumours	NF	1	1	5				
	F	1	0	1				
	ALL	2	1	6	ALL	NS	NS	P
Skin/appendage fibroblastic tumours	NF	0	0	2				
	F	0	4	1				
	ALL	0	4	3	ALL	NS	NS	P

F = fatal
NF = non-fatal
L = large sample tests
P = permutational tests

NS = not significant for increasing or decreasing dose response (P>0.05)

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ON ORIGINAL

TABLE 9.2

Inter group comparison of tumour incidence

Tumour incidence in females: Groups 1, 4 and 5 analysed
Results of tests for increasing and decreasing dose response

Tumour type		Number of tumour bearing Animals in Group 1	4	5	Included in analysis	Groups 1v5	Groups 1+5v4	Tests
Pituitary adenoma	NF	0	1	2	NF	NS	NS	P
ZLR lymphoid tumours	NF	4	1	5				
	F	4	11	4				
	U	1	0	1	ALL (U as F)	NS	NS	L
	ALL	9	12	10	ALL (U as NF)	NS	NS	L
Liver hepatocellular tumours	NF	2	2	3	NF	NS	NS	P
Lung alveolar epithelial tumours	NF	8	5	3				
	F	0	1	0				
	ALL	8	6	3	ALL	NS	NS	L
Mammary gland epithelial tumours	NF	1	0	1				
	F	0	0	1				
	ALL	1	0	2	ALL	NS	NS	P
Uterus stromal tumours	NF	3	3	3				
	F	0	0	1				
	ALL	3	3	4	ALL	NS	NS	L
Uterus smooth muscle tumours	NF	2	1	2				
	F	0	1	0				
	ALL	2	2	2	ALL	NS	NS	P
Blood vessel tumours	NF	2	0	1				
	F	1	0	0				
	U	0	1	0	ALL (U as F)	NS	NS	P
	ALL	3	1	1	ALL (U as NF)	NS	NS	P
Histiocytic sarcoma	NF	4	0	1				
	F	1	0	1				
	ALL	5	0	2	ALL	NS	NS	P
Osteogenic tumours	NF	1	0	0				
	F	1	1	1				
	ALL	2	1	1	ALL	NS	NS	P
Skin/appendage fibroblastic tumours	NF	3	0	2				
	F	3	1	2				
	ALL	6	1	4	ALL	NS	NS	L

F = fatal
NF = non-fatal
U = uncertain
L = large sample tests
P = permutational tests

NS = not significant for increasing or decreasing dose response (P>0.05)

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ON ORIGINAL

D. Genetic Toxicology Studies

1. Reverse Mutation Assays (Vol 1.36)

Three different Ames reverse mutation assays were performed using the identical procedures. The following is a list of the studies, initiation and completion dates and batch numbers of test substance used for each study.

CHE Report Nos:	Study Initiated	Study Completed	Batch Number	Purity
ARL 115/921040*	7/14/92	8/6/92	LAS 31416	99.5%
655/65-1052	5/23/96	6/29/96	J003	**
655/35	12/1/93	12/22/93	C273M	**

* [redacted]

** Purity not stated.

Performing Laboratory: [redacted]

Quality Assurance: Statements of compliance with GLP is included for all 3 studies.

Bacterial strains used: For Study No. ARL 115/921040, strains used were *S typhimurium* TA1535, TA1537, TA1538, TA98 and TA100. For Study Nos. 655/65-1052 and 655/15, strains used were *S typhimurium* TA1535, TA1537, TA98, TA100 and TA102.

Procedure: In the preliminary dose finding tests, doses of 0, 50, 500 and 5000 ug/plate for all 5 strains in the first study, or doses 8, 40, 200, 1000 and 5000 ug/plate for all 5 strains were used in the second and third studies, and no toxicity was observed. Therefore, 5000 ug/plate was chosen as the maximum concentration in all 3 studies. In the definitive tests, doses of 0, 50, 150, 500, 1500 and 5000 ug/plate were used in the first study, whereas doses of 312.5, 625, 1250, 2500 and 5000 ug/plate were used in the second and third studies. All tests were performed in the absence and presence of S9 obtained from [redacted] induced rat livers. The definitive tests were performed with triplicate plates for all concentrations. Concurrent positive controls were included for each strain, both with and without S9.

Results: No evidence of mutagenic activity was seen in all three studies.

2. Induction of Chromosomal Aberrations in Cultured Human Lymphocytes (Vol 1.37)

HE Study No. 655/39

Performing Laboratory: [redacted]

Dates Study Performed: 4/20/94-5/31/94

Quality Assurance: A statement of GLP compliance is included

Test Substance: Batch I001 (purity not indicated).

Procedure: Human lymphocytes were obtained from a healthy, non-smoking female (used in Experiment 1) and male (used in experiment 2) for two independent but similar tests. In experiment 1, for determination of cell toxicity, duplicate cultures (designated as A and B) ranged between [redacted] (concentrations expressed as free base). Treatment in the absence of S9 was continuous for 20 hours (20+0), or only for 3 hours in the presence of S9 followed by a 17 hour recovery period (3+17). In Experiment 2, duplicate cultures ranged between [redacted] Treatment periods without S9 were 20 and 44 hours (20+0 and 44+0), and with S9 they were 3 hours followed by 17 and 41 hours of recovery (3+17 and 3+41). Solvent controls were similarly treated for each treatment regimen in both experiments. Negative controls were incubated in quadruplicate (designated as A, B, C and D) and harvested at 20 or 3+17 hours. Positive control consisted of 4-nitroquinoline 1-oxide (NQO) without S9 and cyclophosphamide (CPA) with S9, treated for 20+0 or 3+17 hours, respectively. Slides from solvent control cultures C and D were scored only if required to resolve an equivocal result.

Criteria for selection of doses for cytogenetic analysis: In both experiments, the highest dose selected for the 20 hour or 3 + 17 hour treatments was to be the one which caused a 50-80% reduction in mitotic index, or 3352 ug/mL (10 mM), the highest dose incubated. Slides from this and the next 2 lower doses were taken for microscopic analysis. If negative or equivocal results were obtained in Experiment 1, a single dose from the delayed harvest (44 or 3 + 41 hour) was scored in Experiment 2 corresponding to the maximum dose in Experiment 1.

At least 25 cells from positive control cultures and 100 cells from each negative control or drug-treated culture were analyzed and scored in a blinded fashion. Aberrant cells were categorized for cells with 1) gaps, 2) structural aberrations excluding gaps and 3) polyploidy, endoreduplicated or hyperdiploidy. Only cells with 44-46 chromosomes were considered acceptable for analysis of structural aberration. This excluded polyploidy, endoreduplicated or hyperdiploidy, which were recorded separately. Probability values of ≤ 0.05 or better, compared to control, were accepted as significant (Fisher's exact test).

Criteria for a positive response were:

1) a statistically significant increase of cells with structural aberrations (excluding gaps) occurred at 1 or more concentrations,
2) proportion of cells with structural aberrations at such doses exceeded the normal range, and
3) the results were confirmed in Experiment 2.

Increases in number cells with gaps or increases in the proportion of cells with structural aberrations not exceeding the normal range or occurring only at very high or very toxic concentrations were likely to be concluded as "equivocal". Cells with exchange aberrations or cells with greater than one aberration were to be considered of particular biological significance. A positive result only at the delayed harvest in Experiment 2 was to be taken as evidence of clastogenicity provided criteria 1 and 2 were met.

Results (compound related effects):

The following summarizes doses selected for analysis and mitotic inhibition at the highest dose.

		Mitotic inhibition †
20+0 hours, - S-9:	335.6, 447.5 and 596.6 µg base/ml	76%
3+17 hours, + S-9:	1886, 2514 and 3352 µg base/ml	24%
44+0 hours, - S-9:	251.7 µg base/ml	65%
3+41 hours, + S-9:	3352 µg base/ml	4%

† At (highest) analysed dose

Sponsor's Tables 1 to 4 (pages 55 and 56) summarizes the results of Experiments 1 and 2. A statistically significant increase in aberrant cells (excluding gaps) was observed in Experiment 2 at 335.6 and 447.5 ug/mL (but not at 596.6 ug/mL) in the absence of S9 with 20+0 incubation, not in the presence of S9 (Tables 3 and 4). An increase in aberrant cells was not observed in Experiment 1 (Tables 1 and 2). Sponsor indicates, "These increases (in Experiment 2) were not considered biologically important as they were seen against a zero background of aberrant cells in solvent control cultures, not seen in a dose dependent manner and historical negative control ranges were not exceeded in the majority of the LAS 31416-treated cultures at either dose. Single cultures, in Experiments 1 and 2, did exhibit aberrant cell frequencies just outside the normal range, however, since no reproducible effect was seen in concurrent replicate cultures the effect was not considered of any biological importance".

The normal range for structural aberrations excluding gaps in historical controls falls between 0-3 (See page 57). Therefore, we question sponsor's claim that because a positive response against a zero background it is not considered biologically important. Based on the third criterion for a positive response, we consider the clastogenic activity of almotriptan in the absence of S9 to be equivocal because a positive response was obtained in Experiment 2 but not in Experiment 1.

TABLE 1

Cells with structural aberrations: Experiment 1

20+0 hours treatment, + S-9 — Test chemical: LAS 31416
Donor sex: female

Treatment (µg base/ml)	Replicate	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Significance $	Mitotic index (mean)
Solvent	A	100	4	3		5.1
	B	100	1	1		5.3
	Totals	200	5	4		(5.2)
276.1	A	100	2	2		1.7
	B	100	1	1		1.5
	Totals	200	3	3	NS	(1.6)
394.4	A	100	4	4		1.8
	B	100	3	2		1.8
	Totals	200	7	6	NS	(1.8)
563.4	A	100	3	2		1.4
	B	100	1	1		1.0
	Totals	200	4	3	NS	(1.2)
NQO, 2.5	A	25	10	10		
	B	25	12	10		
	Totals	50	22	20	p ≤0.001	

$ Statistical significance (Appendix 5a)

NS = not significant

Numbers highlighted exceeded historical negative control ranges (Appendix 7)

TABLE 2

Cells with structural aberrations: Experiment 1

3+17 hours treatment, + S-9 — Test chemical: LAS 31416
Donor sex: female

Treatment (µg base/ml)	Replicate	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Significance $	Mitotic index (mean)
Solvent	A	100	3	3		5.1
	B	100	1	0		6.0
	Totals	200	4	3		(5.6)
1643	A	100	3	2		4.9
	B	100	1	0		6.1
	Totals	200	4	2	NS	(5.5)
2347	A	100	3	1		4.2
	B	100	2	2		5.1
	Totals	200	5	3	NS	(4.7)
3352	A	100	3	2		4.0
	B	100	2	2		3.5
	Totals	200	5	4	NS	(3.8)
CPA, 25	A	25	10	10		
	B	25	12	10		
	Totals	50	22	20	p ≤0.001	

$ Statistical significance (Appendix 5a)

NS = not significant

Numbers highlighted exceeded historical negative control ranges (Appendix 7)

TABLE 3

Cells with structural aberrations: Experiment 2

20+0 hours treatment, - S-9 Test chemical: LA6 31416
Donor sex: male

Treatment (µg base/ml)	Replicate	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Signifi-cance $	Mitotic index (mean)
Solvent	A	100	1	0		6.6
	B	100	2	0		5.4
	Totals	200	3	0		(6.0)
335.6	A	100	0	0		2.5
	B	100	4	[illegible]		2.4
	Totals	200	4	4	p ≤0.05	(2.5)
447.5	A	100	3	3		2.1
	B	100	3	3		2.1
	Totals	200	6	6	p ≤0.01	(2.1)
596.6	A	100	2	1		1.4
	B	100	0	0		1.5
	Totals	200	2	1	NS	(1.5)
MQO, 2.5	A	25	[illegible]	[illegible]		
	B	25	[illegible]	[illegible]		
	Totals	50	19	15	p ≤0.001	

$ Statistical significance (Appendix 5b)

NS = not significant

Numbers highlighted exceeded historical negative control ranges (Appendix 7)

TABLE 4

Cells with structural aberrations: Experiment 2

3+17 hours treatment, + S-9 Test chemical: LA5 31416
Donor sex: male

Treatment (µg base/ml)	Replicate	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Signifi-cance $	Mitotic index (mean)
Solvent	A	100	3	2		3.6
	B	100	4	3		3.4
	Totals	200	7	5		(3.5)
1886	A	100	6	1		3.1
	B	100	4	1		4.2
	Totals	200	10	2	NS	(3.7)
2514	A	100	3	2		3.0
	B	100	4	0		4.0
	Totals	200	7	2	NS	(3.5)
3352	A	100	2	1		2.2
	B	100	4	0		3.1
	Totals	200	6	1	NS	(2.7)
CPA, 25	A	25	6	6		
	B	25	15	11		
	Totals	50	21	17	p ≤0.001	

$ Statistical significance (Appendix 5b)

NS = not significant

Numbers highlighted exceeded historical negative control ranges (Appendix 7)

APPENDIX 7

Historical solvent control data for human lymphocyte cultures*

Sampling time (hours)	Aberrant cells per 100 scored	Sex	- S-9 Cells scored	- S-9 Mean	- S-9 Calculated normal range	+ S-9 Cells scored	+ S-9 Mean	+ S-9 Calculated normal range
20	Structural aberrations including gaps	Male	6800	1.4		6800	1.7	
		Female	6200	1.7		6200	1.9	
	Structural aberrations excluding gaps	Male	6800	0.5		6800	0.7	
		Female	6200	0.7		6200	0.8	
	Numerical aberrations	Male	6800	0.5		6800	0.5	
		Female	6200	0.6		6200	0.4	
44	Structural aberrations including gaps	Male	3200	3.0		3000	1.6	
	Structural aberrations excluding gaps	Male	3200	1.2		3000	0.6	
	Numerical aberrations	Male	3200	0.6		3000	0.7	

* Calculated on the basis of 43 recent consecutive experiments at 17 March 1993

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3. Mutation at the Thymidine Kinase (tk) Locus in Mouse Lymphoma L5178Y Cells Using the Microtitre Fluctuation Technique (Vol 1.37)

HE Study No. 655/40-1052

Performing Laboratory [redacted]

Dates Study Performed: 4/20/94-9/30/94

Quality Assurance: A statement of GLP compliance is included

Test Substance: Batch I001 (purity not indicated).

