| Treatment | Precipitate at Time of Harvest | Cytotoxicity as Trypan Blue Exclusion: Absolute Viability (Percent) | Cytotoxicity as Trypan Blue Exclusion: Relative Viability (Percent) |
|---|---|---|---|
| Negative Control | - | 83 | 100 |
| L-748,731 | | | |
| 10 µM | - | 82 | 99 |
| 25 µM | - | 69 | 83 |
| 50 µM | - | 70 | 84 |
| 100 µM | - | 69 | 83 |
| 150 µM | - | 74 | 89* |
| 200 µM | + | 59 | 71* |

* Cell blebbing

In study TT #94-8220, DNA strand breaks occurred at ≥ *125* µM L-748,731. Slopes were 0.098 and 0.058 at 125 µM and 0.251 and 0.356 at 150 µM; however, a precipitate was seen during the viability counts at ≥100 µM. Aflatoxin $B_1$ produced an induced elution slope [mean treatment slope (-) mean negative control slope] of 0.116, with the ATP content at 89%. The assay was repeated with several modifications of the SOP.

In the repeat test, the concentrations tested were 70, 115, and 150 µM. The modifications that were done were in the harvesting of cultured cells, in the cytotoxicity evaluation after treatment, and in the determination of DNA strand breaks. The results showed that the induced elution slope was not greater than 0.005 at ≤ 115 µM and the positive control slope was 0.242 –0.226. The discrepancy in the TT #94-8220 assay was due to precipitate on the filter papers. [*SOP variation resulted in reversibility of the induction of DNA strand breaks at 150 µM, observed during standard protocol, in one duplicate but not the other. Therefore, this modification did not completely reverse the drug-induced DNA strand break. The Sponsor concludes that this concentration "is a borderline insoluble dose level at which drug precipitation can occur at 37° C". No precipitate was observed at this dose during viability counts. "Drug precipitate alone on filters at the time of cell lysis produces a qualitatively and quantitatively similar result with untreated control cells."]*

In conclusion, L-748,731 did not produce any DNA damage due to soluble drug in the rat hepatocyte alkaline elution assay, as judged by the limitations of the study.

### 5.2 *In Vivo* Assays

#### 5.2.1. *In Vivo* Alkaline Elution/Rat Liver DNA Damage Assay in Male Rats [*Vol. 1.32: p. D-171*

Study No: TT #94-8222
Compound: L-748,731-000R009
Formulation: Suspension in 0.5% methylcellulose in water
Strain: Sprague-Dawley, Crl:CD®(SD)BR, 179-213 g body wt., 8 weeks old
Number 4/group
Dosage: 200, 667, and 2000 mg/Kg . *Dose selection was based on [1] "the highest dose level required by the FDA for lethality studies" and [2] lack of toxicity in the acute oral studies at 2000 mg/kg.*
Route: Oral gavage at 10.0 ml/Kg
Duration of Exposure: 4 hours
Negative Control: 0.5% methylcellulose in water
Positive Control: N-nitrosodimethylamine [DMN], at 2.5 mg/Kg in 0.5% methylcellulose in water
Study Site: Merck, West Point, PA
Date: September 20, 1994
GLP/QAU: Both present and signed

The objective of the study was to determine if orally administered L-748,731 can induce strand breaks in DNA of rat liver cells without concomitant induction of hepatocellular necrosis. For the alkaline elution assay, the animals were sacrificed at 4 hours after drug administration. Two 0.5 ml aliquots of liver cell

nuclei suspension (5 x $10^5$ nuclei and cells) were loaded onto separate 2.0 micron polycarbonate filters, lysed, and the DNA eluted according to SOP. Other animals were evaluated for hepatotoxicity 24 hours after drug administration.

The criteria for a positive result are:

1) Dose related effect.
2) A mean elution slope of ≥ 0.050 in at least one treatment group not associated with clinical or histopathologic evidence of an acute hepatotoxic effect at 24 hours post-treatment.

The study design is indicated below.

Alkaline Elution

| Group: | Treatment | Dose |
|---|---|---|
| 1 | Vehicle/Negative control | 10.0 mL/Kg |
| 2 | L-748,731 | 200 mg/Kg |
| 3 | L-748,731 | 667 mg/Kg |
| 4* | L-748,731 | 2000 mg/Kg |
| 5 | DMN/Positive control | 2.5 mg/Kg |

Hepatotoxicity Evaluation

| Group: | Treatment | Dose |
|---|---|---|
| 1 | Vehicle/Negative control | 10.0 mL/Kg |
| 2 | L-748,731 | 200 mg/Kg |
| 3 | L-748,731 | 667 mg/Kg |
| 4* | L-748,731 | 2000 mg/Kg |

* An additional 4 rats were dosed to be used as replacements if necessary.

