Statistical Analysis - Sponsor conducted analysis - The statistical analysis is the same as that described for Study # TT#95-076-0: L-748,731: One Hundred-Six-Week Oral Carcinogenicity Study in Rats.

Additional analysis - Dr. Baldeo Taneja provided a statistical consult. It was requested that Dr. Taneja conduct the following statistics: [1] comparison of the individual tumor types to each control separately as well as combined and [2] combination of the following tumors for analysis: a. lung adenoma and adenocarcinoma; b. hepatocellular adenoma and adenocarcinoma; c. uterine leiomyoma and leiomyosarcoma; and d. all sarcomas - large intestine leiomyosarcoma, liver hemangiosarcoma, uterus hemangiosarcoma, leiomyosarcoma and endometrial stroma sarcoma, testis hemangiosarcoma, skin fibrosarcoma, hemangiosarcoma, osteosarcoma, spleen hemangiosarcoma, cranial and facial osteosarcoma, eye and tail rhabdomyosarcoma, and primary site undetermined osteosarcoma, hemangiosarcoma, and histiocytic sarcoma. Dr. Taneja indicated that combining the controls increased the sensitivity of the analysis and, therefore, comparisons were made to combined controls only. Tumor and mortality data were analyzed by methods described for Study # TT#95-076-0: L-748,731: One Hundred-Six-Week Oral Carcinogenicity Study in Rats.

Results

Clinical Observations - Neither a narrative description nor a tabular presentation of clinical observations was provided. However, the Sponsor reports that there were no drug-related clinical signs. The Sponsor also reports that no clinical signs or nonspecific clinical signs were observed in premature decedents.

Mortality – According to the Sponsor, there was a statistically significant increase in mortality with increasing dose in males. Although not statistically significant, the increase in mortality with increasing dose in females was considered biologically relevant. This is in agreement with the statistical analysis conducted by Dr. Taneja.

Drug related mortality was observed at ≥10 mg/kg/day. The table below indicates total mortality, and drug and non-drug related mortality [according to the Sponsor]. Drug-related mortality [1] generally showed a dose-dependent relationship; [2] tended to be higher in males; [3] generally was secondary to intestinal ulceration and/or peritonitis; and [4] generally was associated with no clinical signs or nonspecific clinical signs prior to death or sacrifice.

Dose Group	Total Mortality		Drug-Related Mortality		Mortality Unrelated to Drug	
	Female	Male	Female	Male	Female	Male
Vehicle 1	18 [36%]	21 [42%]	-	-	18 [36%]	21 [42%]
Vehicle 2	27 [54%]	21 [42%]	-	-	27 [54%]	21 [42%]
5 mg/kg/day	20 [40%]	20 [40%]	0	0	20 [40%]	20 [40%]
10 mg/kg/day	20 [40%]	28 [54%]*	1 [2%]	4 [8%]	19 [38%]	24 [48%]
20 mg/kg/day	18 [36%]	32 [64%]*	1 [2%]	7 [14%]	17 [34%]	25 [50%]
30 mg/kg/day	25 [50%]	27 [54%]*	4 [8%]	10 [20%]	21 [42%]	17 [34%]

*Indicate statistically significant change compared to mean of the two controls.

Mortality should ideally be <50%. The level of mortality shown in this study was, in general, considered acceptable, as there were a minimum of 20 animals remaining at the end of the study. The exception was for males administered 20 mg/kg/day, in which there were 18 animals that survived to the end of the study.

The timing of these unscheduled deaths or sacrifices is indicated in the table below.

Dose Group	Weeks 1-52		Weeks 53-72		73-89		Weeks 90-105/6	
	Female	Male	Female	Male	Female	Male	Female	Male
Vehicle 1	3	4	4	1	6	7	5	9
Vehicle 2	3	2	6	0	10	3	8	16
5 mg/kg/day	1	5	3	5	6	3	10	7
10 mg/kg/day	1	7	4	4	4	7	11	10
20 mg/kg/day	1	6	2	10	6	5	9	11
30 mg/kg/day	3	7	4	4	6	9	12	7

The most common causes of death, excluding those related to GI toxicity, included: [1] urinary obstruction in males [31 mice]; [2] skin abscessation and/or ulceration in males and females [13 and 10 mice respectively]; [3] lung adenocarcinoma in males and females [10 and 11 mice, respectively]; and [4] lymphoma in males and females [11 and 26 mice, respectively]. The other causes of death were sporadic and/or occurred at the same frequency across dose groups.

Body weight – There were no drug-related changes in body weight or body weight gain.

Ophthalmic Examination – The Sponsor reports that there were no drug-related changes. A tabulation of these data were not provided.

Hematology – Hematology was reported for 3 animals.

Gross Necropsy - Drug-related lesions included large intestinal ulceration with/without perforation and generally a secondary peritonitis. These lesions correlated with the increase in mortality. Other findings were considered incidental. The Sponsor states that any clinical signs seen in this study were commonly observed in mice in their facility. A tabulation of these signs was not provided.

Histopathology – Non-neoplastic Changes - No statistical analysis was conducted on these data. The table below delineates histopathological findings that were considered treatment related or potentially treatment related.