Test System: L5178Y TK $^{+/-}$ mouse lymphoma cells obtained from American Type Culture Collection.

Nature of Test: Almotriptan malate was assayed for its ability to induce mutations at the *tk* locus (5-trifluorothymidine resistance {TFT}) in mouse lymphoma cells.

Procedure: The study consisted of a cytotoxicity range-finder, followed by 3 independent experiments. Two experiments were conducted in the presence of S9 (+S9) ([redacted] induced rat liver) and in the absence of S9 (-S9) and a third experiment was conducted only +S9. Incubation periods were for 3 hours. Osmolality and pH measurements on post-treatment media were made.

In the cytotoxicity range finder study, doses tested ranged between [redacted] (2-fold increases between doses; concentrations expressed in terms of the base). Single cultures were used and there were no positive controls. In Experiments 1 and 2, test chemical solutions were dissolved in purified water and negative control was purified water. In Experiment 3, because of the perceived occurrence of false positives, attributed to low pH found in post-treatment media (pH 6.8 and 6.4, respectively for 2500 and 5000 ug/mL, respectively, vs pH 7.2 for controls in Experiment 1), chemical solutions were prepared by dissolving test compound in phosphate buffered saline, then adjusting to pH 7.25, and the control was buffered media. In Experiments 1-3, all concentrations were tested in duplicate, except positive controls were single cultures. For positive controls, 4-nitroquinoline-1-oxide (NQO) -S9 and benzo(a)pyrene (BP) +S9 were used.

In the cytotoxicity test, cells survived at all doses. At the maximum dose (5000 ug/mL), there was 8.5% relative survival –S9 and 16.2% survival +S9. Accordingly, the 5 maximum doses were selected for Experiment 1. Incubation (expression) period was for 2 days. The maximum dose yielded 73.2 and 20.0 % relative survival –S9 and +S9, respectively. Due to low viabilities in the presence of S9 in Experiment 1, "indicating

incomplete recovery from treatment", a 3 day expression period was used for Experiments 2 and 3.

An assay was considered valid if the following criteria were met:

1) the mutant frequencies in the negative (solvent) control cultures fell within the normal range (not more than 3 times the historical mean value)
2) at least 1 concentration of each of the positive control chemicals induced a clear increase in mutant frequency (the difference between the positive and negative control mutant frequencies was greater than half the historical mean value).

The substance was considered mutagenic if:

1) the assay was valid
2) the mutant frequency at 1 or more doses was significantly greater than that of the negative control
3) there was a significant dose-relationship as indicated by the linear trend analysis
4) the effects described above were reproducible.

Results: Sponsor's summary of results for all 3 experiments is shown on page 62.

In Experiment 1, a dose related, statistically significant increase in mutant frequency was observed at 2500 and 5000 ug/mL (dose related) in the presence of S9. At the end of the incubation period, low pH was observed in the incubation media at these 2 doses (pH 6.8 and 6.4, respectively, vs. pH 7.2 for controls), and "very low viability counts", measured in terms of relative survival (RS), were found. The RS found were 16.3 and 20.0 at 2500 and 5000 ug/mL, respectively (See page 62).

In Experiment 2, a statistically significant increase in mutant frequency was observed again at 2500 ug/mL in the presence of S9; the 5000 ug/mL dose was rejected due to low relative survival. Again, the increase in mutant frequency was attributed to with low pH (pH 6.7 at 2500 mg/mL vs. pH 7.3 for controls), and "very low viability counts".

The investigators claimed, "It is well known that low pH ($\leq$6.8) can induce increases in mutant frequencies in this assay, especially in the presence of S9 (Cifone et al, Mutation Res. **189:** 39-46, 1987). Therefore, the third experiment was conducted only in the presence of S9 and the pH was adjusted to pH 7.2 in a buffered medium.

In experiment 3, no statistically significant increase in mutant frequency was observed at any dose level, including 2500 ug/mL. The pH of the test chemical solution was neutralized to pH 7 before adding to the culture media and the pH in the post-treatment media from the highest dose, 2500 mg/mL, was 7.2, vs. 7.3 for controls. At 5000 ug/mL, a precipitate was observed upon test chemical addition which was still evident at the end of the incubation period, and the RS was 0.0. No statistically significant increase in mutant frequency was observed at any dose level up to 2500 ug/mL.

The proportion of small colony mutants in the negative controls in the absence of S9 ranged from 52 to 70%, but a high proportion (64-77%) was found for positive controls, both –S9 and +S9 (See sponsor's Appendix 7 on page 63). In Experiment 1, at doses of

almotriptan where significant increases in mutant colonies were seen, both small and large colony counts were high, but the proportion of small colony mutants was similar to positive control at the 5000 ug/mL dose. In Experiment 2, only the small colony counts were high at 2500 ug/mL than control.

Sponsor concluded that almotriptan is not mutagenic at the *tk* locus of L5178Y mouse lymphoma cells. However, we found it difficult to accept such a conclusion on the basis of only one experiment; i.e. Experiment 3, even though the possibility of mutagenic activity by almotriptan was suggested in the first 2 experiments. We requested the consult of Dr. Martha Moore of EPA, who is well known as an expert and a pioneer in the development of this assay. Numerous flaws were found in sponsor's interpretation of Experiments 1 and 2, and Experiment 3 was considered to be completely deficient and unacceptable. We informed the sponsor that we considered the results of this study as equivocal. The reasons given for our conclusions were as follows.

1. There is no justification for a 3-day expression period. The only situation where it may be a good idea is when there is a negative response after a 2 day expression period and we wish to determine if there is a possibility of a positive response. With a few compounds, a more clear-cut positive response is obtained after 3 days. However, decreases in small colony frequencies may be observed with increased expression time. Thus, a 3 day expression period more often will confound the results and may get rid of a positive response (Moore et al. Environ Molec Mutagenesis **35**: 185-190, 2000; Clive et al . Environ Molec Mutagenesis **25**: 165-168, 1995; Moore and Clive Environ Mutagenesis **4**: 499-519, 1982).

2. In Experiment 1, a dose related, statistically significant increases in mutant frequencies were observed at 2500 and 5000 ug/mL in the presence of S9. Low pH was observed at these 2 doses (pH 6.8 and pH 6.4). Based on the data of Cifone et al., pH 6.5 appears to be the borderline for a false positive response. Therefore, we believe that the response seen at pH 6.8 is more likely a true positive response. The RS for the 2500 and 5000 ug/mL doses were 16.3 and 20.0, respectively, which falls between the usually recommended and accepted RS for the highest doses (RS between 10 and 20%). Sponsor's claim that low viability contributed to the positive effect may not be valid because a relative total growth (RTG) between 10 and 20% are required at the highest dose.

3. In Experiment 2, a statistically significant increase in mutant frequency was observed at 2500 ug/mL; the 5000 ug/mL dose was rejected due to low relative survival (RS was 4.6). The increases in mutant frequencies were associated with a pH of 6.7 and the RS value listed in the table was 72.1. Therefore, the effect observed at 2500 ug/mL might also be considered a possible true positive response.

We recommended that sponsor perform a study similar to that of Cifone et al. Using the same media as that used for negative controls, they should try testing between pH 6.4 and 7.3. That is, add additional negative controls in which the pH is lowered between 6.4 and

7.3 by adding acid. In our telecom of June 9, 2000, sponsor preferred not doing this study. Our reply was that it was an optional request.

In Experiment 3, the RS was 41.6 and 0% for 2500 and 5000 ug/mL, respectively. According to Dr. Moore, **"This is a no test. They haven't reached an adequate cytotoxicity level."** They need to test doses between 2500 and 5000 ug/mL to get a satisfactory RS. For example, try 300 ug/mL increments; 2500, 2800, 3100, 3400.....5000 ug/mL.

According to Dr. Moore, in all of the above experiments, 2-fold increases between doses may not be acceptable, particularly at the higher doses, such as between 2500 and 5000 ug/mL. Lots of doses can be tested between 2500 and 5000 ug/mL, but it is not necessary to test at the lower doses where the laboratory has already demonstrated a negative response. There is no benefit to continuing to repeat testing at doses shown to be negative.

We asked Dr. James MacGregor for his opinion on the requests we made for further studies by the sponsor with the mouse lymphoma assay. He agreed that our requests were reasonable and that Martha Moore is one of the most knowledgeable people on this assay.

Additional suggestions, sent to the sponsor by FAX on the same day, were as follows:

1. Use a 3 and 4 hour incubation period with S9. By using a 4-hour treatment period with S9, you may be able to test lower concentrations in the presence of S9 because you may get RS of 10 or 20% with lower concentrations.

2. Conduct a study testing 4 hour and 24 hour incubation periods in the absence of S9, covering the entire dose range.

See if a dose can be found that causes 10 or 20% survival, both in the presence and absence of S9.

[redacted]

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Experiment 1

Treatment (µg/mL)	-S-9 %RS	-S-9 Mutant frequency#	Treatment (µg/mL)	+S-9 %RS	+S-9 Mutant frequency#
0	100.0	99.61	0	100.0	81.40
312.5	95.8	80.65 NS	312.5	90.3	94.44 NS
625	85.8	87.89 NS	625	102.2	90.98 NS
1250	76.6	86.52 NS	1250	93.0	84.59 NS
2500	84.6	82.93 NS	2500	16.3	313.89 *
5000	73.2	111.71 NS	5000	20.0	353.60 *
Linear trend		NS	Linear trend		***
NQO			BP		
0.05	62.8	555.26	2	85.7	396.54
0.1	54.7	724.00	3	64.6	889.78

Experiment 2

Treatment (µg/mL)	-S-9 %RS	-S-9 Mutant frequency#	Treatment (µg/mL)	+S-9 %RS	+S-9 Mutant frequency#
0	100.0	123.93	0	100.0	165.23
312.5	89.9	132.11 NS	312.5	103.6	124.49 NS
625	80.8	114.95 NS	625	86.2	131.19 NS
1250	62.2	128.15 NS	1250	75.8	138.01 NS
2500	72.1	123.61 NS	2500	12.7	326.67 *
5000 $	1.4		5000 $	4.6	
Linear trend		NS	Linear trend		***
NQO			BP		
0.05	75.3	1125.61	2	88.0	641.12
0.1	50.8	852.72	3	61.2	651.21

Per 10^6 viable cells
$ Dose rejected from analysis due to low relative survival
NS Not significant
*,**,*** Significant at 5%, 1% and 0.1% level respectively

Experiment 3

Treatment (µg/mL)	+S-9 %RS	+S-9 Mutant frequency#
0	100.0	93.42
312.5	112.0	94.54 NS
625	108.1	96.54 NS
1250	116.9	71.69 NS
2500	41.6	115.67 NS
5000 $	0.0	
Linear trend		NS
BP		
2	93.8	915.41
3	71.2	834.85

$ Not plated for viability / 5-TFT resistance

APPENDIX 7

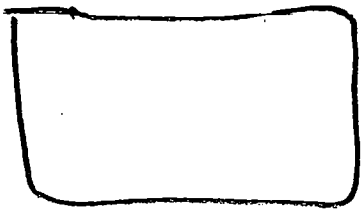
Small and large colony mutant frequencies for negative and positive controls and doses of LAS 31416 showing a significant increase in mutant frequency

Experiment	Concentration (μg/mL)	S-9	Mutant frequency*		Proportion small colony mutants
			Small colony	Large colony	
1	0	-	55.3	40.7	0.58
	NQO 0.05		361.5	125.0	0.74
	NQO 0.1		466.0	199.1	0.70
	0	+	45.1	34.0	0.57
	2500		176.7	131.9	0.57
	5000		250.1	90.9	0.73
	BP 2		280.7	87.2	0.76
	BP 3		597.3	175.9	0.77
2	0	-	61.4	57.6	0.52
	NQO 0.05		621.6	288.5	0.68
	NQO 0.1		525.7	193.0	0.73
	0	+	86.8	69.5	0.56
	2500		236.3	77.3	0.75
	BP 2		386.2	166.6	0.70
	BP 3		424.4	147.1	0.74
3	0	+	63.8	26.9	0.70
	BP 2		504.0	231.8	0.68
	BP 3		417.3	239.3	0.64

* Per 10^6 viable cells

4. **Induction of Micronuclei in Bone Marrow of Mice** (Vol 1.37)

Study No. 655/41

Performing Laboratory: 

Dates Study Performed: 4/21/94-5/23/94

Quality Assurance: A statement of GLP compliance is included.

Test Substance: Batch I001

Procedure: CD-1 mice, from Charles River, UK, were used. A range-finder study with doses between 357 and 2000 in 3/sex/group was conducted to determine highest dose that could be administered. For the main study the animals were 31-38 days old; males weighed 25-31 g, females weighed 22-26 g. Test substance was given by gavage for 2 consecutive days in a 1% methyl cellulose suspension to 15/sex (10/sex were used for the study, 5 extra in case of deaths) at 2000 mg/kg/day. Negative controls (10/sex) received vehicle, positive controls (5/sex) received cyclophosphamide. Bone marrow from negative controls for cytogenetic analysis was obtained from 5/sex/group at 24 and 48 hours after dosing (only at 24 hours from positive controls). Total numbers of polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE) and micronuclei from each animal were counted. The individual and group frequencies of micronuclei in 1000 PCE, based on approximately 2000 PCE, were determined and compared to control and background incidence. The ratio of PCE/NCE was obtained to determine if there was a decrease in treated animals, which could be taken as evidence of bone marrow toxicity.

Results: Sponsor's summary of results is shown on the page that follows.