There were no deaths in any of the animals used for the liver toxicity evaluation. Mean serum AST, ALT, and alkaline phosphatase indicated no increases over the control. The liver wt to body wt (LW/BW) mean values indicated a slight dose related increase in the mean values of 3.76 (G1), 3.70 (G2), 3.88 (G3), and 4.14 (G4). No histopathologic examinations were done on any organ. The results of the alkaline elution assay are indicated in the following table.

Table 1. L-748,731: *In Vivo* Alkaline Elution/Rat Liver DNA Damage Assay in Male Rats. Summary of DNA Strand Breaks (Elution Slope) TT #94-8222

| Treatment | Animal No. | Alkaline Elution at 4 Hours: Elution Slopes | Animal Mean Elution Slope | Group Mean[1] Elution Slope | Increment[2] Slope |
|---|---|---|---|---|---|
| Negative Control | | | | | |
| 0.5% aqueous-methylcellulose 10 ml/kg | 1 | 0.027, 0.033 | 0.030 | 0.038 ± .017 | 0.000 |
| | 6 | 0.071, 0.054 | 0.063 | | |
| | 11 | 0.034, 0.032 | 0.034 | | |
| | 16 | —[3], 0.026 | 0.026 | | |
| Positive Control | | | | | |
| N-nitrosodimethylamine 2.5 mg/kg | 5 | 0.243, 0.254 | 0.248 | 0.268 ± .030 | 0.230 |
| | 10 | 0.298, 0.319 | 0.309 | | |
| | 15 | 0.344, 0.346 | 0.345 | | |
| | 20 | 0.248, 0.291 | 0.270 | | |
| Irradiated Control Cells[4] | | | | | |
| 3 Gy gamma irradiation | 1 | 0.260, 0.257 | 0.263 | 0.235 ± .011 | 0.217 |
| | 6 | 0.265, 0.249 | 0.257 | | |
| | 11 | 0.276, 0.245 | 0.260 | | |
| | 16 | 0.239, —[3] | 0.239 | | |
| L-748,731 | | | | | |
| 200 mg/kg | 2[5] | 0.019, 0.017 | 0.018 | 0.022 ± .004 | -0.016 |
| | 7[5] | 0.023, 0.019 | 0.021 | | |
| | 12 | 0.028, 0.027 | 0.027 | | |
| | 17 | 0.023, 0.020 | 0.022 | | |
| 667 mg/kg | 3 | 0.053, 0.043 | 0.048 | 0.031 ± .014 | -0.007 |
| | 8 | 0.027, 0.040 | 0.033 | | |
| | 13 | 0.031, 0.030 | 0.030 | | |
| | 18 | 0.013, 0.015 | 0.014 | | |
| 2000 mg/kg | 4 | 0.026, 0.023 | 0.025 | 0.028 ± .009 | -0.010 |
| | 9 | 0.019, 0.017 | 0.018 | | |
| | 14 | 0.025, 0.037 | 0.031 | | |
| | 19 | 0.046, 0.032 | 0.039 | | |

1. Values are mean ± standard deviation.
2. Arithmetic difference between the mean elution slope value for a treatment group and the mean elution slope for the negative control article.
3. Data lost due to technical error.
4. A portion of control nuclei/cells exposed to 3 Gy gamma irradiation in a $^{137}Cs$ irradiator, on ice, just prior to alkaline elution.
5. Mis-timed/Excision resulted in the late sacrifice (30 minutes) of animal #2 and early sacrifice (25 minutes) of animal #7.

The drug did not produce a mean elution slope greater than seen in the negative control. A slope of 0.268 ± 0.030 was calculated for the positive control and irradiated controls had a group mean elution slope

of 0.255 ± 0.011. In conclusion, oral administration of ≤2000 mg/Kg L-748,731 or its metabolites did not produce detectable DNA strand breaks in liver cells exposed up to 4 hours.