Lesion	Dose/mg/kg/day											
	0		0		5		10		20		30	
	F	M	F	M	F	M	F	M	F	M	F	M
Stomach	[50]*	[50]	[49]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]
Glandular mucosa erosion/ulcer/scar/ perforation	-	-	-	2	-	1	1	5	3	3	3	4
Proliferative gastritis	5	12	7	11	8	5	4	6	3	8	7	9
Large Intestine	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]
Focal infarction	-	-	-	-	1	-	-	-	-	-	-	-
Perforation, ulcer and/or scar	-	-	-	-	-	-	2	5	1	9	8	10
Pancreas	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]
Purulent inflammation	-	-	-	-	-	-	-	-	-	2	1	1
Peritoneum	[4]	[4]	[5]	[2]	[6]	[0]	[10]	[8]	[5]	[8]	[14]	[11]
Peritonitis	-	-	-	-	-	-	2	6	1	8	7	10
Liver	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]
Extramedullary hematopoiesis	2	-	4	-	2	3	3	4	2	4	6	4
Spleen												
Extramedullary hematopoiesis	15	6	18	4	9	3	15	14	6	14	13	18
Periarteriolar region, lymphocytic necrosis	-	2	-	1	1	1	-	4	-	4	1	4

Lesion	Dose/mg/kg/day											
	0		0		5		10		20		30	
	F	M	F	M	F	M	F	M	F	M	F	M
Lymph node	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]
Extramedullary hematopoiesis	5	8	3	5	5	4	4	12	3	12	3	9
Lymphoid hyperplasia	5	9	8	5	14	6	7	4	10	6	4	10
Plasma cell hyperplasia	4	11	4	6	-	8	6	14	4	11	5	15
Bone marrow	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]
Increased granulopoiesis	15	20	17	13	16	15	22	24	13	21	18	24
Kidney	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]
Papillary necrosis**	-	3	-	1	-	3	1	5	1	4	3	3
Tubular dilatation	5	3	2	1	2	4	3	4	3	5	2	1
Proximal convoluted tubule, hyaline droplets	1	-	3	1	4	-	3	-	2	-	1	-
Proximal convoluted tubule, multifocal necrosis	-	-	-	-	-	-	1	1	1	-	-	1
Tubular hyperplasia	2	-	1	2	2	-	1	-	1	-	2	1
Chronic nephritis	6	5	6	5	7	9	3	4	6	4	5	7

*Number in parentheses indicates number of animals evaluated for a given tissue

** Severity scores for renal papillary necrosis – 1=very slight, 2=slight, 3=moderate, 4=marked, 5=severe

	Females	Males
VH1	-	3,2,3
VH2	-	2
5 mg/kg	-	1,3,4
10 mg/kg	3	2,2,3,3,4
20 mg/kg	4	1,2,3,5
30 mg/kg	3,3,3	3,4,5

The stomach lesions were combined since they were considered a continuum from erosion to perforation and scarring. Analyzed in this fashion, the data indicates that there may be a slight increase in the incidence of glandular mucosal lesions.

In general, large intestinal ulceration/perforation and peritonitis were observed concomitantly. With the exception of the renal lesions, the other histopathological findings were considered likely to be secondary to the large intestine ulceration/perforation.

In Study TT#95-610-0: Fourteen-Week Oral Range-Finding Study in Mice, the Sponsor classified very slight renal papillary necrosis, which was observed in a single female in the low dose, as drug-related based on similar observations in rats administered L-748,731 as well as the fact that this lesion has been observed in rodents following administration of nonspecific COX inhibitors. In the present study, there was a slight increase in the incidence of the lesion in females. In males, a dose dependent relationship for the incidence of papillary necrosis was not observed, but severity tended to be increased. Papillary necrosis was frequently observed in addition to one of the following renal lesions in the male dose groups: [1] urinary obstruction; [2] renal amyloidosis; [3] chronic nephritis; and [4] acute and chronic pyelonephritis. These data do not demonstrate a definitive causal relationship between drug administration in mice and the induction of renal papillary necrosis. However, a drug-related relationship can not be ruled out. Tubular lesions, specifically tubular basophilia, have been observed in rats and mice following oral administration of L-748,731 [at higher doses], and therefore, the incidence of tubular hyperplasia are included in the above table.

There was considerable autolysis in the gastrointestinal tract for this study, especially in the small intestine.

Although the Sponsor states that "autolysis does not preclude examination for neoplastic and obvious non-neoplastic changes", this finding in a target organ could potentially obscure lesions. In addition, it may account for the fact that small intestinal ulceration was not observed more frequently in this study. The table below outlines the incidence of autolysis observed in all groups.

Dose Group*	Autolysis -# [%]	
	Female	Male
Group 1 - Vehicle	5 [10%]	4 [8%]
Group 2 - Vehicle	4 [8%]	8 [16%]
Group 3 - 5 mg/kg/day	4 [8%]	6 [12%]
Group 4 - 10 mg/kg/day	1[2%]	8[16%]
Group 5 - 20 mg/kg/day	7 [14%]	9 [18%]
Group 6 - 30 mg/kg/day	4 [8%]	13 [26%]

*N = 50

Neoplastic and associated non-neoplastic changes – The following summary table indicates the incidence of the most commonly observed neoplastic lesions in this study. Also included are selected proliferative/inflammatory lesions observed in organs that were sites for the more commonly occurring neoplasias.