It was concluded that almotriptan was unable to induce micronuclei in the polychromatic erythrocytes of bone marrow in mice treated on 2 consecutive days at 2000 mg kg (expressed as base), a dose, which was known to cause, limited mortality.

TABLE 1

Summary of group mean data for

test chemical, vehicle and positive controls

Data for LAS 31416

Treatment (mg base/kg)	Kill time (hours)	Sex	Mean ratio PCE/NCE	Group mean frequency of micronucleated PCE (per 1000)	
				per sex	per treatment group
Vehicle control	24	M	1.14	0.40	0.50
		F	1.09	0.60	
	48	M	1.02	0.40	0.50
		F	1.36	0.60	
2000	24	M	1.54	0.90	0.75
		F	1.08	0.60	
	48	M	0.90	0.70	0.70
		F	0.88	0.70	
CPA, 80+	24	M	0.67	16.77	21.86
		F	0.67	26.95	

+ Administered as a single dose

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E. Reproductive Toxicity Studies

1. Fertility, Pre and Postnatal Development Study in Rats (Segment I)

(Vol 1.32)

Report No: 655/58

Performing Laboratory: [redacted]

Dates Performed: Treatment initiated 1/15/96, necropsies completed 5/22/96

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

Doses: 0 (control), 25, 100 and 400 mg/kg/day (dose level expressed in terms of base). The dose volumes were 10 mL/kg, based on individual body weight. Vehicle used was [redacted]

Rationale for Dose Selection: Sponsor claims they were based on previous toxicity studies in rats.

Procedure: Crl:CD(SD)BR strain rats (Charles River, UK), 48/sex/group, were used. At initiation of treatment, males were 10-12 weeks of age with mean body weights of 310.8 to 393.2 g, females were 12 to 14 weeks old and weighed 222.8 to 299.1 g. Treated rats received LAS 31416 (DL hydrogen malate) (Lot no. J003; purity not specified) once daily by oral gavage. The animals were housed 4 or 5 per cage, except during mating, pregnancy and lactation. The P generation males were dosed daily for 4 weeks prior to mating with females of the same treatment group that had been treated for 2 weeks prior to mating. Dosing of mated females continued throughout pregnancy and lactation, until postpartum day (PPD) 22. Half the females in each group were C-sectioned on gestation day (GD) 13 for determination of pregnancy data. The remaining half of P females was allowed to litter and suckle their offspring to PPD 22. At weaning, 20 male and 20 female F_1 offspring from each group were selected for further study; non-selected were killed and examined on PPD 22.

The P males which had not sired a pregnancy during the initial mating period were paired after 11 weeks of treatment with untreated females. The mated females were C-sectioned on GD 13 and examined for pregnancy. Dosing of the males from all groups continued throughout both pairing periods until the day before necropsy in Week 15 of treatment. The P females were dosed until the day before necropsy in Week 19.

The F_1 animals were retained without further treatment for 10 weeks after which time their physical and behavioral development was assessed. They were then paired within parental treatment groups for up to 15 days and the outcome of pregnancy was assesses on GD 13. F_1 males were killed following evaluation of F_1 females.

During the entire course of the study, all animals were examined daily for morbidity, mortality and clinical observations. Body weights and food intake (when possible) were recorded weekly in non-pregnant animals, twice weekly during pregnancy and lactation. Estrous cycles by vaginal washings were monitored for P females from 15 days prior to treatment until mating had occurred.

Five P females and the respective males that showed no evidence of mating were re-mated with proven, untreated males or with untreated females. Treatment-related effects on pregnancy were evident, but in order to ascertain if this effect was due to one or both sexes, the males that had mated but did not sire a pregnancy were mated to additional untreated females. All untreated females were examined for evidence of mating and subsequently C-sectioned to determine if pregnancy had occurred.

Half the P females in each group were C-sectioned on GD 13. C-section data included pregnancy status, corpora lutea count, number and intra-uterine positions of implantations, live embryos, early and late intrauterine deaths. Uteri of apparently non-pregnant animals were immersed in 10% NH_4S.

The remaining females were allowed to litter and on PPD 4, the litters were culled to 8/litter (4/sex/litter if possible). P litter (F_1 offspring) data included duration of gestation, number of live and dead pups at birth, daily litter size and sex, daily clinical signs, pup weights on PPD 1, 4, 7, 14 and 21, and necropsy findings of dead and culled pups. Developmental parameters included days of pinna unfolding, incisor eruption and eye opening. Functional tests included surface righting reflex on PPD 1, air righting reflex on PPD 17, grip strength, pupillary reflex, auditory response and visual placing response on PPD 21. Physical development tests included vaginal opening starting from PPD 30 and balano-preputial separation starting on PPD 40, learning ability (swimming maze) during Weeks 1 and 2 and motor activity in an open field arena was measured during Week 3.

Post-Mortem: Testes from all P males were fixed in Bouin's fluid and embedded in wax. The left epididymis of males that mated with females allocated to caesarian section was examined for seminology. The testes and left epididymis from additional selected males were similarly processed. Samples of the following organs from all animals were fixed in 10% buffered formalin: ovaries, uterus, cervix, vagina, pituitary, testes, epididymides, seminal vesicles, prostate, coagulating gland and lesions.

Seminology included sperm counts, viability and morphology from up to 25 males in each group from the P generation. Histopathology was performed on control and high dose P females and males with which they mated. A higher number of pale kidneys were seen in treated animal, at necropsy. To evaluate them further, tissues from all pale kidneys were microscopically examined. Testicular histopathology evaluation in all P

males included staging of the spermatogenic cycle. Reproductive organs from 5 high dose F_1 males that did not mate were processed for histopathology and testicular evaluation.

Results, P Generation (with the exception of Mortality, limited to compound related effects)

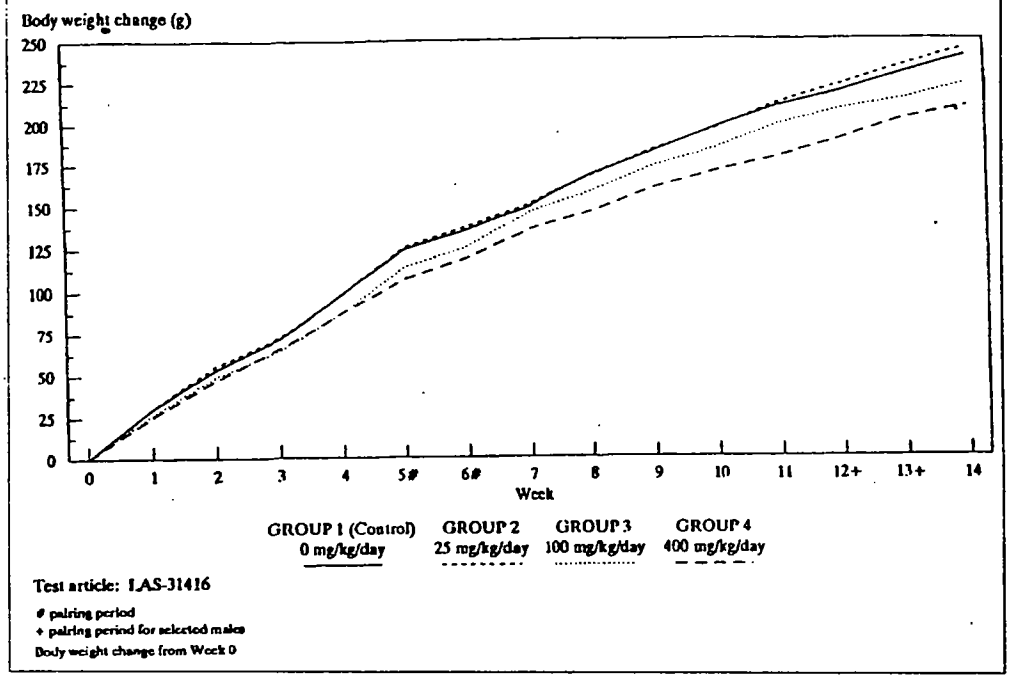
Mortality and Morbidity: 2 mid dose males (on Days 26 and 66 of treatment), 1 mid dose female (on Day 15 due to a gavage dosing error), and 2 high dose males (the latter 2 were both killed in extremis on Days 68 and 105 of treatment). There were no consistent gross pathology effects that would suggest that the deaths were compound related. Sponsor considered the deaths to be unrelated to treatment.

Clinical Observations: From Day 1, squinting of eyes first seen 0.5 hours post-dosing, still seen at 4 hours but no longer evident by 24 hours post-dosing, salivation, paddling of fore-limbs, red extremities in 17 high dose males only on Day 17. At high dose, 25 males appeared hyperactive in Week 2, also seen in Weeks 3 to 10 but less frequently.

Body Weight: (See sponsor's Figures 1.1, 1.2 and 1.3 on the pages that follow). In males, a slight dose-related reduction in body weight gain occurred in mid and high dose males (statistically significant between Weeks 3 and 5, Weeks 7 and 8), such that by Week 14, mean body weights were 3.5 and 6% lower than controls, respectively. In females, body weight gain at mid and high doses were higher than controls ($P<0.05$ and 0.01, respectively by Week 4 of treatment), During pregnancy and lactation, body weight of mid and high dose groups tended to be lower than controls.

Food Intake: At mid and high doses, food intake of both the males and females before pairing were lower than controls ($P<0.001$ for first week and $P<0.05$ for dose relationship during second week). There was also a pattern of slightly reduced but dose related food intake at mid and high doses during pregnancy and lactation.

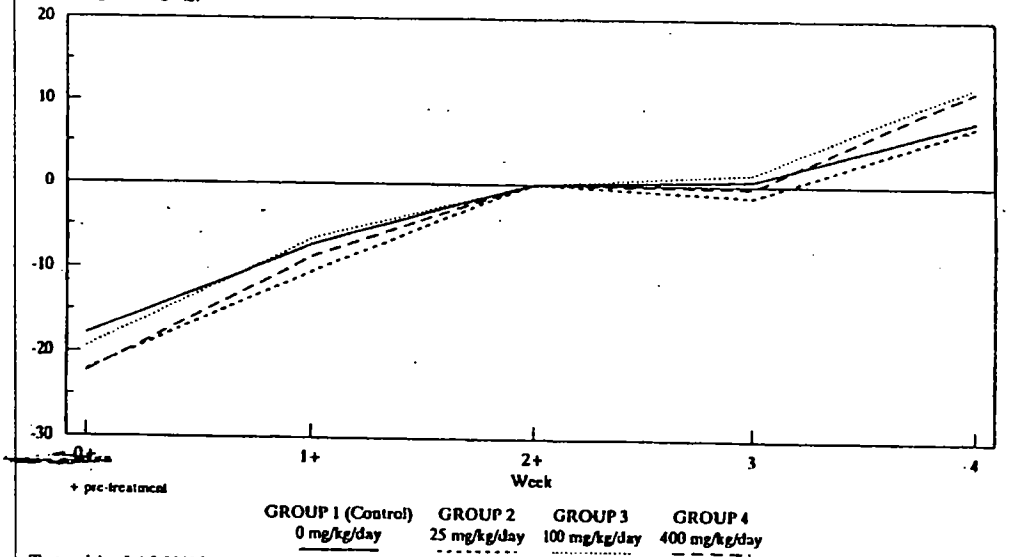
FIGURE 1.1
Group mean body weight change (g) - P generation
Males



Test article: LAS-31416

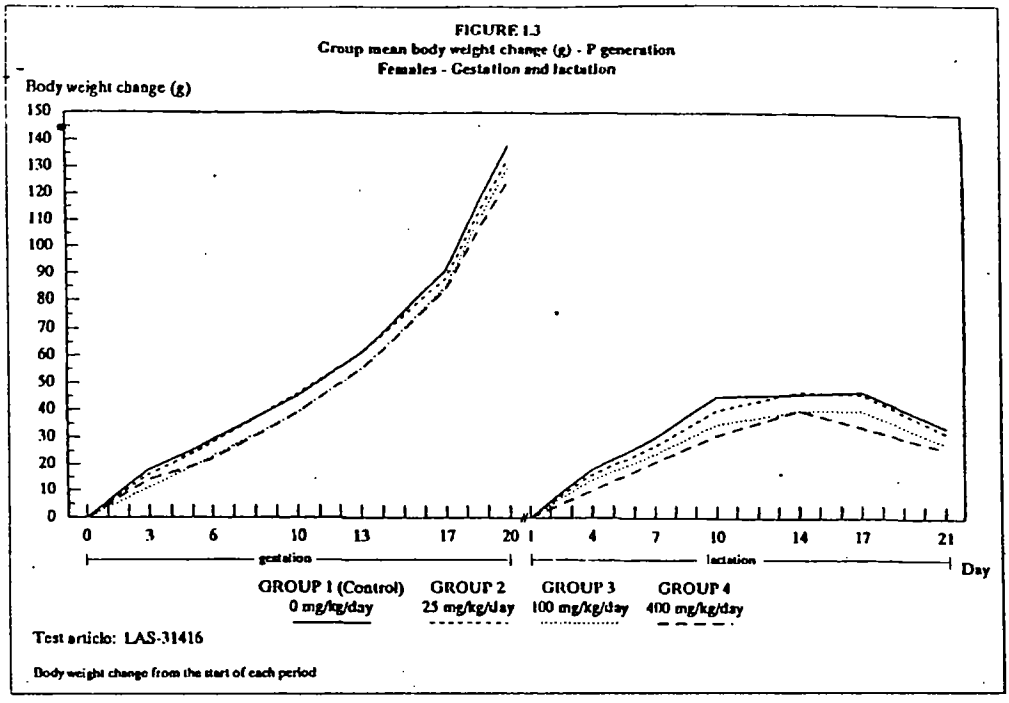
pairing period
+ pairing period for selected males
Body weight change from Week 0

FIGURE 1.2
Group mean body weight change (g) - P generation
Females - Pre-pairing



Test article: LAS-31416

Body weight change from the start of treatment



Estrous Cycles: The mid and high dose females were in the estrous stage for a greater number of days than controls; consequently less estrous cycles ($P<0.05$ at high dose).