*5.1.2 In Vivo* Alkaline Elution/Rat Liver DNA Damage Assay in Female Rats *[Vol. 1.34, p. D-214]*

Study No.: TT #94-8223
Compound: L-748,731-000R009
Formulation: Suspension in 0.5% methycellulose in water
Strain: Sprague-Dawley, Crl:CD®(SD)BR, 141-176 g body wt., 8 weeks old
Number: 4/group
Dosage: 200, 667, and 2000 mg/Kg at 10.0 mL/Kg
Route: Oral gavage
Duration of Exposure: 4 hours
Negative Control: 0.5% methylcellulose in water
Positive Control: N-nitrosodimethylamine (DMN)
Study Site: Merck, West Point, PA
Date: September 16, 1994
GLP/QAU: Both present and signed.

The objective of the study was to determine if orally administered L-748,731 can induce strand breaks in DNA of rat liver cells without concomitant induction of hepatocellular necrosis. The study design and criteria for a positive result were similar to the above study in male rats.

Results and Discussion

No mortality was reported in the rats used for evaluating liver toxicity. AST ALT, and alkaline phosphatase values showed only minor variations. Liver wt to body wt showed the same slight dose related increase as in the males. No histopathologic examinations were done on the liver

The alkaline elution assay data did not show group mean elutions greater than the negative control (0.021). N-nistrosodimethylamine had an elution slope of 0.243 ± 0.020 and irradiated control cells had a group mean elution slope of 0.32. In conclusion, the drug or its metabolites did not produce detectable DNA strand breaks in rat liver cells when the animals were exposed orally up to 2000 mg/Kg for four hours. Based on the results in both male and female rats, L-748,731 is not considered genotoxic when evaluated under the conditions of this assay.

5.2.3. MK-0966: Assay for Chromosomal Aberrations *In Vivo*, in Mouse Bone Marrow [Vol. 1.33; D-403]

**Study Identification:** TT #96-8614 and TT #96-8624
**Site:** Merck Research Laboratories, West Point, PA
**Study Dates:** Feb. 28 - May 6, 1996 and April 17- June 21, 1996
**Formulation:** L-748,731-000R027;
Negative control - 0.5% methylcellulose
Positive control - Mitomycin C
**Certificate Analysis: No (X)**
**Final Report (X) Dec. 9, 1996**
**GLP and QA Statements Signed: Yes (X)**
**Objective:** "The objective of these studies was to determine whether MK-0966 induced chromosomal aberrations in bone marrow cells of female and male Crl:CD-1®(ICR) BR mice.

| Test Material/ Group Designation | Dose* | | | | Sex** | N | Species/Strain |
|---|---|---|---|---|---|---|---|
| | mg/kg | ml/10 g | Route | # days dosed | | | |
| Group 1 - vehicle | - | 0.1 | oral gavage | 1 | M/F | 12 | Crl:CD-1 BR mice |
| Group 2 - MK-0966 | 150 | | | | | 8 | |
| Group 3 - MK-0966 | 300 | | | | | 8 | app. 4 weeks |
| Group 4 - MK-0966 | 600 | | | | | 8# | F - 17.1-24.2 g g |
| Group 5 - Mitomycin C | 3.5 | | ip | | | 4 | M - 18.2 - 28.3 |
| Group 6 - Mitomycin C | 1.0 | | | | | 8 | group housed 4-12/cage |

*Dose was selected based on a single dose oral toxicokinetic study [TT #95-617-0] - Reviewed under Pharmacokinetic/Toxicokinetic Studies

**both males and females were evaluated due to differences in the PK profile between genders; e.g. males tend to have a higher exposure

#10 dosed, 8 evaluated

Colchicine [2 mg/kg] was administered to all animals approximately 3 hours prior to sacrifice.

| Parameter Evaluated | Timing |
|---|---|
| Clinical Observations | "At selected times after drug administration, and before each sacrifice" |
| Bone marrow smears [May-Gruenwald and Giemsa solutions] - generally 50 cells/mous were scored - % mitotic cells, total number of aberrant cells, % aberrant cells, total number of aberrations, frequency of aberrations/100 cells | 6, 24 and 48 hours after dosing for the negative control and test article groups 24 hours for the positive control group |

Sponsor's criteria for a positive - a significant increase in percent of cells with aberrations at ≥2 doses when compared to controls; "either 2 doses at a given sacrifice time or one dose at each of two sacrifice times"