Lesion	Dose/mg/kg/day											
	0		0		5		10		20		30	
	F	M	F	M	F	M	F	M	F	M	F	M
Liver	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]
Neoplastic lesion												
Hepatocellular adenoma	1	4	-	11	-	4	-	2	1	4	-	6
Hepatocellular carcinoma	-	3	-	2	-	4	-	1	-	3	-	4
Non-neoplastic lesion												
Basophilic Focal Cellular Alteration	-	7	1	2	-	3	-	3	-	1	1	1
Eosinophilic Focal Cellular Alteration	-	6	-	6	-	4	1	4	4	1	-	2
Necrosis	-	3	4	4	4	1	5	1	4	4	4	4
Adrenal	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]
Neoplastic lesion												
Cortex, adenoma	1	7	-	12	-	7	-	13	1	9	-	8
Benign spindle cell tumor	2	1	-	3	1	1	-	2	1	1	-	2
Malignant spindle cell tumor	-	-	1	-	-	-	-	-	-	-	-	-
Non-neoplastic lesion												
Cortex, Focal hyperplasia	5	14	5	25	5	21	4	17	2	22	4	11
Cortex, Focal hypertrophy	-	13	1	7	2	10	1	5	2	5	1	6
Spindle cell formation	43	24	40	18	44	18	48	20	43	15	45	23
Ovary –**Neoplastic lesion**	[50]		[50]		[50]		[50]		[50]		[50]	
Cystadenoma	4	-	2	-	1	-	2	-	5	-	3	-
Benign luteoma	3	-	2	-	2	-	2	-	-	-	3	-
Malignant luteoma	-	-	-	-	-	-	-	-	-	-	1	-
Uterus	[50]		[50]		[50]		[50]		[50]		[50]	
Neoplastic lesion												
Leiomyoma	-	-	-	-	1	-	-	-	5	-	1	-
Leiomyosarcoma	-	-	-	-	1	-	1	-	1	-	1	-
Polyp	1	-	1	-	2	-	2	-	-	-	2	-
Non-neoplastic lesion												
Cystic dilatation	-	-	4	-	1	-	3	-	2	-	-	-
Endometrial cystic hyperplasia	30	-	33	-	30	-	33	-	30	-	32	-

Lesion	Dose/mg/kg/day											
	0		0		5		10		20		30	
	F	M	F	M	F	M	F	M	F	M	F	M
Lung	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]
Neoplastic lesion												
Adenocarcinoma	2	5	3	9	6	4	5	2	3	8	6	10
Adenoma	7	7	7	8	8	8	5	6	6	4	3	6
Non-neoplastic lesion												
Alveolar epithelial hyperplasia	-	3	1	2	-	2	1	1	1	-	-	-
Eye	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]
Neoplastic lesion												
Harderian gland adenocarcinoma	-	-	-	-	1	-	1	-	-	-	-	1
Harderian gland adenoma	2	2	4	3	2	4	5	5	1	5	10	4
Non-neoplastic lesion												
Harderian gland, focal hyperplasia	-	-	-	1	-	1	-	3	-	1	-	1
Primary site undetermined	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]
Lymphoma	3	8	9	5	9	-	10	2	4	5	12	3

*Number in parentheses indicates number of animals evaluated for a given tissue.

Tumors with comparable incidences across groups included pituitary adenoma, mammary gland adenocarcinoma, splenic hemangiosarcoma, and histiocytic sarcoma,. The other neoplasias observed occurred sporadically [≤2% overall incidence/sex] and/or at a similar frequency across groups. The Sponsor states that " the nature of neoplasms found in this study was similar to the kind seen in untreated mice of this strain maintained in these laboratory conditions." The other histopathological lesions observed in this study were comparable in incidence and severity across all groups and were consistent with changes associated with age.

According to the Sponsor, prior to adjustment for multiplicity of statistical tests, a statistically significant increase was observed in the incidence of [1] Harderian gland adenoma in females at 30 mg/kg/day [p=0.009]; [2] uterine leiomyoma in females at 20 mg/kg/day [p=0.013]; and [3] lung adenocarcinoma in males at 30 mg/kg/day [p=0.011]. The Sponsor concludes that these are not treatment related because [1] "there is no known mechanism of action to suggest that these tissues are a target organ; [2] "this type of finding could easily arise purely by chance due to the number of statistical tests performed"; and [3] these findings were not observed in Study TT #96-605-0:1; One Hundred-Four-Week Oral Carcinogenicity Study in Mice, in which a higher dose was administered [e.g. 60 mg/kg/day]. In addition, the uterine leiomyoma did not demonstrate a dose dependent relationship, and there was no increase in males in the incidence of Harderian gland adenoma. Following adjustment for multiplicity of tests, there was no statistically significant trend for any single tumor type. This is in agreement with Dr. Taneja's analysis.

Demonstration of a dose-dependent response does provide support to a conclusion that an effect is toxicologically relevant even in the absence of statistical significance. However, the argument that the Harderian gland adenoma and leiomyomas in females and lung adenocarcinomas in males were not treatment related due to lack of a dose dependent relationship does not take into consideration the possibility that the mechanisms involved in the induction of this tumor may be overwhelmed at higher doses [e.g. a bell shaped dose response]. However, as noted by the Sponsor, due the narrow range of the doses [e.g. 2-8 mg/kg/day] a bell-shaped curve would be unusual.