Mating Performance and Pregnancy Rate: (Sponsor's Table 5 that follows). All 47 or 48 females in each group mated, except for 1 on high dose. At high dose, 10/47 paired animals that mated failed to produce a pregnancy, resulting in substantial reductions in fertility and fecundity indices in females compared to controls ($P<0.05$; see sponsor's Table 5.4 that follows). When the high dose males that failed to sire a litter were mated again with untreated females, all the females became pregnant, indicating that the fertility effect was clearly due to treatment of the females. There appeared to be no individual correlation between the effects on the estrous cycles and effects on fertility.

Uterine Data (Sponsor's Table 6 which follows): Corpora lutea and implantation data (mean number per female) were similar in all groups, but pre-implantation loss as well as mean number of early and late intrauterine deaths per female were all higher in mid dose females. Since there was no dose relationship or statistical significance, sponsor considered this as not compound related.

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TABLE 5

Group smearing and mating data - P generation

Test article	Control	LAS-31416		
Group	1	2	3	4
Level (mg/kg/day)	0	25	100	400

5.4 Mating indices

	Group 1	Group 2	Group 3	Group 4	Statistics
Number of paired males	48	48	47	48	X
Number of paired females (treated)	48	48	47	48	X
Number of paired females (untreated, first pairing)	3		1	1	X
Number of paired females (untreated, second pairing)	2	1	3	10	X
Number of mated males	48	48	47	48	X
Number of mated females (treated)	48	48	47	47	X
Number of mated females (untreated, first pairing)	3		1	1	X
Number of mated females (untreated, second pairing)	2	1	3	10	X
Number of males inducing pregnancy	48	48	47	47	X
Number of pregnant females (treated)	46	47	44	37	X
Number of pregnant females (untreated, first pairing)	3		1	1	X
Number of pregnant females (untreated, second pairing)	2	1	3	9	X
Mating index % (treated)	90.6	98.0	97.9	94.0	F
Fertility index % : male	100.0	100.0	100.0	97.9	F
: female (treated)	95.8	97.9	93.6	77.1*	F
Fecundity index % : male	100.0	100.0	100.0	97.9	F
: female (treated)	95.8	97.9	93.6	78.7*	F

X = Not analysed
F = Cochran-Armitage and Fisher-Irwin tests

* P<0.05

TABLE 6

Group mean uterine/implantation data - P generation

Test article	Control	LAS-31416		
Group	1	2	3	4
Level (mg/kg/day)	0	25	100	400

	Group 1	Group 2	Group 3	Group 4	Statistics
Number of pregnant females with live embryos at Day 13 gestation	23	24	22	17	X
Number of corpora lutea	374	377	349	273	
Mean number per female	16.3	15.7	15.9	16.1	J
Number of implantations	350	352	299	238	
Mean number per female	15.2	14.7	13.6	14.0	J
% pre-implantation loss	6.3	6.3	13.9	12.2	F
Number of early intrauterine deaths	11	13	20	8	
Mean number per female	0.5	0.5	0.9	0.5	F
Number of late intrauterine deaths	8	9	16	4	
Mean number per female	0.3	0.4	0.7	0.2	F
% post-implantation loss	5.2	6.3	12.0	5.2	F
Number of live embryos	331	330	263	226	
Mean number per female	14.4	13.8	12.0	13.3	J
% of implantations	94.8	93.7	88.0	94.8	X

J = Kruskal-Wallis, Terpstra-Jonckheere and protected Wilcoxon
F = Cochran-Armitage and Fisher-Irwin tests
X = Not analysed

Litter Data (See table 7 below and on the following page)

Mean duration of pregnancy was very slightly prolonged at high dose compared to controls ($P<0.05$). There were slightly lower numbers live pups/litter at birth in treated groups compared to control (3.8, 3.8 and 9.2% for low, mid and high dose groups, respectively), which apparently resulted in a smaller group size on PPD 4, before culling. There was no further decrease in live pups/litter after PPD 4. However, there were statistically significant decreases in mean pup weights/litter at high dose, which were evident on PPDs 7, 14 and 21.

Semenology: No effect on motility, numbers or morphology.

Testicular Staging: No effect

F_1 Generation: After weaning, there were no effects that were compound related on mortality, physical or maturational development, performance, mating or on uterine/implantation data.

TABLE 7

• Group mean litter data - P generation

Test article	Control	LAS-31416		
Group	1	2	3	4
Level (mg/kg/day)	0	25	100	400

7.1 Pup numbers - females rearing young to weaning

	Group 1	Group 2	Group 3	Group 4	Statistics
Number of females with live pups at birth	23	23	21	19	X
Number of females with live pups at Day 21 *post-partum*	23	23	21	18	X
Mean duration of gestation (days)	22.2	22.4	22.4	22.7*	F
Number of implantation sites Mean number per female	318 13.8	321 14.0	300 14.3	235 13.1	J
Number of pups born Mean number per female	298 13.0	287 12.5	263 12.5	213 11.8	J
Number of pups alive Day 1 Mean number per female	291 12.7	283 12.3	253 12.0	205 11.4	X
Percentage male pups Day 1	50.2	45.6	52.3	53.0	J
Number of pups alive Day 4 before culling Mean number per female	281 12.2	282 12.3	248 11.8	203 11.3	X
Number of pups culled Day 4 Mean number per female	97 4.2	102 4.4	84 4.0	69 3.8	X
Number of pups alive Day 4 after culling Mean number per female	184 8.0	180 7.8	164 7.8	134 7.4	X
Number of pups alive Day 7 Mean number per female	183 8.0	180 7.8	164 7.8	132 7.3	X
Number of pups alive Day 14 Mean number per female	183 8.0	180 7.8	159 7.6	131 7.3	X
Number of pups alive Day 21 Mean number per female	183 8.0	180 7.8	159 7.6	131 7.3	X

J = Kruskal-Wallis, Terpstra-Jonckheere and protected Wilcoxon
F = Cochran-Armitage and Fisher-Irwin tests
X = Not analysed

* $P<0.05$

TABLE 7

Group mean litter data - P generation

Test article	Control	LAS-31416		
Group	1	2	3	4
Level (mg/kg/day)	0	25	100	400

7.2 Gestation and neonatal survival indices - females rearing young to weaning

	Group 1	Group 2	Group 3	Group 4	Statistics
Gestation index %	100.0	100.0	95.5	95.0	F
Post-implantation survival index %	93.7	89.1	87.2	91.5	J
Live birth index %	97.5	98.4	96.9	96.7	F
Viability index 1 %	96.7	99.7	98.1	99.0	F
Viability index 2 %	99.5	100.0	100.0	98.6	F
Viability index 3 %	100.0	100.0	97.0	99.2	F
Viability index 4 %	100.0	100.0	100.0	100.0	F

J = Kruskal-Wallis, Terpstra-Jonckheere and protected Wilcoxon
F = Cochran-Armitage and Fisher-Irwin tests

TABLE 7

Group mean litter data - P generation

Test article	Control	LAS-31416		
Group	1	2	3	4
Level (mg/kg/day)	0	25	100	400

7.3 Mean pup weights (g)

		Group 1	Group 2	Group 3	Group 4	Statistics
Mean weight (g) Day 1:	male	6.7	6.7	6.6	6.3	A
	female	6.3	6.4	6.0	6.1	A
	combined	6.5	6.6	6.3	6.2	A
Mean weight (g) Day 4:	male	9.6	9.5	9.4	9.1	A
	female	9.1	9.2	8.7	8.8	A
	combined	9.4	9.4	9.1	8.8	A
Mean weight (g) Day 7:	male	15.6	15.5	15.2	14.6 DR*	A
	female	14.9	15.0	14.4	14.1 DR*	A
	combined	15.3	15.2	14.8	14.0*	A
Mean weight (g) Day 14:	male	33.4	32.0	31.6	29.9***	A
	female	32.1	31.2	30.1	28.8**	A
	combined	32.7	31.6	30.9	29.5***	A
Mean weight (g) Day 21:	male	52.9	52.3	51.3	48.6*	A
	female	50.9	51.0	48.8	47.0*	A
	combined	52.0	51.6	50.2	47.9*	A
% weight change (combined) Days 1-21•		707	685	697	676	A

• calculated from individual values
A = ANOVA, regression and Dunnett's

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

-2. Oral Gavage Developmental Toxicity Study in Rats (Vol 1.33)

Report No: T.31416.14 (Report translated from Spanish)

Performing Laboratory: [redacted]

Dates Performed: Treatment initiated 7/4/94, necropsies completed 7/20/94

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

Doses: 0 (control), 250, 500 and 1000 mg/kg/day (dose level expressed in terms of base). The dose volumes were 10 mL/kg, based on individual body weight. Vehicle used was [redacted]

Rationale for Dose Selection: In a dose-finding study (report no. T31416.13) with 5 pregnant rats/group at 0, 600, 800 and 1000 mg/kg/day, toxicity to the dams (decreased mean body weight at all 3 doses, labored breathing at mid and high doses, and abnormal quietness and dyskinesis at high dose) were observed.

Procedure: Crl:CD(SD)BR strain rats (Charles River, UK), 25 mated females/group, were used. At initiation of treatment, the dams were 10-12 weeks of age with mean body weights of 233 to 296g. Treated rats received LAS 31416 (DL hydrogen malate) (Lot no. I002; purity not specified) once daily by oral gavage, between GD 6 and 15. Observations of the dams included mortality, clinical signs, body weight and food consumption. C-sections were done on GD 20 and included data on numbers of corpora lutea, implantations, early and late resorptions, live and dead fetuses, fetal weights and sex. About 2/3 of the fetuses from each rat were examined for skeletal abnormalities according to the method of Dawson. The remaining fetuses were fixed in Bouin's fluid and examined for visceral anomalies according to the method of Wilson.

A satellite study included an additional 10 dams/group that received 250, 500 and 1000 mg almotriptan/kg/day from GD 6 to GD 13. On GD 13, blood samples for toxicokinetics were obtained at 1, 2, 4, 8 and 24 hours after administration. Results for pregnant rats are shown on pages 82 and 89.

Results (~~includes~~ only mortality and compound related effects):

Mortality: none

Clinical Signs: Palpebal ptosis in most at low dose, in all at mid and high doses, salivation in all treated animals from 2nd or 3rd day of treatment until last day of treatment, dirty fur on several days at high dose, urine spots around genitalia in 4 and 23 rat, at mid and high doses, respectively, chromodiacryorrhea in 4 and 16 rats at mid and

high doses, cloñic contractions of hind limbs in 10 rats at high dose in the first days of treatment.

Mean Body Weight: Significantly lower at high dose compared to control between GD 7 and GD 20; dose related decrease in other groups as well (see sponsors Table 2 which follows).

Food Consumption: Dose related statistically significant decreases between GD 6 and GD 18.

PESO CORPORAL MEDIO POR GRUPO (g) ± DS
GROUP MEAN BODY WEIGHT (g) ± SD

Día de gestación *Day of gestation*	Grupo. *Group* 1 Control	Grupo. *Group* 2 250 mg/kg	Grupo. *Group 3* 500 mg/kg	Grupo. *Group* 4 1000 mg/kg
0	261 ±12	261 ±16	266 ±14	262 ±14
6	301 ±15	300 ±20	308 ±19	304 ±21
7	303 ±16	300 ±20	301 ±19	291* ±19
8	308 ±16	302 ±19	301 ±19	284*** ±19
9	312 ±15	308 ±21	308 ±20	287*** ±21
10	320 ±14	312 ±21	314 ±18	297*** ±20
11	325 ±17	317 ±20	317 ±18	300*** ±20
12	329 ±19	318 ±20	319 ±18	305*** ±22
13	335 ±18	323 ±22	324 ±17	311*** ±21
14	341 ±20	329* ±21	328* ±18	318*** ±23
15	348 ±21	335* ±22	335* ±19	324*** ±22
18	394 ±33	379 ±29	379 ±24	365** ±30
20	425 ±40	411 ±38	414 ±28	396* ±38
6-15	47 ±13	35** ±15	27*** ±12	21*** ±10
6-20	124 ±33	111 ±32	106* ±23	92*** ±23

* = LSD 5%
** = LSD 1%
*** = LSD 0.01%

Fetal Data: At high dose, more resorptions and post-implantation loss were seen than in control; although not statistically significant, these effects are considered compound related. Mean weight of male and female fetuses and of both sexes combined were lower at high dose than control (P<0.001). No effects noted on live or dead fetuses per litter.