**Results -**

**Clinical observations and mortality** - There were no treatment related clinical signs or mortality.

**Chromosomal aberrations** - There were no statistically significant increases in chromosomal aberrations and "no evidence of an increasing trend in aberration frequency with dose at any of the sacrifice times" following administration of L-748,731 to either male or female mice at any time point. The percentage of cells with aberrations in males tended to be increased by 2, 7, and 4X at 6 hours; 3, 3, and 6X at 24 hours; and 1, 2, and 3X at 48 hours at 150, 300, and 600 mg/kg, respectively. However, all values were within the historical control ranges. There was, however, significant increase in chromosomal aberrations in the positive control animals. The table below delineates these results.

| TT# Sex | Sacrifice Hour | % Cells with Aberrations | | | | | |
|---|---|---|---|---|---|---|---|
| | | Vehicle Control | MK-0966 (mg/kg) | | | Positive Control Mitomycin C (mg/kg) | |
| TT 096-8614 Females | | 0.5% aq methocel | 150 mg/kg | 300 mg/kg | 600 mg/kg | 1.0 | 3.5 |
| | 6 | 1.33 | 0.25 | 0.75 | 0.25 | n/a[1] | n/a[1] |
| | 24 | 0.83 | 1.00 | 2.00 | 1.00 | 6.75 | 26.29 |
| | 48 | 0.83 | 0.50 | 1.75 | 1.00 | n/a[1] | n/a[1] |
| | | | | | | | |
| TT 096-8624 Males | | 0.5% aq methocel | 150 mg/kg | 300 mg/kg | 600 mg/kg | 1.0 | 3.5 |
| | 6 | 0.33 | 0.75 | 2.25 | 1.25 | n/a[1] | n/a[1] |
| | 24 | 0.17 | 0.50 | 0.50 | 1.00 | 4.25 | 25.14 |
| | 48 | 0.50 | 0.50 | 1.00 | 1.50 | n/a[1] | n/a[1] |

[1] n/a = not applicable; Mitomycin C is used in 24 hr timepoint only.

**Reviewer's Comment - Study Design and Data Presentation** - The highest dose selected did not result in signs of toxicity.

**Sponsor's Conclusions (numbered) and Reviewer's Comments**

1. Under these experimental conditions, L-748,731 was negative for chromosomal aberrations in mouse bone marrow assay for both males and females. **Reviewer's Comment** - The Reviewer concurs.

Summary of Genotoxicity - The following *in vitro* assays were considered negative: [1] bacterial mutagenicity assay; [2] chromosomal aberrations in Chinese hamster ovary (CHO) cells; [3] V-79 mammalian cell mutagenesis assay; and [4] alkaline elution/rat hepatocyte assay. When initially tested in the *in vitro* alkaline elution/rat hepatocyte assay, there was an increase in DNA strands resulting in a positive test at ≥115 μM. However, at ≥100 μM, a precipitate was observed during viability counts. When modifications were made to the Standard Operating Procedure, DNA strand breaks were observed in only 1/2 replicates at the highest concentration used [150 μM]. The Sponsor states that "drug precipitate on the filter alone at the time of cell lysis produces a qualitatively and quantitatively similar result with untreated cells. The following *in vivo* assays were considered negative: [1] alkaline elution/rat liver DNA damage assay in male and female rats; and [2] chromosomal aberrations in mouse bone marrow in male and female mice.

The Genetic Toxicology Committee had the following concerns: [1] in the mammalian mutagenicity assay, the Sponsor did not test in the insoluble range despite a dose-related cytotoxicity as recommended in current ICH guidelines; [2] the level of cytotoxicity recommended in the ICH guidelines [e.g. >50%, optimal cytotoxicity at which chromosomal aberrations are detected] was not achieved in the CHO chromosomal aberrations assay; and [3] the determination of the high dose used in the *in vivo* mouse micronucleus assay was based on AUC 'plateauing' of the parent compound and this figure should be calculated both on the parent compound and metabolites. Despite these concerns, the Committee concurred that the data generated in these assays "provided convincing evidence that MK-0966 has been shown to be non-genotoxic under the conditions tested".

**5. Carcinogenicity** - ***The following studies were reviewed by the current Pharmacology/Toxicology Reviewer***

## 6.1 CARCINOGENICITY – RAT STUDY

**6.1.1. L-748,731: One Hundred-Six-Week Oral Carcinogenicity Study in Rats: [Vol. 1.35; p. E-1464, Vol. 1.36; p. E-399]**