The fact that an increase in the incidence of Harderian gland adenomas was not observed in males does not eliminate the potential for gender related differences. The incidence of Harderian gland adenoma in female mice described in historical control data [1983-1990; 21 month studies: Appendix 1: p. 3; Submission date April 1, 1999], for studies conducted in the same facility in France in which this study was conducted, ranged from 0-4% [overall incidence was 6/300 (2%)]. The incidence of Harderian gland adenoma in female mice, described for the U.S. facility historical control data [1989-present; 24 month

studies: Appendix 1: p. 6; Submission date April 1, 1999] ranged from 0-12% [overall incidence was 34/800 (4.2%)]. A treatment-related effect can not be ruled out. However, an analogous structure does not exist in humans. Therefore, this finding is difficult to extrapolate to humans. Following adjustment for multiplicity of tests, the increase was not statistically significant. This is in agreement with the analysis conducted by Dr. Taneja.

The incidence of endometrial hyperplastic lesions in aged mice is common. It has been suggested that endometrial hyperplasia may progress to leiomyomas or leiomyosarcomas.[3] The incidence of hyperplasia in the 20 mg/kg/day group was comparable in incidence and severity to both of the controls. The incidence of uterine leiomyoma in this study was 0, 0, 2%, 0, 10%, 2% at 0, 0, 5, 10, 20, and 30 mg/kg/day. The incidence of uterine leiomyoma described in historical control data [1983-1990; 21 month studies: Appendix 1: p. 3; Submission date April 1, 1999], for studies conducted in the same facility in France in which this study was conducted, ranged from 0-4% [overall incidence was 5/300 (1.7%)]. The incidence of uterine leiomyoma, described for the U.S. facility historical control data [1989-present; 24 month studies: Appendix 1: p. 6; Submission date April 1, 1999] ranged from 0-6% [overall incidence was 19/800 (2.4%)]. Dr. Taneja's analysis for the tumor combination of leiomyoma and leiomyosarcoma indicated a statistically significant positive linear trend [p = 0.0167]. The incidence of uterine leiomyoma and leiomyosarcoma combined in this study was 0, 0, 4%, 2%, 12%, and 4% at 0, 0, 5, 10, 20, and 30 mg/kg/day. The incidence of uterine leiomyoma/leiomyosarcoma described in historical control data [1983-1990; 21 month studies: Appendix 1: p. 3; Submission date April 1, 1999], for studies conducted in the same facility in France in which this study was conducted, ranged from 0-10% [overall incidence was 12/300 (4%)]. The incidence of uterine leiomyoma/leiomyosarcoma, described for the U.S. facility historical control data [1989-present; 24 month studies: Appendix 1: p. 6; Submission date April 1, 1999] ranged from 0-6% [overall incidence was 23/800 (2.8%)]. Although the mid-dose incidence of uterine leiomyomas is outside of the historical control values, the following considerations argue against the biological significance of this finding; [1] lack of a dose dependent relationship and [2] lack of an increase in endometrial hyperplasia. Therefore, the incidence of leiomyomas as well as leiomyoma/leiomyosarcoma combined in female mice administered 20 mg/kg/day of L-748,731 is considered of questionable significance.

Lung adenocarcinomas are commonly observed tumors in this strain of mice. The incidence of lung adenocarcinoma in male mice in this study was 10%, 18%, 8%, 4%, 16%, 20% at 0, 0, 5, 10, 20, and 30 mg/kg/day. The incidence of lung adenocarcinoma in male mice, described in historical control data [1983-1990; 21 month studies: Appendix 1: p. 3; Submission date April 1, 1999], for studies conducted in the same facility in France in which this study was conducted, ranged from 4-18% [overall incidence was 37/300 (12%)]. The incidence of lung adenocarcinoma in male mice, described for the U.S. facility historical control data [1989-present; 24 month studies: Appendix 1: p. 6; Submission date April 1, 1999] ranged from 0-22%% [overall incidence was 81/800 (10%)]. Therefore, this finding is comparable to these historical controls as well as one of the concurrent controls in this study. Therefore, this finding is considered of questionable significance.

In addition, statistical analysis by Dr. Taneja of all sarcomas combined as well as hepatocellular adenoma/adenocarcinoma did not demonstrate a significant trend.

Study Design and Data:

1. The mortality was high but considered acceptable.

2. The level of autolysis was considered excessive especially since it occurred predominantly in a target organ for toxicity, specifically the gastrointestinal tract. This may have resulted in underreporting of intestinal lesions, especially those affecting the small intestine.

---

[3] Maita, K. et. al. 1988. Mortality, major cause of moribudity, and spontaneous tumors in CD-1 mice. *Toxicol Pathol.* **16[3]**:340-349.

3. Based on the GI toxicity, the MTD was achieved in this study. Based on achievement of the MTD and the level of mortality, the doses selected were considered acceptable.

Sponsor's Conclusions [numbered] and Reviewer's Comments –

1. "Oral administration of L-748,731 to mice for approximately 106 weeks at dose levels of 5, 10, 20, or 30 mg/kg/day showed no evidence of a statistically significant [p≤0.05] trend for any tumor type in either sex." **Reviewer's Comment** - Prior to adjustment for multiplicity of tests, there was a statistically significant increase in [1] Harderian gland adenoma in females at 30 mg/kg/day; [2] uterine leiomyoma in females at 20 mg/kg/day; and [3] lung adenocarcinoma in males at 30 mg/kg/day. There was a statistically significant increase in the incidence of leiomyoma and leiomyosarcoma combined. As discussed above, the biological relevance of these findings is questionable. The Executive Carcinogenicity Assessment Committee [E-CAC], which met on March 23, 1999, recommended that the full CAC should meet to discuss the biological significance of the incidence of these tumors. The conclusions of the full CAC are presented in the Carcinogenicity Summary below.