DATOS DE LA CESÁREA
CESARIAN DATA

DATOS SOBRE LAS IMPLANTACIONES UTERINAS *UTERINE IMPLANTATIONS DATA*	**Grupo. *Group* 1** Control	**Grupo. *Group* 2** 250 mg/kg	**Grupo. *Group* 3** 500 mg/kg	**Grupo. *Group* 4** 1000 mg/kg
Número de hembras con implantaciones el día 20 de gestación *Number of females with implantations at day 20 of gestation*	21	25	23	20
Número de cuerpos lúteos. *Number of corpora lutea*	368	435	418	379
Número medio por hembra. *Mean number per female*	17.5	17.4	18.2	19.0
Número de implantaciones. *Number of implantations*	321	374	364	341
Número medio por hembra. *Mean number per female*	15.3	15.0	15.8	17.1
% Pérdidas pre-implantación. *% pre-implantation loss*	15.6	17.7	14.1	10.3
Número de reabsorciones tempranas. *Number of early resorptions*	12	6	20	26
Número de reabsorciones tardías. *Number of late resorptions*	0	11	0	4
Número medio de reabsorciones. *Mean number of resorptions*	0.6	0.7	0.9	1.5
Número total de reabsorciones. *Total number of resorptions*	12	17	20	30
Número de fetos muertos. *Number of dead foetuses*	1	0	0	0
% Pérdidas post-implantación. *% post-implantation loss*	3.4	5.5	5.9	8.8
·o de fetos vivos. *Number of live foetuses*	308	357	344	311
ɔ medio de fetos vivos. *Mean number of live foetuses*	14.7	14.3	15.0	15.6

DATOS DE LA CESÁREA
CESARIAN DATA

DATOS DE LOS FETOS *CESARIAN DATA*	**Grupo. *Group* 1** Control	**Grupo. *Group* 2** 250 mg/kg	**Grupo. *Group* 3** 500 mg/kg	**Grupo. *Group* 4** 1000 mg/kg
Número de fetos machos. *Number of male foetuses*	161	174	190	171
Número de fetos hembras. *Number of female foetuses*	147	183	154	140
% de fetos machos. *% male foetuses*	52.3	48.7	55.2	55.0
Peso medio de la camada (g). *Mean litter weight (g)*	53.5 ±16.7	50.7 ±19.2	54.0 ±15.7	48.7 ±14.7
Peso medio de los fetos (g). *Mean foetal weights (g)*	3.7 ±0.3	3.6 ±0.4	3.7 ±0.3	••• 3.1 ±0.3
Peso medio de los fetos machos (g). *Mean male foetal weights (g)*	3.8 ±0.3	3.7 ±0.3	3.8 ±0.3	••• 3.2 ±0.4
Peso medio de los fetos hembras (g). *Mean female foetal weights (g)*	3.6 ±0.3	3.5 ±0.4	3.5 ±0.3	••• 3.0 ±0.3

••• = LSD 0.1 %

adrenals		ŝ
animal identification		
aorta		
brain		ŝ
caecum		ŝ
colon		ŝ
duodenum		ŝ
eyes		ŝ
femur with bone marrow and articular surface		
gall bladder		ŝ
gross lesions		ŝ
Harderian glands	d	
head		
heart		ŝ
ileum		ŝ
jejunum		ŝ
kidneys		ŝ
lacrimal glands	d	
larynx		
liver		ŝ
lungs with mainstem bronchi		ŝ
mammary	f	ŝ
mandibular lymph nodes		ŝ
mesenteric lymph nodes		ŝ
muscle (quadriceps)		ŝ
nasal turbinates	d	
nasopharynx	d	

oesophagus		ŝ
optic nerves		ŝ
ovaries		ŝ
pancreas		ŝ
pituitary		ŝ
prostate		ŝ
rectum		ŝ
salivary glands		ŝ
sciatic nerves		ŝ
seminal vesicles		ŝ
skin		ŝ
spinal cord cervical		ŝ
spinal cord lumbar		ŝ
spinal cord thoracic		ŝ
spleen		ŝ
sternum with bone marrow		ŝ
stomach		ŝ
testes + epididymides		ŝ
tissue masses		ŝ
thymus		ŝ
thyroids + parathyroids		ŝ
tongue		
trachea		ŝ
urinary bladder		ŝ
uterus		ŝ
vagina		ŝ
Zymbal glands	d	

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

The original protocol called for a study duration of 80 weeks but following a discussion with the sponsor, they agreed to a 104 week term. However, each group remained in the study until survival reached approximately 15 in the group. Consequently, the compound was administered to group 2M for 96 weeks, to groups 1M, 3M, 4M, 5M and 4F for 98 weeks and to groups 1F, 2F, 3F and 5F for 103-104 weeks.

For toxicokinetics, blood samples were taken, when possible, from 3/sex/group, at around 1, 3, 6 and 24 hours post dosing, in weeks 26, 52 and 91.

Results

Mortality: The following table is sponsor's summary of survival and mortality during the 103-week study period.

[redacted]

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A summary of all fetal abnormalities found follows.

Control:
One fetus with dome-shaped head and nose malformed, abnormal rotation of the right hind limb. The same fetus had bone anomalies including maxillae shortened nasals shortened and zygomatic arches malformed.

250 mg/kg:
One fetus with cervical vertebral arches fused.

500 mg/kg:
One fetus with the right superior lung lobe reduced in size.
In a different litter, 7 fetuses with sacral vertebrae absent.

1000 mg/kg: :
One fetus with immature lung lobes.
One fetus with bilateral artophthalmia, another in the same litter with umbilical hernia.
One fetus with lumbar vertebrae absent.

Variations

There was a delay in the ossification of sternebrae, cervical and thoracic centra, anterior and posterior phalanges and metatarsals in the group treated with 1000 mg/kg. The delay in the ossification, limited to sternebrae 5 and 6, was observed in the 2 remaining treatment groups.

There was also an increase in asymmetrically ossified sternebrae 1 - 4 asymmetrically ossified in the groups treated with 500 and 1000 mg/kg.

The visceral examination of the fetuses presented no treatment-attributable variations.

It was concluded that there were no compound related effects on external, visceral or skeletal anomalies.

-3. Oral Gavage Developmental Toxicity Study in Rats (Vol 1.34)

Report No: T.31416.19

Performing Laboratory: [redacted]

Dates Performed: Treatment initiated 11/18/96, necropsies completed 12/4/96

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

Doses: 0 (control) and 125 mg/kg/day (dose level expressed in terms of base). The dose volumes were 10 mL/kg, based on individual body weight. Vehicle used was [redacted]

Rationale for Dose Selection: The objective of the study was to determine if the dose of 125 mg/kg/day could be considered a NOAEL.

Procedure: Crl:CD(SD)BR strain rats (Charles River, France), 25 mated females/group, were used. At the time of mating, the females were 9-10 weeks of age with mean body weights of 210 to 270g. Compound batch no. K001 (purity not specified) was used. The procedure was the same as in the previous study.

Toxicokinetics: A satellite study included an additional 15 dams/group that received 0 and 125 almotriptan/kg/day, which were treated from GD 6 to GD13.. On GD 13, blood samples for toxicokinetics were obtained at 1, 2, 4, 8 and 24 hours after administration. Summaries of results for pregnant rats are found on pages 82 and 89.

Results:

Dams: There were no deaths. Palpebal ptosis and dirty fur around the mouth was observed in most treated animals. Clonic contracture was observed in 1 treated rat on GD 7. A slight decrease in mean body weight (1.6%; n.s.) and body weight gain (15.4%; $P<0.05$) were noted.

C-Section Observations: There were apparently no effects on fetuses in the treated group compared ~~to controls~~.

Sponsor's Conclusion: The NOAEL for dam and fetal effects is 125 mg/kg/day. They attributed the slightly lower mean body weight in treated dams to a lower number of fetuses/litter, which was apparently due to higher preimplantation loss, therefore a "fortuitous effect" not due to treatment.

-4. Oral Gavage Developmental Toxicity Study in Rabbits (Vol 1.35)

Report No: ALM/25/95

Performing Laboratory: [redacted]

Dates Performed: Treatment initiated 11/18/96, necropsies completed 12/4/96

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

Doses: 0 (control), 5, 20 and 60 mg/kg/day (dose level expressed in terms of base). The dose volumes were 2 mL/kg, based on individual body weight. Vehicle used was [redacted]

Rationale for Dose Selection: In a dose-finding study (report no. ALM/24/94) with 5 pregnant rabbits/group at 0, 25, 50, 100 and 150 mg/kg/day, clinical signs in the does (red liquid on tray and reduced loose stools) at the 2 highest dose levels, reduced and loose fecal output was observed.; also decreased mean body weight and a trend toward decreased food intake at the 2 highest doses and decreased body weight gain at 50 mg/kg/day, were observed. In the uterus, there was an increase in post-implantation loss and a reduction in live fetuses at the 2 highest dose.

Procedure: New Zealand White rabbits [redacted] 20 time-mated females/group, were used. At the time of mating, the females were around 4 months of age with mean body weights of 3-4 kg. Treated rabbits received LAS 31416 (DL hydrogen malate; Lot no. I001; purity not specified) once daily by oral gavage, between GD 6 and 18. Observations on the dams included mortality, clinical signs, body weight and food consumption. C-sections were done on GD 28 and included data on numbers of corpora lutea, implantations, early and late resorptions, live and dead fetuses, fetal weights and sex and necropsy findings in the does. Viscera of all fetuses were examined by dissection, heads were examined by razor blade cuts along the fronto-parietal suture, skeletal examination was done after staining with Alizarin red S.

Toxicokinetics were obtained on GD 6 and GD 18 (after the 1st and 13th dose) from 3/sex/group designated as satellite animals in the dose-finding study in plasma obtained at predose ~~(GD 6 only)~~ 1, 2, 4, 8 and 24 hours after administration. The results are summarized on pages 83 and 90.

Results (includes only mortality and compound related effects):

Mortality: None

Clinical Signs: Slightly higher incidence than controls of does with reduced quantity/loose-liquid stools at mid and high doses.

Body Weight: During the initial 2 days of treatment, there was a slight decrease in body weight at high dose. Although the body weights of treated groups were slightly lower than controls during the treatment period and body weight gain between GD 6 and GD 18 was slightly lower ($P<0.05$), there was no statistically significant effect on mean body weight at any time.

Food Intake: There was a reduction in food intake at high dose during the treatment period, but not statistically significant.

Figure 1

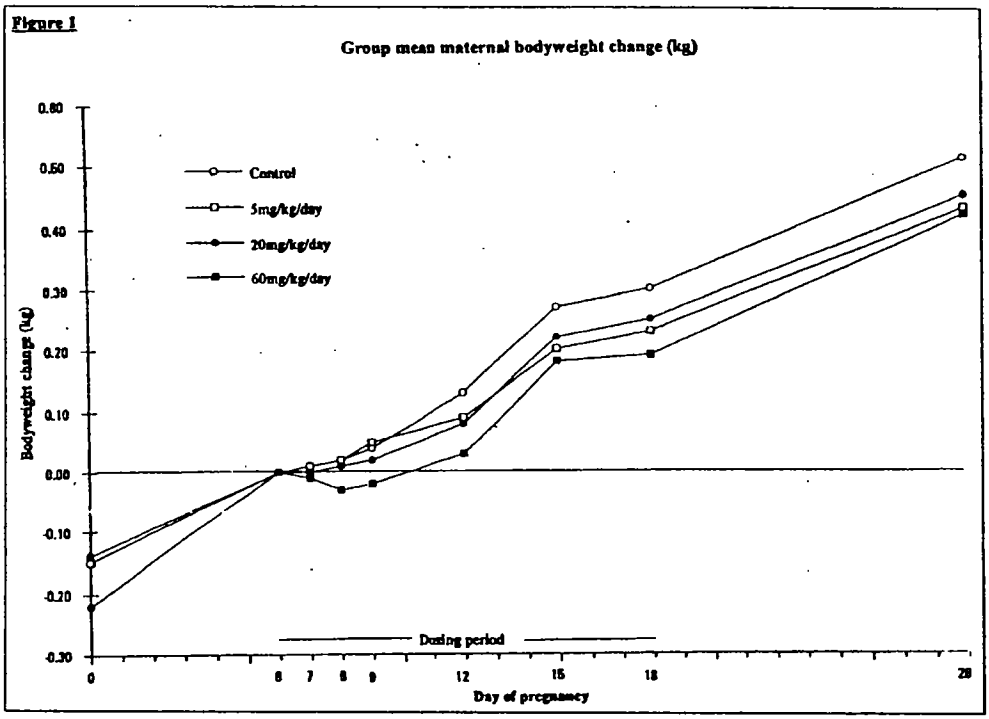

TABLE 1

Group mean maternal bodyweights (kg) ± S.D.

Group	:	1	2	3	4
Treatment	:	Control		LAS 31416	
Dosage (mg/kg/day)	:	0	5	20	60

Group	N	Day of pregnancy 6	7	8	9	12	15	18	28
1	20	3.83 ± 0.19	3.98 ± 0.33	3.99 ± 0.34	4.00 ± 0.35	4.02 ± 0.35	4.11 ± 0.37	4.25 ± 0.38	4.28 ± 0.40	4.49 ± 0.43
2	19	3.70 ± 0.29	3.85 ± 0.30	3.86 ± 0.31	3.87 ± 0.30	3.90 ± 0.30	3.94 ± 0.30	4.05 ± 0.29	4.08 ± 0.29	4.28 ± 0.37
3	17	3.73 ± 0.24	3.95 ± 0.34	3.95 ± 0.34	3.96 ± 0.36	3.97 ± 0.36	4.03 ± 0.37	4.17 ± 0.39	4.20 ± 0.39	4.40 ± 0.43
4	20	3.72 ± 0.27	3.86 ± 0.32	3.85 ± 0.33	3.83 ± 0.31	3.84 ± 0.33	3.89 ± 0.33	4.04 ± 0.34	4.05 ± 0.34	4.28 ± 0.29
Analysis of variance		NS	NS	NS	NS	NS	NS	NS	NS	NS

N = number of animals included in the mean

Fetal Effects: There was an increase in postimplantation loss ($P<0.05$) and a decrease in live fetuses/doe at high dose (n.s.). There was also an increase in pre-implantation (n.s.) at high dose, which may have contributed to the decrease in live fetuses/doe. No effect on other fetal parameters and no increase in external, visceral or skeletal abnormalities or variations were observed.

TABLE 1

Group mean pregnancy data

Group	:	1	2	3	4		
Treatment	:	Control		LAS 31416			
Dosage (mg/kg/day)	:	0	5	20	60		

Group	Number pregnant on day 28 (N)	Mean no. of corpora lutea ± S.D.	Mean no. of implantations ± S.D.	Mean no. of live foetuses ± S.D.	Mean pre-implantation loss (%)	Mean post-implantation loss (%)	Sex ratio M : F
1	20	11.0 ± 1.9	10.2 ± 2.1	9.3 ± 2.3	7.2	8.7	48 : 52
2	20	10.8 ± 1.9	9.8 ± 2.4	8.6 ± 2.5	9.4	12.2	47 : 53
3	17	11.8 ± 2.4	10.4 ± 2.6	9.2 ± 2.5	10.9	11.1	53 : 47
4	20	11.5 ± 1.8	9.9 ± 1.7	7.6 ± 2.2	13.4	22.7+	47 : 53
Analysis of variance		NS	NS	NS			
Kruskal-Wallis test					NS	$p < 0.05$	NS

N = number of animals included in the mean.
+ = statistically different from the control, $p < 0.05$, Dunn's multiple comparison test

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F. Toxicokinetics for All Repeat-Dose Studies (Vol 1.9 pages 67-77 & Vols 1.14-1.15).

Sponsor has provided summary tables of toxicokinetic data for almotriptan in non-pregnant mice, rats, dogs and rabbits obtained in most of the repeat dose toxicology studies and in pregnant rats and rabbits. They include range-finding, subchronic, chronic, oncogenicity and developmental toxicity studies. Summary results of these studies are shown in the tables that follow (pages 82 and 83).

Dose-dependent exposure after oral dosing was confirmed in all species studied at all time periods. C_{max} and AUC increased greater than in proportion to dose in mice, rats and rabbits (suggestive of saturation in liver metabolism), but in dogs, C_{max} and AUC increased only in proportion to the dose. In rats, plasma values of C_{max} and AUC were increased on Day 22 and during Week 13 of treatment, compared to Day 1, with some further possible increases by Week 26 at doses of 20 and 100 mg/kg/day but not at 500 mg/kg/day; no further increases in C_{max} and AUC by weeks 52 and 104 (males) or 95 (females). In the mouse carcinogenicity study, the earliest time period for measurement of plasma C_{max} and AUC levels was at Week 26. It was not possible to determine if C_{max} and AUC values increased with time between Day 1 and Week 26. The data suggest that AUC, but not C_{max}, increased with time of treatment between Week 26 and Week 52 in mice. By Week 91, further increases were seen only at the 2 lower doses (40 and 100 mg/kg/day) but not at the highest dose (250 mg/kg/day). In dogs, on the other hand, there were small but questionable increases in plasma C_{max} and AUC levels on Days 14 and 28, compared to Day 1, but no apparent increases in Weeks 26 or 52, compared to Day 1.

The sponsor concluded that there were generally no differences in AUC level between males and females in all animals.

Toxicokinetic studies were also performed in pregnant rats and rabbits. In rats, C_{max} and AUC increased linearly, and dose proportionately. There were little or no increases in plasma C_{max} or AUC values on Day 7 of treatment (GD 13) compared to Day1 (GD 6). On the other hand, in pregnant rabbits, C_{max} and AUC increased greater than in proportion to dose and were considerably higher on Day 13 than on Day 1 of dosing.

In every study and at all time periods after dosing in rats, mice and dogs, plasma levels of almotriptan, by 24 hours after dosing, were non-detectable or present at very low plasma concentrations; equivalent to C_{min} levels. This suggests that bioaccumulation was not occurring, in spite of increasing values of C_{max} and AUC with continued time of treatment. ~~Sponsor~~ indicated that there were no consistent gender differences and no induction of almotriptan metabolism was observed.

Summary of C_{max} and AUC Data in All Mouse, Rat, and Dog Studies, Including Pregnant Rats and Pregnant Rabbits

Table 2. Summary of Almotriptan Repeated-Dose Pharmacokinetic Data.*

Species	Dose/day	Mean Cmax (µg/mL)						Mean AUC0–∞ (µg·h/mL)					
	Mg/kg FBE	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Mice PO				Week 13						Week 13			
	20			0.062	0.064					0.102	0.112		
	160			2.03	0.86					4.55	3.61		
	320			4.82	3.75					19.8	16.7		
		Week 26		Week 52		Week 91		Week 26		Week 52		Week 91	
	40	0.130	0.221	0.202	0.258	0.231	0.352	0.250	0.410	0.486	0.472	0.465	0.600
	100	1.67	0.293	2.20	1.38	2.99	1.63	3.92	1.40	4.00	2.89	5.92	3.17
	250	2.92	5.01	4.41	6.67	5.13	5.86	15.1	24.1	24.5†	27.3	15.1	25.2
Rats PO		Day 1		Day 22				Day 1		Day 22			
	6	0.100	0.105	0.113	0.068			0.258‡	0.284‡	0.374‡	0.287‡		
	60	1.35	2.57	2.72	4.06			4.16	6.88	10.4†	13.4†		
	600	13.0	18.1	21.5	24.8			198	215	247†	338†		
		Day 1		Week 13		Week 26		Day 1††		Week 13†		Week 26†	
	20	0.197	0.160	0.859	1.08	1.00	1.43	0.842	0.590	2.67	2.83	2.86	3.03
	100	2.46	2.90	4.84	4.90	5.92	6.87	14.9	19.8	25.3	34.9	38.3	41.5
	500	9.13	9.05	39.2	26.1	21.5	32.7	130	140	406	322	261	300
		Week 26		Week 52		Week 104	Week 95	Week 26†		Week 52†		Week 104†	Week 95†
	10	0.359	0.294	0.337	0.276	0.512	0.280	1.26	0.780	1.12	0.833	1.42	1.37
	27	1.60	2.90	1.74	1.94	1.18	2.16	7.31	10.5	8.07	6.39	6.24	10.3
	75	6.09	7.74	6.55	6.41	6.82	5.23	41.7	54.0	39.8	38.3	39.7	37.4
				Day 7 (Day 13 of gestation)						Day 7 (Day 13 of gestation) †			
	250				9.00						114		
	500				16.4						254		
	1000				28.9						479		
		Day 1(Day 7 of gestation)		Day 7 (Day 13 of gestation)				Day 1(Day 7 of gestation)†		Day 7(Day 13 of gestation)†			
	125		7.66		6.54				40.4		48.5		

Data were rounded to 3 significant digits; † AUC0-24; ‡ AUC0-8; § AUC0.5-8; ¶ AUC0.5-4; **Day 1 at each dose level, rising dose; †† Day 1 at each dose level, decreasing dos
‡ n=1; §§ AUC0-4
Abbreviations: AUC = area under the concentration-time curve; C_{max} = maximum drug plasma concentration; FBE = free base equivalents; IV = intravenous; PO = oral; SC = subcutaneous

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PO		Day 1		Week 26				Day 1†		Week 26†		
	2	0.278	0.387	0.313	0.355			1.34	1.81	1.35	1.23	
	5	0.795	0.823	0.784	1.04			3.30	3.45	3.18	3.45	
	12.5	2.06	1.73	2.49	1.99			9.88	10.2	9.48	8.92	
		Day 1		Week 26		Week 52		Day 1†		Week 26†		Week 52†
	2	0.296	0.250	0.327	0.351	0.347	0.336	1.37	1.18	1.37	1.34	1.33
	5	0.685	0.851	0.703	1.02	0.824	0.871	3.72	4.21	4.00	3.93	3.29
	12.5	1.67	1.44	2.25	2.41	2.67	2.20	10.9	11.1	11.6	10.7	10.0
		Day 1**						Day 1**				
	3	0.790	0.590					2.04§	1.32¶			
	6	1.22	1.07					4.39§	3.33§			
	12	3.10	2.85					9.88§	10.3§			
		Day 1		Day 14				Day 1§		Day 14§		
	12	1.18	2.54	1.92	3.67			6.23	9.54	7.25	10.0	
		Day 1		Day 28				Day 1		Day 28		
	2	0.392	0.379	0.332	0.417			1.26‡	0.931§§	1.16‡	1.16‡	
	5	1.22	0.817	1.29	1.18			3.91‡	3.08‡	4.35‡	3.40‡	
	12.5	1.63	1.91	3.19	2.84			9.23‡	8.09‡	10.05‡	10.07‡	

Data were rounded to 2 significant digits. AUCs and AUCs

Rabbits PO		Day 1**						Day 1‡**				
	50		0.548						1.61			
	100		1.30						3.93			
	200		12.8						59.0			
		Day 1		Day 13				Day 1†		Day 13†		
	200		18.2		25.4				89.4		138	
		Day 1		Day 13				Day 1†		Day 13†		
	25		0.174		0.753				0.537		1.53	
	50		0.668		2.31				3.38		10.7	
	100		7.24		10.8				18.9		48.6	
	150		8.38		17.3				47.6		157	

Toxicokinetic parameters derived from the 104/95 week study in rats, the 91 Week study in mice and the 52 Week study in dogs are shown on the 3 pages that follow. T_{max} was around 1 hour at all 3 doses in rats and mice of both sexes. In dogs, T_{max} generally varied between 1.8 and 2.2 hours during the first 26 weeks but lower values (0.8-1.5 hours) were found after 52 weeks. In all 3 species, C_{min} or plasma levels found by 24 hours after dosing were non-detectable or very low, which suggests that bioaccumulation does not occur at the dose levels used.

Toxicokinetic Data in the 95/104 Week Rat Carcinogenicity Study (CLE Report No: 655/68-D6154)

Table XIII.- Pharmacokinetic parameters of LAS 31416 obtained in Sprague-Dawley rats in weeks 26, 52 and 95 (females) or 104 (males) of treatment with oral doses of 10, 27 or 75 mg/kg/day.

PHARMACOKINETIC PARAMETERS OF LAS 31416						
DOSE (mg/kg/day)	SEX	PARAMETER	UNITS	WEEK 26	WEEK 52	WEEK 95/104 (*)
10	M	C_{max}	µg/ml	0.359	0.337	0.512
		t_{max}	h	1.0	1.0	1
		C_{min}	µg/ml	0.009	ND	ND
		$t_{½}$	h	1.3	1.3	1.4
		AUC(0-24)	µg.h/ml	1.264	1.118	1.424
	F	C_{max}	µg/ml	0.294	0.276	0.280
		t_{max}	h	1.0	1.0	1.0
		C_{min}	µg/ml	ND	0.011	ND
		$t_{½}$	h	1.2	1.3	1.6
		AUC(0-24)	µg.h/ml	0.780	0.833	1.368
27	M	C_{max}	µg/ml	1.604	1.738	1.178
		t_{max}	h	1.0	1.0	1
		C_{min}	µg/ml	0.053	ND	ND
		$t_{½}$	h	1.5	1.6	2.0
		AUC(0-24)	µg.h/ml	7.307	8.068	6.242
	F	C_{max}	µg/ml	2.903	1.941	2.156
		t_{max}	h	1.0	1.0	1.0
		C_{min}	µg/ml	0.033	0.029	0.008
		$t_{½}$	h	1.8	1.0	2.9
		AUC(0-24)	µg.h/ml	10.45	6.389	10.29
75	M	C_{max}	µg/ml	6.087	6.551	6.624
		t_{max}	h	1.0	1.0	1
		C_{min}	µg/ml	0.232	0.291	0.008
		$t_{½}$	h	3.0	2.3	2.3
		AUC(0-24)	µg.h/ml	41.68	39.80	39.67
	F	C_{max}	µg/ml	7.744	8.413	5.230
		t_{max}	h	1.0	1.0	1.0
		C_{min}	µg/ml	1.350	0.045	0.021
		$t_{½}$	h	2.1	2.0	2.8
		AUC(0-24)	µg.h/ml	53.96	38.29	37.40

M : male
F : female
(*) : males: week 104; females: week 95
ND : not detected (<0.005 µg/ml)

Toxicokinetic Data in the103 Week Mouse Carcinogenicity Study (CLE Report No: 655/67-D6154)

Table XVII- Pharmacokinetic parameters of LAS 31416 obtained in male mice in weeks 26, 52 and 91 of treatment with oral doses of 40, 100 or 250 mg/kg/day.

PARAMETER		WEEK	DOSE (mg/kg/day)		
			40	100	250
C_{max}	(µg/ml)	26	0.130	1.666	2.921
t_{max}	(h)		1.0	1.0	1.0
C_{min}	(µg/ml)		ND	ND	ND
$t_{1/2}$	(h)		0.8	1.2	3.0
AUC(0-6)	(µg.h/ml)		0.220(*)	3.766	10.98
AUC	(µg.h/ml)		0.250	3.916	15.05
C_{max}	(µg/ml)	52	0.202	2.196	4.414
t_{max}	(h)		1.0	1.0	1.0
C_{min}	(µg/ml)		ND	ND	0.008
$t_{1/2}$	(h)		1.0	1.0	2.5
AUC(0-6)	(µg.h/ml)		0.476	3.913	15.53
AUC	(µg.h/ml)		0.486	3.997	24.52(**)
C_{max}	(µg/ml)	91	0.231	2.989	5.125
t_{max}	(h)		1.0	1.0	1.0
C_{min}	(µg/ml)		ND	ND	ND
$t_{1/2}$	(h)		0.9	0.8	1.5
AUC(0-6)	(µg.h/ml)		0.398(*)	5.865	14.048
AUC	(µg.h/ml)		0.465	5.920	15.08

ND: not detected (< 0.005 µg/ml); (*): AUC(0-3); (**): AUC(0-24)

Table XVIII- Pharmacokinetic parameters of LAS 31416 obtained in female mice in weeks 26, 52 and 91 of treatment with oral doses of 40, 100 or 250 mg/kg/day.