2. A statistically significant trend for mortality secondary to drug treatment [e.g. peritonitis secondary to ulceration of the large intestine] was observed in males only at ≥10 mg/kg/day. **Reviewer's Comment:** Although the increase in drug-induced mortality [e.g. due to peritonitis] was not statistically significant in females, it was biologically relevant.

Additional Reviewer Comments - As noted by the Sponsor, males tended to be more sensitive to the drug-induced mortality and gastrointestinal changes than females. This may reflect, in part, the increased level of exposure in males when compared to females at comparable doses.

6.2.2. L-748,731: One Hundred-Four-Week Oral Carcinogenicity Study in Mice: Vol. 1.39; p. E-1464, Vol. 1.40; p. E-1828]

Study Identification: TT#96-605-0;-1

Site: Laboratoires Merck Sharp & Dohme-Chibret, Centre De Recherche, Riom France; "Portions of the histologic preparation and histopathologic evaluation were conducted at Merck Research Laboratories, West Point, PA."

Study Dates [In-life]: Feb. 26, 1996 - Feb. 20, 1998

Formulation: Test article - L-748,731 Vehicle - 0.5% aqueous methylcellulose

Lot No. L-748,731-000R032; [redacted] mixed daily, "has been previously documented to be stable under the conditions of use in this study"

Certificate Analysis Submitted: No (X) assayed for uniformity Drug week 1; assayed for concentration Weeks 1, 7, 20, 26, 39, 52, 56, 65, 78, 91, 96, and 104 with results within acceptable limits

Final Report (X) July 23, 1998

GLP and Quality Assurance Statements Signed: Yes (X) [See comments below under Protocol/GLP Adherence]

Objective: "To evaluate the carcinogenic potential of L-748,731 [MK-0966] when administered chronically for approximately 104 weeks to mice by the oral route."

Test Material/ Group Designation	Dose*				Sex	N**	Species/Strain
	mg/kg	ml/kg	Route	# days dosed			
Group 1 - Vehicle	-	10	oral, gavage	F = 724 -725 M = 619 -620	M	50	-Crl:CD-1® [IBR] BR albino mice - - 38 days at start of study -F – 18.5 to 24.2 g; M – 21.1 -housed 2-3/box
Group 2 - Vehicle					F	50	
Group 3 - L-748,731	60						
Group 4 - L-748,731	100			F = 498 M = 498			
Group 5 - L-748,731	300						

*administered SID, mice had free access to food and drinking water

**an additional 5 mice/sex/treatment were dosed to be used for replacements for mice dying secondary to caused unrelated to drug administration during the first 8 weeks of the study.

Parameter Evaluated	Time Point(s)
Clinical observations/mortality **-Palpated for masses**	Daily, q4wks beginning Week 26
Body weight	pretest, 1X/week for Week 1, then generally 2X/week through Week 13, 1X/week from Week 14 to end of study
Ophthalmic Examination - indirect, slit lamp biomicroscopy as needed, per protocol	pretest, Week 52, Weeks 88 and 95
Hematology* - RBC count, WBC count and differential, Hb, Hct, MCH, MCHC, MCV, and platelet count	
Necropsy	Premature deaths, unscheduled and scheduled terminal sacrifice**
Histopathology *** - The following tissues were evaluated in all control and low dose animals, premature decedents – salivary gland, esophagus, stomach, small intestine, large intestine, liver, gall bladder, pancreas, adrenal, parathyroid [when present in thyroid section], pituitary, thyroid, kidney, urinary bladder, ovary, uterus, testis prostate, skin, mammary gland [when present in skin section], lung, heart, spleen, lymph node, thymus, bone marrow, bone, skeletal muscle, brain, spinal cord, nerve [sciatic], eye [including optic nerve]	Premature deaths, unscheduled and scheduled terminal sacrifice**

*at the discretion of the pathologist

** mice which were sacrificed prematurely during Week 72 [100 and 300 mg/kg/day] were discarded without further examination

***this represents a complete list of tissues evaluated

Dose Selection - The Sponsor's apparent rationale for dose selection was to ensure that the MTD was achieved in the mouse studies. The maximum dose for the initial study was 30 mg/kg/day. The Sponsor initiated the study prior to a review by the ECAC as to the appropriateness of their dose selection. The ECAC, which met on April 2, 1996, recommended that the high dose should be 60 mg/kg/day instead of 30 mg/kg/day. The Sponsor had selected the 60 mg/kg/day dose as their low dose for this second study.

Protocol/GLP Adherence – All females and males receiving 100 and 300 mg/kg/day were sacrificed by Week 72 due to what the Sponsor considered "high drug-related mortality". The Agency concurred with this decision [May 19, 1997]. Males that were administered 60 mg/kg/day as well as the 2 concurrent controls were also sacrificed prematurely during Week 89 due to "high drug-related mortality". The Agency concurred with this decision [Sept. 9, 1997]. Those animals in the mid and high dose groups which died or were sacrificed prior to Week 72 underwent necropsy and histopathological evaluation. Those which were sacrificed Week 72 were discarded without further evaluation. The Sponsor did not include an amendment to the protocol. Consequently, this study was not conducted in compliance with GLP guidelines.