PARAMETER		WEEK	DOSE (mg/kg/day)		
			40	100	250
C_{max}	(µg/ml)	26	0.221	0.293	5.008
t_{max}	(h)		1.0	1.0	1.0
C_{min}	(µg/ml)		ND	ND	ND
$t_{1/2}$	(h)		0.8	2.4	3.3
AUC(0-6)	(µg.h/ml)		0.369(*)	1.159	16.173
AUC	(µg.h/ml)		0.410	1.400	24.07
C_{max}	(µg/ml)	52	0.258	1.378	6.670
t_{max}	(h)		1.0	1.0	1.0
C_{min}	(µg/ml)		ND	ND	ND
$t_{1/2}$	(h)		0.9	1.6	2.4
AUC(0-6)	(µg.h/ml)		0.464	2.577	22.05
AUC	(µg.h/ml)		0.472	2.894	27.29
C_{max}	(µg/ml)	91	0.352	1.631	5.859
t_{max}	(h)		1.0	1.0	1.0
C_{min}	(µg/ml)		ND	ND	ND
$t_{1/2}$	(h)		0.6	1.1	2.1
AUC(0-6)	(µg.h/ml)		0.566(*)	3.067	21.81
AUC	(µg.h/ml)		0.600	3.171	25.23

ND: not detected (< 0.005 µg/ml); (*): AUC(0-3)

Toxicokinetic Data in the 52-Week Dog Study (CLE Report No: 655/69-D6154)

Males

PARAMETER	WEEK	DOSE (mg/kg/day)		
		2	5	12.5
C_{max} (µg/ml)	1 (day 1)	0.296 ± 0.025	0.685 ± 0.092	1.671 ± 0.533
t_{max} (h)		1.5 ± 0.6	2.1 ± 1.4	4.0 ± 2.2
C_{min} (µg/ml)		ND	0.006 ± 0.004	0.027 ± 0.014
$t_{1/2}$ (h)		1.9 ± 0.4	2.2 ± 0.8	2.4 ± 0.8
AUC(0-24) (µg.h/ml)		1.366 ± 0.217	3.724 ± 0.333	10.91 ± 1.506
C_{max} (µg/ml)	26	0.327 ± 0.095	0.703 ± 0.283	2.246 ± 0.341
t_{max} (h)		1.9 ± 1.5	3.6 ± 3.3	2.5 ± 1.2
C_{min} (µg/ml)		ND	ND	0.012 ± 0.004
$t_{1/2}$ (h)		1.9 ± 0.2	2.5 ± 0.5	2.5 ± 0.5
AUC(0-24) (µg.h/ml)		1.371 ± 0.093	4.002 ± 0.466	11.55 ± 2.467
C_{max} (µg/ml)	52	0.347 ± 0.047	0.824 ± 0.107	2.671 ± 0.305
t_{max} (h)		1.1 ± 0.6	0.9 ± 0.3	0.6 ± 0.3
C_{min} (µg/ml)		ND	ND	0.011 ± 0.002
$t_{1/2}$ (h)		2.1 ± 0.1	2.0 ± 0.2	2.2 ± 0.6
AUC(0-24) (µg.h/ml)		1.329 ± 0.153	3.289 ± 0.669	10.02 ± 1.798

Females

PARAMETER	WEEK	DOSE (mg/kg/day)		
		2	5	12.5
C_{max} (µg/ml)	1 (day 1)	0.250 ± 0.031	0.851 ± 0.151	1.435 ± 0.387
t_{max} (h)		2.8 ± 1.5	2.0 ± 0.0	2.7 ± 1.5
C_{min} (µg/ml)		ND	0.006 ± 0.005	0.017 ± 0.005
$t_{1/2}$ (h)		1.6 ± 0.5	2.2 ± 0.6	3.1 ± 0.4
AUC(0-24) (µg.h/ml)		1.182 ± 0.246	4.206 ± 0.711	11.12 ± 1.613
C_{max} (µg/ml)	26	0.351 ± 0.080	1.018 ± 0.122	2.410 ± 1.150
t_{max} (h)		1.4 ± 0.8	1.0 ± 0.7	2.3 ± 2.9
C_{min} (µg/ml)		ND	ND	0.014 ± 0.007
$t_{1/2}$ (h)		1.8 ± 0.2	1.9 ± 0.2	2.2 ± 0.5
AUC(0-24) (µg.h/ml)		1.344 ± 0.233	3.927 ± 0.492	10.68 ± 0.971
C_{max} (µg/ml)	52	0.336 ± 0.080	0.871 ± 0.082	2.198 ± 0.308
t_{max} (h)		0.8 ± 0.3	0.9 ± 0.8	1.5 ± 1.4
C_{min} ~~(µg/ml)~~		ND	ND	0.022 ± 0.014
$t_{1/2}$ (h)		1.9 ± 0.1	2.0 ± 0.1	2.3 ± 0.9
AUC(0-24) (µg.h/ml)		1.205 ± 0.217	3.439 ± 0.552	9.461 ± 1.200

G. Aceeptability of Carcinogenicity, 52-Week Dog and Reproductive Toxicity Studies

Species comparisons for non-pregnant mice, rats and dogs to maximal allowable human doses, based on mg/kg, body surface area (mg/m^2) and systemic exposure to almotriptan based on plasma AUC levels, are shown in the table on the page that follows, The sponsor had recommended 50 mg as the highest clinical dose within a 24 hour period. On August 16, 2000, the reviewing medical officer informed us that clinical data were not sufficient to support a dose of 50 mg but that it did support 25 mg as the highest dose within 24 hours. As a result, the columns comparing animal to human dosage, based on mg/kg, mg/m^2, and animal:human systemicAUC exposure ratios had to be recalculated and are not the same as these values submitted to the executive CAC.

Doses selected for the carcinogenicity tests were based on the "AUC option". Systemic AUC exposures at the highest dose (250 mg/kg) in mice were around 40 during weeks 26 and final week, and around 52 during week 52. After corrections for differences in protein binding which is lower in mice than in humans, exposure ratios to parent, unchanged compound was around 45 during weeks 26 and final week, and at week 52. In a previously performed pharmacokinetic study, it was found that metabolic products were considerably higher in mice that received a single dose of 100 mg/kg than in humans. Thus, total drug level (parent compound and metabolites combined) were higher than 25 at all 3 time periods. The dosage based on mg/m^2 was around 41.6 fold higher in mice than in humans. At the highest dose of 75 mg/kg/day in rats, systemic AUC exposure to parent, unchanged compound was $\geq$77 or at all 3 time periods which would make the rat study acceptable even before corrections for lower protein binding and higher level of circulating metabolites in rats than in humans. Based on mg/m^2, the dosage was about 22.8 fold higher in high dose rats of the carcinogenicity study than in humans receiving the drug at maximum allowable dose. Both the mouse and rat carcinogenicity studies are acceptable if they can be based on the AUC option. If they cannot be acceptable based on the AUC option, doses selected for female groups in the mouse study are considered acceptable, based on the MTD endpoint (mortality).

Almotriptan showed no evidence of genotoxicity in the reverse mutation assay in 6 different strains of *S typhimurium* or in the mouse *in vivo* bone marrow micronucleus test. However, equivocal results were obtained in a study on induction of chromosome aberrations in cultured human blood lymphocytes and in a mouse lymphoma L5178Y cells at the *tk* locus (see report to the Executive CAC on 7/19/00 in Appendix IV). Because the highest doses selected for the rat and mouse carcinogenicity tests were based on the "AUC option", the acceptability of the rat and mouse carcinogenicity tests in support of this NDA are in question.

In the 52 week dog study, systemic plasma AUC exposures to unchanged at the highest dose (12.5 mg/kg) were around 19- to 22-fold higher on Day 1, Weeks 26 and 52 of treatment than in humans receiving the maximum allowable clinical dose. When corrected for differences in protein binding, systemic AUC exposures were 23- to 27-times higher in dogs. Based on mg/m^2, this dose was about 12.5-fold higher in dogs than

in humans. There was no compound related effect on body weight or body weight gain, but at high dose, there were a number of transient clinical signs, such as tachycardia, increased blood pressure, dilated pupils, splayed/stiff legs and abnormal gait. Subsequently cataract development were observed in 1 of 8 dogs (1 of 12 dogs in the high dose group) in each of the 3 treated groups. The cataract observed in the high dose treated dog appeared to be reversible because it was no longer present at week 56, after a 4-week treatment-free period. One of 6 females at high dose died unexpectedly during week 39, due to cardiovascular complications, which may be a drug-related effect. The study is considered acceptable in support of this NDA.

Comparison of Dosages in Animals to humans Based on mg/kg, mg/m^2 and Systemic Exposure

Species	Dose mg/kg	Ratio[4] mg/kg	Ratio[4] mg/m^2	Week 26 AUC[5] ug.hr/mL	**Week 26 Ratio of AUC[4]**	Week 52 AUC[5] ug.hr/mL	**Week 52 Ratio of AUC[4]**	**Final Week[6]** AUC[5] ug.hr/mL	**Final Week[6] Ratio of AUC[4]**
Mouse[1]	40	80	6.6	0.330	0.66	0.479	0.958	0.535	1.070
	100	200	16.6	2.298	4.60	3.446	6.892	4.546	9.092
	250	500	41.6	19.560	39.12	25.905	51.810	20.155	40.310
Rat[2]	10	20	2.86	1.022	2.044	0.974	1.948	1.396	2.792
	27	54	7.72	8.879	17.758	7.229	14.458	8.266	16.532
	75	150	22.82	47.820	95.640	39.045	78.090	38.535	77.070
Dog				Day 1 AUC ug.hr/mL	**Day 1 Ratio of AUC[6]**	Week 26 AUC ug.hr/mL	**Week 26 Ratio of AUC[6]**	Week 52 AUC ug.hr/mL	**Week 52 Ratio of AUC[6]**
Dog[3]	2	4.0	2.0	1.358	2.716	1.267	2.534	1.267	2.534
	5	10.0	5.0	3.965	7.930	3.364	6.728	3.364	6.728
	12.5	25.0	12.5	11.115	22.230	9.741	19.482	9.741	19.482
Man[4]*	0.5	1.0	1.0	0.5	1.0	0.5	1.0	0.5	1.0

[1] Based on the mouse carcinogenicity study with means for males and females combined
[2] Based on the rat carcinogenicity study with means for males and females combined
[3] Based on the 52-week dog study with means for males and females combined
[4] Based on a maximum dose of 25 mg for a human weighing 50 kg.
[5] Mean for male and female; there was no consistent differences between sexes in any of the animal studies.
[6] Week 91 in the mouse, week 95 or 104 in the rat and week 52 in the dog

Pregnant Rat Studies: The tables that follow summarize the findings in pregnant rats.

Based on GD 13 measurements (7th day of treatment), plasma AUC and C_{max} levels were generally dose proportional. At 125 mg/kg/day dose (the only dose level at which plasma measurements were made at 2 time periods during pregnancy) there was no increase in AUC_{0-24} or C_{max} on GD 13, the 7th day of treatment, compared to and GD 6, the 1st day of treatment), and no indication of bioaccumulation.

Doses listed in the tables for rats in the two developmental toxicity tests combined (125, 250, 500 and 1000 mg/kg/day) were obtained in satellite studies, performed simultaneously with the main studies, and are the exact same doses used in the main studies. At the 4 dose levels tested, systemic exposures based on AUC were 97- to 950-fold higher in pregnant rats than in humans receiving the maximum allowable clinical dose. Based on mg/m^2, the doses were about 36- to 286-fold higher in pregnant rats than in humans. Doses used in the fertility and pre and postnatal development study were

only 25, 100 and 400 mg/kg/day, but the treatment period was considerably longer. The procedure for that study was basically the same as in a pre-1994 Segment I study. At the 3 dose levels tested, systemic AUC exposures were estimated to be around 8.8- to 185-fold higher in pregnant rats than in humans receiving a maximum allowable clinical dose.

All of the rat reproductive toxicity studies are considered acceptable.

Summary of Toxicokinetics in Pregnant Rats

Dose (mg/kg)	AUC_{0-24} (ug.hr/mL) Gestation Day 6	13	C_{max} (ug/mL) Gestation Day 6	13
125	40.4	48.5	7.66	6.54
250		114		9.0
500		254		16.4
1000		479		28.9

Comparisons of Pregnant Rat to Human Doses and Exposures*

Dose mg/kg	**Ratio Based on mg/kg**	**Ratio Based on mg/m^2**	**Ratio Based on AUC**
125	250	35.7	97
250	500	71.4	228
500	1000	142.9	508
1000	2000	285.7	950

Pregnant Rabbit Study:

In the tables that follow, all comparisons of animal to human doses and AUCs are based on a 50 kg man receiving the maximal allowable clinical dose. In the first table, the results were obtained in a satellite developmental toxicity study, performed simultaneously with the dose-finding study, not with the main study. The doses in the main study, were only 5, 20 and 60 mg/kg/day; considerably lower than the doses in the satellite dose-finding study. Therefore, rabbit:human AUC exposure ratios for the main study (3rd table) had to be extrapolated from results obtained in the dose-finding study (1st table).

Systemic exposures on both GD 6, the 1st day of treatment, and GD 19, the 13th day of treatment, were considerably greater than dose proportional, which suggested saturation of metabolic enzymes. AUC values on GD 19 were 2.6 to 3.3 times higher than on GD 6, which may suggest bioaccumulation. Systemic exposures based on AUC were 0.2- to 7.4-fold higher on GD 6 and 0.6- to 23.2-fold higher on GD 13 in pregnant rabbits than in humans that received the maximum allowable clinical dose. Based on mg/m^2, these doses were 3.3, 13.3 and 40 times human dose level. Although dose-limiting toxicity was not apparent in the does, the study is considered adequate because dose-limiting toxicity (decreased body weight) was observed at 100 mg/kg/day in the dose-finding study.

Summary of Toxicokinetics in Pregnant Rabbits

Dose (mg/kg)	AUC_{0-24} (ug.hr/mL) Gestation Day 6	19	C_{max} (ug/mL) Gestation Day 6	19
25	0.54	1.53	0.17	0.75
50	3 .39	10.7	0.67	2.31
100	18.9	48.6	7.24	10.8
150	47.6	157	8.38	17.3

GD 6 and GD 19 correspond to 1st and 13th day of treatment, respectively

Comparisons of Pregnant Rabbit to Human Doses and Exposures in the Dose-Finding Study*

Dose mg/kg	Ratio Based on mg/kg	Ratio Based on mg/m^2	Ratio Based on AUC on GD6	on GD 19
25	50	16.7	1.08	3.06
50	100	33.3	6.78	21.40
100	200	66.7	37.80	97.20
150	300	100.0	95.20	304.0

By extrapolation from above, the following doses and exposures were found for the definitive study.

Comparisons of Pregnant Rabbit to Human Doses and Exposures in the Definitive Study*

Dose mg/kg	Ratio Based on mg/kg	Ratio Based on mg/m^2	Ratio Based on AUC on GD6	on GD 19
5	10	3.3	0.2	0.6
20	40	13.3	0.8	2.4
60	120	40.0	7.4	23.2

* Based on a maximum dose of 25 mg in a human weighing 50 kg

IV. OVERALL SUMMARY AND EVALUATION

Axert tablets, containing 8.75 and 17.5 mg of the D,L Hydrogen malate salt of almotriptan, equivalent to 6.25 and 12.5 mg almotriptan, is being developed for use in acute treatment of migraine headaches. The recommended dose is a single 6.25 or 12.5 mg tablet with a maximum allowable dose of 25 mg within 24 hours. In the present NDA submission, the sponsor had recommended 50 mg as the highest clinical dose within a 24 hour period. On August 16, 2000, the medical officer, Dr. Armando Oliva, informed us that clinical data were not sufficient to support a 50 mg dose but that it did support 25 mg as the highest dose within a 24 hour period. The data originally presented to the executive CAC for assessment of the carcinogenicity study (dated July 19, 2000), the minutes of the executive CAC (July 26, 2000) and the correction to the file that followed (August 10, 2000) were all based on a maximum dose of 50 mg in humans. However, this should not change the present recommendations by the committee on pharmacology requirements for final acceptability of the present NDA because human AUC had been derived from plasma levels in men that received a 25 mg dose.

Pharmacology

Like other triptans of its class, almotriptan is considered to be a selective agonist at the 5-HT_{1B} and 5-HT_{1D} receptor sites; it also binds selectively to the 5-HT_{1F} receptors. It is believed to act selectively on extracerebral, intracranial arteries to inhibit excessive dilation of these vessels in migraine.

A series of *in vitro* and *in vivo* studies relating to the proposed indication have been conducted with almotriptan. This drug was found to be a potent vasoconstrictor in isolated cerebral arteries and vasculature considered to be involved in migraine expression. In cat and dog models, i.v. doses of almotriptan and other triptans produced dose-related constriction of carotid artery vascular bed, increases in carotid vascular resistance and concomitant reductions in carotid blood flow. However, the drug caused constriction of ophthalmic artery preparations (as a test for possible ischemic optic neuropathy) but it had little effect on induction of coronary artery vasospasms. Intravenous infusion of as little as 1 mg/kg of almotriptan in 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. When studied in conscious telemetered cynomolgus monkeys, almotriptan caused a small but 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. In dogs, there were dose-related increases in heart rate within 1 hour after oral dosing (with as little as 2 mg/kg), and by the subcutaneous or intravenous routes. It also caused shortening of the PR and QT intervals after oral administration in dogs.

Absorption, Distribution, Metabolism, Excretion and Pharmacokinetics

Oral bioavailability was 37% in rats, 82% in dogs, and 19% in monkeys, suggesting incomplete absorption. . The volume of distribution 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). Almotriptan was rapidly eliminated with a short half-life of 0.8, 2.0 to 2.3 and 1.3 hours after i.v. administration in rats, dogs and monkeys, respectively. 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.

Dose dependent systemic exposures were observed in all species studied, after single or repeated oral dosing. Systemic exposure (based on C_{max} and AUC) increased greater than in proportion to dose at all time periods in mice, rats, dogs and pregnant rabbits, (attributed to saturation of liver enzymes) and there were no consistent gender differences. Plasma AUC levels were generally observed to increase with time of dosing up to around Week 13 or 26 in rats and Week 52 in mice, but in dogs little or no increases were observed at any time period, even after 52 weeks, compared to Day 1. Following every dose at all time periods after dosing in all three species, plasma levels were either not detectable or at very low levels by 24 hours after dosing. It was suggested that there was no bioaccumulation and no induction of almotriptan metabolism were observed after repeated oral dosing.

In mice and rabbits, M2 metabolite levels were markedly higher than parent compound, whereas in dogs, M2 levels were about half those of almotriptan. In rats, M2 metabolite was markedly higher in plasma when administered at 5 mg/kg but markedly lower than at 100 mg/kg. This is considered as evidence for saturation of liver enzymes in rats and rabbits but not in dogs. Studies were also performed in pregnant rats and rabbits. C_{max} and AUC increased linearly with dose in pregnant rats (based on results of a single study) and increased greater than in proportion to dose in pregnant rabbits.

Almotriptan was extensively metabolized in all animal species and man. The major routes of metabolism 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. Based on findings in plasma, urine and feces, qualitative similarity in metabolite profiles was observed across all species, including man, with no evidence of differences based on gender or route of drug administration. All metabolites observed in human plasma were also observed in animals.

In tissue distribution studies with albino rats, radioactivity was widely distributed 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. Unchanged parent compound and metabolites were excreted in milk in rats. In studies with pigmented rats, concentrations of almotriptan in the eyes were very high and declined very slowly (half-life of 22 days), consistent with melanin binding, commonly observed for other triptans. Generally, no notable accumulation of almotriptan was noted in other tissues, or in the eyes of albino rats.

Protein binding in all species tested was low. By ultrafiltration, mean unbound fraction was 41.1, 45.3 and 17% in rats, dogs and humans. By equilibrium dialysis, mean unbound fractions were between 66.3 and 75.2% in animals (mouse, rat, rabbit and dog) and between 54.8 and 59.9 in humans. Almotriptan does not bind extensively to serum proteins.

Toxicology

Acute toxicity studies, with doses up to 2000 mg/kg, were performed in mice and rats. In both species, the highest non-lethal dose was 1000 mg/kg and deaths occurred within 48 hours after oral dosing. Clinical signs which generally preceded death included palpebral ptosis, tremors, abnormal gait, mydriasis (only in rats), and clonic convulsions.

Doses for a 4-week rat study were 0, 6, 60 and 600 mg/kg/day (n=10/sex/group) and for the 26-week rat study doses were 0, 20, 100 and 500 mg/kg/day (n=20/sex/group). In the 4 week study, there was no mortality. In the 26 week study, there were 10 deaths, including 2 control males at weeks 13 and 26, one low dose female at week 25, two mid dose males at weeks 13 and 19, four high dose males at weeks 5, 13, 18 and 25 and one high dose female at week 13. Cause of deaths in all but 2 was considered to be eye lesions due to bleeding or misintubation. However, the 2 high dose males had massive necrosis of the liver. In both studies, clinical signs, consisting of salivation, paddling and ptosis, were observed following dosing. Effects on clinical pathology at high dose included increases in ALP in both studies and in AST only in the 4-week study. Urinalysis revealed an increase in urinary protein in both sexes at high dose. Increased liver weights and increased follicular cell height of the thyroids observed microscopically were seen at doses of 500 and 600 mg/kg/day. Other findings at the 500 mg/kg/day dose of the 26 week study included increased adrenal and kidney weights in both sexes and increased heart weight in females, histopathology changes also included centrolobular hypertrophy ~~or fib~~rosis 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 weight gain in the 4 or 26-week studies. (See figures 1 and 2 that follow for body weights in the 26-week study.)

Doses given in the 4, 26 and 52 week dog studies were 2, 5 and 12.5 mg/kg/day, resulting in AUC systemic exposures at the highest dose that were between 8.9 to 10.1 times human AUC levels in humans receiving a 25 mg dose. Clinical signs observed at all 3 doses (all transitory, lasting about an hour after each dose) included stiff or splayed legs, vocalization, and in the 4 week study at the 2 higher doses, it included aggressiveness, excitability, hyperactivity, nervousness and tremors. Effects on the heart were observed in all 3 studies and included increased heart rate at all 3 doses, decreased PR and QT intervals at the 2 higher doses. In the 52-week study, 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. 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. One female at high dose had a sudden death; microscopic findings in the heart of this animal included hemorrhage in the myocardium and coronary groove, coronary arteritis and prominent vascular engorgement in other tissues, suggesting a pre-terminal cardiovascular event. After the 4 week treatment free period (Week 55 of the study), heart rate of the animals (high dose) were decreased, compared to controls and pre-dose values, which suggests that the dogs had not recovered from the compensatory mechanisms involved in the cardiac toxicity.

Effects on the eyes, observed each day within the first hour of treatment at all 3 doses included dilated pupils. At the Week 51 ophthalmology exam, three males, one at each of the 3 dose levels, had unilateral, "slight" opacities of the cornea. The eyes of these dogs had been clear when previously 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. However, this finding cannot be ignored as a possible effect due to a prolonged treatment in dogs. Corneal opacities in dogs are also known to occur due to chronic treatment with other triptans, such as sumatriptan.

Doses selected for the mouse carcinogenicity test (based on the AUC option), were 40, 100 and 250 mg/kg/day, resulting in systemic plasma AUC exposures to parent, unchanged drug at high dose that were 18 and 24 times higher than for humans at weeks 26 and 52 and end of the study. When corrected for protein binding and metabolic products, systemic plasma AUC levels were $\geq$25 at all 3 time periods. Because of low survival, several groups in the mouse study had to be terminated by 96 or 98 weeks instead of 104 weeks, including all treated and control males and high dose females. A statistically significant increase in mortality was noted in high dose females when compared to the combined control groups, and an increasing trend in mortality was seen across the ~~female~~ groups. There were no statistically significant differences in incidences of tumors in high dose males or females compared to controls. There were also no treatment-related effects on gross or microscopic pathology. With the possible exception of increased mortality in high dose females, there were no drug-related effect of any kind noted and virtually no indication of toxicity or of any effect, even at the highest dose. There was no specific effect that could relate to the higher incidence of mortality in high dose females. At the meeting of 7/18/00, the executive-CAC concluded that female groups in the mouse carcinogenicity test were adequate, based on MTD (mortality).

Doses selected for the rat carcinogenicity study, (based on the AUC option) were 10, 27 and 75 mg/kg/day, resulting in systemic plasma AUC exposures were ≥35 times greater than for humans at all 3 time periods. Female rats in all control and treated groups had to be terminated at week 84 instead of at scheduled sacrifice of 104-weeks because of low survival. No dose limiting or compound related effect of any kind was noted in the rat carcinogenicity study. This study would be considered to be acceptable based on the AUC option if negative results are obtained in the genotoxicity tests that are presently in progress.

Negative results for mutagenic and clastogenic activities were obtained in an Ames test with 5 strains of *S typhimurium*, in a and in bone marrow cells of a mouse micronucleus test. However, equivocal results were obtained in a test for chomosomal aberrations in human lymphocytes in the absence of S9 metabolic activation (not in the presence of S9) and in a test with the mouse lymphoma L5178Y cell line at the *tk* locus. Consequently, because dose levels for the carcinogenicity tests were based on the AUC option and the AUC option may not be valid for rats of both sexes and male mice. If it is affirmed that the drug is genotoxic, additional studies may be required by the CAC.

In the rat developmental toxicity studies, doses tested were125, 250, 500 and 1000 mg/kg./day; resulting in plasma systemic AUC exposures were around 44, 104, 231 and 435 times higher for rats than for humans. An increased number of dead fetuses/litter (n.s.) and a statistically significant decrease in mean fetal weight at the highest dose of 1000 mg/kg/day were seen. Although the increase in fetal loss was not statistically significant, the sponsor considered it to be compound related, even indicating it in their proposed labeling. There was a delay in ossification of sternebrae in all treated groups, and at the high dose delayed ossification also occurred in the sternebrae, cervical and thoracic centra, anterior and posterior phalanges and metatarsals. A second developmental toxicity study was performed with a single dose of 125 mg/kg/day because toxicity to the dams (as evidenced by clinical signs, decreased mean weight and body weight gain of the dams) had been observed even at the lowest dose of 250 mg/kg/day. At the 125 mg/kg dose, some clinical signs and decreased weight gain (attributed to preimplantation loss and therefore not considered compound related by the sponsor) were evident in the dams, but no compound related fetal effect was noted.

In the rabbit developmental toxicity study, doses tested were 5, 20 and 60 mg/kg/day; resulting in systemic exposures after the first dose (GD 6) in pregnant rabbits were around 0.13, 0.4 and 4.1 times higher for rabbits than for humans; after the 13th dose (GD 19) they ~~were 0.3,~~ 1.1 and 11.6 times higher in rabbits because of bioaccumulation resulting from repeated dosing. An increase in post-implantation loss compared to control was observed even at the lowest dose of 5 mg/kg/day, but this effect was significant ($P<0.05$) only at the highest dose. An accompanying decrease in mean number of live fetuses per doe was observed (n.s.) only at the high dose.

A fertility, pre and postnatal development study was carried out in rats; generally similar to the pre-1994 required "Segment 1" test. Males and females were treated before

mating, during mating, throughout pregnancy and lactation with doses of 25, 100 and 400 mg/kg/day, resulting in plasma systemic AUC exposures were calculated to be around 19.5, 78 and 406 times higher for rats than for humans. There was an apparent disruption of the estrous cycles such that mid and high dose females were in estrus (based on vaginal smears) for a greater number of days than controls, resulting in longer lasting estrous cycles ($P<0.05$ at high dose). In females at high dose, there was an 18.7% reduction in fertility rate ($P< 0.05$; based on number of females that were paired but did not become pregnant) and a 17.1% reduction in fecundity rate ($P<0.05$; based on decrease in number of females that had mated but did not become pregnant) compared to controls. There were no decreases in corpora lutea count/dam, but pre-implantation loss was higher at mid and high doses compared to control (n.s.). Mean duration of pregnancy was slightly prolonged and mean number of live pups at birth was slightly (9.2%) lower at high dose. Mean pup weights at high dose were significantly lower than controls throughout the lactation period.

V. RECOMMENDATIONS

This NDA should be approved if the results of the repeated genotoxicity studies are clearly negative. On August 24, sponsor indicated by telephone that the unaudited reports of the in vitro human lymphocyte and the in vitro mouse study studies, which they were asked to repeat, should be received by CDER by September 11, 2000.

3 page(s) of revised draft labeling has been redacted from this portion of the review.