Exposure comparisons: The table below delineates the relationship between the selected doses and anticipated maximum human exposure, based on a body weight and surface area dose equivalency, as well

as AUC. The maximum human dose for osteoarthritis is 25 mg/day and for dysmenorrhea and acute pain is 50 mg/day for a maximum of 5 days. For a 60-kg individual this represents a dose of 0.4 and 0.8 mg/kg/day, respectively. The AUC figures were obtained from Study TT #95-611-0: Five Week Oral Toxicokinetic Study in Mice for mice. The steady state value for humans at 25 and 50 mg/day was 4.02 [Study #P043] and 11.48 µg•hr/ml [Study #P042], respectively.

Dose [mg/kg/day]	Exposure [XMHD]			
	Body mass	Surface Area	AUC	
			Female	Male
60	150[75]X	14[7]X	*	*
100	250[125]X	23[11]X	6[2]X	10[3]X
300	750[375]X	68[34]X	12[4]X	16[5]X

*The Sponsor extrapolated this figure based on linear kinetics over 30-100 mg/kg/day from Study TT #95-611-0. The estimated exposure at 60 mg/kg/day was 29.7 µg•hr/ml which represents approximately an 7[3]X human exposure at 25[50] mg/day based on AUC. It is not known whether this figure is based on AUC in males only or on a mean AUC in males and females. The multiples of human exposure based on female mouse AUC data would be less than 7[3]X. The multiples of human exposure based on female mouse AUC data would be greater than 7[3]X if the value is a mean of male and female AUC.

Statistical Analysis: Sponsor Analysis –The statistical analysis is the same as that described for Study # TT#95-076-0: L-748,731: One Hundred-Six-Week Oral Carcinogenicity Study in Rats.

Additional analysis - Dr. Baldeo Taneja provided a statistical consult. It was requested that Dr. Taneja conduct the following statistics: [1] comparison of the individual tumor types to each control separately as well as combined and [2] combination of the following tumors for analysis: (a) lung adenoma and adenocarcinoma: and (b) Harderian gland adenoma and adenocarcinoma. Dr. Taneja indicated that combining the controls increased the sensitivity of the analysis and, therefore, comparisons were made to combined controls only. Tumor and mortality data were analyzed by methods described for Study # TT#95-076-0: L-748,731: One Hundred-Six-Week Oral Carcinogenicity Study in Rats.

Results

Clinical Observations - Neither a narrative description nor a tabular presentation of clinical observations was provided. However, the Sponsor reported that there were no drug-related clinical signs.

Mortality - There was a statistically significant increase in mortality with increasing dose in males. The statistical analysis by Dr. Taneja indicated a significant increase in mortality in the low dose male mice.

Drug related mortality was observed at ≥60 mg/kg/day. The drug-related deaths were [1] due to peritonitis secondary to GI toxicity; [2] generally dose-dependent; and [3] higher in males than females. The table below indicates total, drug, and nondrug-related mortality.

Dose Group	Total Mortality		Drug-Related Mortality		Mortality Unrelated to Drug	
	Female	Male	Female	Male	Female	Male
Vehicle 1	19 [38%]	13 [26%]*	-	-	19 [38%]	13 [26%]*
Vehicle 2	18 [36%]	11 [22%]*	-	-	18 [36%]	11 [22%]*
60 mg/kg/day	19 [38%]	27 [54%]*	5 [10%]	13 [26%]*	14 [28%]	14 [28%]*
100 mg/kg/day	9 [18%]**	19 [38%]**	2 [4%]**	15 [30%]**	7 [14%]**	4 [8%]**
300 mg/kg/day	14 [28%]**	23 [46%]**	10 [20%]**	21 [42%]**	4 [8%]**	2 [4%]**

*Through Week 89
**Through Week 72

The timing of these unscheduled deaths or sacrifices is indicated in the table below.

Dose Group	Weeks 1-52		Weeks 53-72		Weeks 73-89		Weeks 90-104	
	Female	Male	Female	Male	Female	Male	Female	Male
Vehicle 1	1	1	1	0	9	12	8	-
Vehicle 2	0	3	3	3	6	5	9	-
60 mg/kg/day	2	15	2	4	2	8	13	-
100 mg/kg/day	1	13	8	6	-	-	-	-
300 mg/kg/day	5	16	9	7	-	-	-	-

The most common causes of death, excluding deaths due to GI toxicity, were [1] urinary obstruction in males, and [2] lymphoma in females. The overall incidence/sex for both causes of death was approximately 7%. The other causes of death were sporadic and/or occurred at the same frequency across dose groups.

Body weight – In Week 72, there was a 19%, 13%, and 10% decrease in body weight in males at 60, 100, and 300 mg/kg/day, respectively. The Sponsor states that this change was slight and not dose dependent, and, therefore, considered incidental. In light of the degree of GI toxicity observed in these studies, the decrease in body weight gain in males is considered by the Reviewer to be potentially related to drug administration. The reason that the differences in body weights had an inverse relationship to dose is not known. Evaluation of those animals that died prematurely revealed a variable effect on weight loss. In the majority of animals at the 100 and 300 mg/kg/day, which died prematurely or underwent unscheduled sacrifice due to GI toxicity, there was no weight loss; however, at the lower dose animals tended to exhibit weight loss. The weight loss was minimal [e.g. generally <10%].

Ophthalmic Examination – There were no drug-related changes. According to the Sponsor, there were a "variety of incidental findings." These findings were neither described nor tabulated.

Hematology – Hematology was reported for 1 animal.

Gross Necropsy - Drug-related changes included [1] large intestinal ulceration with/without perforation; [2] secondary peritonitis; and [3] numerous abdominal adhesions. The Sponsor states that due to the severity of the peritonitis and the adhesions, it was not always possible to observe the ulceration site. According to the Sponsor, all other findings were considered incidental since they were commonly observed in mice in their facility.

Histopathology

– Non-neoplastic Changes– Statistical analysis was not conducted on these data

The table below delineates histopathology findings that were considered treatment-related or potentially treatment-related, most notably ulceration of the small and large intestine as well as peritonitis. Findings, such as those observed in the liver, lymph nodes, and bone marrow, were considered by the Sponsor to be secondary to the GI toxicity and peritonitis.

Lesion	Dose/mg/kg/day									
	0		0		60		100		300	
	F	M	F	M	F	M	F	M	F	M
Stomach	[50]*	[50]	[50]	[50]	[50]	[49]	[9]	[19]	[14]	[23]
Glandular mucosa erosion/ulcer	3	-	-	2	2	2	1	3	2	2
Proliferative gastritis, glandular mucosa	20	22	16	26	12	18	-	-		1
Small intestine							[9]	[19]	[14]	[23]
Ulcer/erosion	2	1	1	-	1	2	-	2	2	4
Large Intestine	[50]	[50]	[50]	[50]	[50]	[50]	[9]	[19]	[14]	[23]
Colon inflammation	-	-	-	-	-	1	-	-	1	-
Ulcer	-	-	1	-	3	11	1	11	4	11
Peritoneum	[11]	[5]	[8]	[1]	[12]	[15]	[9]	[19]	[14]	[23]
Peritonitis	1	1	-	-	6	15	2	15	10	21
Liver	[50]	[50]	[50]	[50]	[50]	[50]	[9]	[19]	[14]	[23]
Multifocal Necrosis	1	1	4	2	3	-	1	5	2	3

*Number in parentheses indicates number of animals evaluated for a given tissue

There was comparable incidence and severity across the 2 control and the 60 mg/kg/day groups for renal lesions [e.g. tubular basophilia] and increased bone marrow granulopoiesis, both of which have been associated with L-748,731 in other toxicology studies, as well as all other histopathological lesions.

There was considerable autolysis in the gastrointestinal tract in this study. Although the Sponsor states that "autolysis does not preclude examination for neoplastic and obvious non-neoplastic changes", this finding in a target organ could potentially obscure lesions.

Dose Group*	Autolysis -# [%]	
	Female	Male
Group 1 - Vehicle	3 [6%]	8 [16%]
Group 2 - Vehicle	6 [12%]	3 [6%]
Group 3 - 60 mg/kg/day	4 [8%]	7 [14%]
Group 4 - 100 mg/kg/day	4 [44%]	2 [10%]
Group 5 - 300 mg/kg/day	0	3 [13%]

N = 50 except for Group 4 [F = 9, M = 19] and Group 5 [F = 14, M = 23]

Neoplastic changes – The following summary table indicates the incidence of the most commonly observed neoplastic lesions.

Primary Neoplasm	Dose/mg/kg/day									
	0		0		60		100		300	
	F	M	F	M	F	M	F	M	F	M
Liver	[50]	[50]	[50]	[50]	[50]	[50]	[9]	[19]	[14]	[23]
Hepatocellular adenoma	2	6	2	7	-	4				
Hepatocellular carcinoma	-	1	-	2	-	1	-	-	-	-
Adrenal										
Cortex, Adenoma	-	4	-	8	-	4	-	-	-	-
Cortex, carcinoma	-	-	1	-	-	-	-	-	-	-
Ovary										
Cystadenoma	5	-	3	-	2	-	-	-	-	-
Benign Luteoma	5	-	3	-	3	-	-	-	-	-
Uterus										
Leiomyoma	3	-	1	-	2	-	-	-	-	-
Testis										
Leydig cell adenoma	-	4	-	-	-	1	-	-	-	-

Primary Neoplasm	Dose/mg/kg/day									
	0		0		60		100		300	
	F	M	F	M	F	M	F	M	F	M
Mammary gland										
Adenocarcinoma	2	-	2	-	1	-	-	-	-	-
Lung										
Adenocarcinoma	2	5	5	2	4	2	-	-	-	-
Adenoma	7	4	8	6	2	6	-	-	-	-
Eye										
Harderian gland adenocarcinoma	-	-	2	-	2	-	-	-	-	-
Harderian gland adenoma	2	7	2	5	3	2	-	-	-	-
Primary site undetermined										
Histiocytic sarcoma	3	2	2	-	2	1	-	-	-	1
Lymphoma	9	6	6	2	7	1	2	-	2	-

*Number in parentheses indicates number of animals evaluated for a given tissue

All other tumors occurred sporadically and/or at a comparable frequency across treatment groups.

There was no statistically or biologically significant increase in the incidence of tumors in either male or female mice at 60 mg/kg/day. This in agreement with the analyses conducted by Dr. Taneja. In addition, no statistically significant trend was observed when the following tumors were combined: [1] lung adenoma and adenocarcinoma and [2] Harderian gland adenoma and adenocarcinoma. The Sponsor also states that the incidence of the various tumors observed were within the range observed in untreated mice of this strain for their laboratory.

Study Design and Data: There were concerns with respect to this study that included:
1. Failure to comply with GLP standards by not performing necropsy and histopathology on the animals dosed at 100 and 300 mg/kg/day which were prematurely sacrificed Week 72. No protocol amendment was included.

2. Excessive autolysis in a target organ.

Sponsor's Conclusions [numbered] and Reviewer's Comments –

1. "Oral administration of L-748,731 to mice for approximately 104 weeks 60 mg/kg/day showed no evidence of a statistically significant [$p \leq 0.05$] trend for any tumor type in either sex." **Reviewer's Comment** - The Reviewer concurs. The full CAC was asked to consider whether, due to the mortality and premature termination of the majority of dose groups, the data from this study should be used to assess the carcinogenic potential of L-748,731 in mice.

2. A statistically significant trend for increase in mortality with increasing dose of drug was observed in males only. **Reviewer's Comment:** Although the increase in drug-induced mortality [e.g. due to peritonitis] with dose was not statistically significant in females, it was biologically relevant. This is in agreement with the analysis conducted by Dr. Taneja.

Additional Reviewer Comments - As noted by the Sponsor, males tended to be more sensitive to the drug induced mortality and gastrointestinal changes than females. This may reflect, in part, the increased level of exposure in males when compared to females at comparable doses.

6.3 SUMMARY OF CARCINOGENICITY STUDIES – Based on GI toxicity [intestinal ulceration/perforation and peritonitis] and mortality, the MTD was reached in both the rat and mouse studies. The exposure in male and female rats at the highest dose evaluated was approximately 5[2]X and 7[2.4]X that in humans receiving a dose of 25[50] mg/day. The exposure in male and female mice

administered 30 mg/kg/day was approximately 5[2]X and 2[<1]X that in humans receiving a dose of 25[50] mg/day. The Sponsor extrapolated an AUC value for 60 mg/kg/day represents based on data obtained in Study TT #95-611-0: Five Week Oral Toxicokinetic Study in Mice. Based on this estimation, this dose represents approximately an 7[3]X human exposure at 25[50] mg/day based on AUC. There was a lack of agreement as to whether the data obtained from the high dose mouse study should contribute to the evaluation of the carcinogenic potential of L-748,731. In general, it was felt that the high dose provided supportive information, especially for control values and females; however, the studies should not be combined for statistical analysis. Due to the concerns raised regarding the high dose, the Reviewer feels that for labeling purposes, only the low dose study should be included. Therefore, a dose of 30 mg/kg/day in mice represents a multiple of the human exposure of 2[1] and 5[3] based on AUC at a human dose of 25[50] mg/day.

The full CAC, which met on April 8, 1999, discussed the carcinogenic potential of L748-731 based on results from the 2-year rodent bioassays. [See meeting minutes for full summary including Sponsor presentation.] There was a concern voiced that, based on some findings in the toxicology studies and the pharmacodynamic activity, L-748,731 could potentially induce immunotoxicity, including both immune suppression and immune enhancement. "It was suggested that immunosuppressive activity could have contributed to the tumor findings, although there was no consensus regarding this point." Additional concern was voiced that the "agency has had concern for rare tumor findings for other agents". However, it was agreed that L-748,731 was not genotoxic and, therefore, a genotoxic mechanism would not be involved. It was further suggested that testing to assess the immunotoxic potential of L-748,731 would be appropriate.

In general for both the rat study and low dose mouse study, it was agreed that the doses used were adequate. There was a lack of agreement as to whether the data obtained from the high dose mouse study should contribute to the evaluation of the carcinogenic potential of L-748,731. In general, it was felt that the high dose provided supportive information, especially for control values and females; however, the studies should not be combined for statistical analysis. Due to the concerns raised regarding the high dose, the Reviewer feels that for labeling purposes, only the low dose study should be included.

There was a consensus that the following tumors which exhibited a statistically significant increase prior to adjustment for multiplicity of tests were not biologically significant, e.g. treatment related: [1] pancreatic islet adenoma and acinar adenoma in male rats; [2] leiomyomas-leiomyosarcomas in female mice; and [3] lung adenocarcinomas in male mice. The Harderian gland adenoma in female mice was generally considered not to be biologically significant, although there was concern that the incidence in the high dose females exceeded historical controls provided by the Sponsor. There was no consensus with respect to the biological significance of brain malignant glioma in female rats. The concern was that this was a rare tumor type that was observed in 2 of the 3 test article groups, and the incidence exceeded the historical controls reported by the Sponsor. It was recommended that the "histology slides for the gliomas [be re-evaluated] so as to subgroup them. . . to get a sense of the true 'rarity' including glioma in the spinal cord". The overall conclusion with respect to the evidence of carcinogenic potential for rofecoxib was that either there was no convincing evidence [9/15] or that the evidence was equivocal [6/15].

The Office of Testing and Research at FDA conducted a structure activity review of L-748,731 for its carcinogenic potential. Their conclusion [report dated April 30, 1999] indicated that, L-748,731 was "not predicted to be a trans-gender or trans-species rodent carcinogenic" based on 8 rodent carcinogenicity database modules and was negative in the MASE QSAR Rodent Carcinogenicity.