**Study Identification:** TT#95-076-0
**Site:** Merck Research Laboratories, West Point, PA
**Study Dates [In-life]:** Nov. 20, 1995 – Nov. 25, 1997
**Formulation:** Test Article - L-748,731 Vehicle - 0.5% aqueous methylcellulose
**Lot No.** L-748,731-000R015; Weeks 1-11; L-748,731-000R027; Weeks 12-106; 99.9% 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, 12, 24, 27, 39, 51, 63, 75, 87, 99, and 105 with results, according to the Sponsor within acceptable limits
**Final Report** (X) July 27, 1998
**GLP and Quality Assurance Statements Signed: Yes (X)**
**Objective:** "To evaluate the carcinogenic potential of L-748,731 [MK-0966] when administered chronically to rats by the oral route."

| Test Material/ Group Designation | Dose* | | | | Sex | N | Species/Strain |
|---|---|---|---|---|---|---|---|
| | mg/kg | ml/kg | Route | # days dosed | | | |
| Group 1 - Vehicle control | - | 5 | oral, gavage | M -728 -731 | M | 50 | -Crl:CD® [SD] BR- Sprague Dawley rats<br>- ~ 36 days at study start<br>-F – 90 to 150 g; M – 95 to 183<br>-individually housed |
| Group 2 - Vehicle control | | | | F – 731-736 | F | 50 | |
| Group 3 - L-748,731 | 2 | | | | | | |
| Group 4 - L-748,731 | 5 | | | | | | |
| Group 5 - L-748,731 | 8 | | | | | | |

*Administered SID, female and male rats were fed approximately 17 and 24 gm, respectively, of PMI Certified Rodent diet, free access to drinking water

| Parameter Evaluated | Time Point(s) |
|---|---|
| **Clinical observations/mortality** | Daily |
| **-Palpated for masses** | q4wks beginning Week 26 |
| **Body weight** | pretest, 1X/week for Week 1, then generally 2X/week through Week 13, then weekly thereafter |
| **Ophthalmic Examination** – indirect ophthalmoscopy as needed, per protocol | pretest, Weeks 52 and 101 |
| **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 - salivary gland, stomach, small intestine, large intestine, liver, pancreas, adrenals, pituitary, thyroids, parathyroids [when present in thyroid section], kidneys, urinary bladder, uterus/prostate, ovaries/testes [with epididymides], skin, mammary gland [when present in skin section], lung, heart, spleen, lymph nodes, thymus, bone, bone marrow, skeletal muscle, brain [including cerebral cortex and subcortical white matter, cerebellum and pons], cervical spinal cord, nerve[sciatic], eye [including Harderian gland and optic nerve], gross lesions | Premature deaths, unscheduled and scheduled terminal sacrifice |

*At the discretion of the pathologist
**this represents the complete list of tissues examined

**Diet** – The Sponsor has conducted two studies to assess the effect of different feeding regimens on gastrointestinal toxicity as well as the plasma and gastrointestinal toxicokinetics of L-748,731 in male and female rats [Study TT #95-022-1: L-748,731: Sixteen-Day Oral Toxicokinetic Study in Female Rats and Study TT #95-022-0: L-748,731: Sixteen-Day Oral Toxicokinetic Study in Male Rats]. In these studies, rats were either allowed feed *ad libitum* or were fed a restricted diet of 24 and 17 g/day for males and females, respectively. These two feeding regimens did not appear to alter [a] absorption; [b] plasma toxicokinetics; or [c] gastrointestinal toxicokinetics of L-748,731. Two *ad libitum* female rats at the high dose [50 mg/kg/day X 15 days] were found to have peritonitis at necropsy. The Sponsor states [p. B-18] that these data indicate that *ad libitum* feeding may potentiate GI toxicity, although "intestinal lesions were observed at equivalent dosages regardless of feeding regimen". Restricted food consumption has been reported to decrease mortality, morbidity, and the incidence of spontaneous tumors. These facts provide a rationale for the feeding regimen used in this study. Based on food consumption in Studies TT #95-022-1 and TT #95-022-0 of approximately 30-35 and 24 gm/day [7 and 8 gm/100 gm body weight] for males and females respectively, the feeding regimen used in this study represents approximately a 30% diet restriction.

**Dose Selection:** The Sponsor based the dose selection on the maximum tolerated dose [MTD] with intestinal ulceration and peritonitis the dose limiting toxicities. Studies that were cited by the Sponsor to support the dose selection were [1] TT # 95-018-0: Fourteen-Week Oral Range-Finding Study in Rats; and [2] TT #95-601-0: Twenty-Seven Week Oral Toxicity Study in Rats. Mortality secondary to GI toxicity [GI ulceration/perforation and peritonitis] was observed at ≥125 and ≥10 mg/kg/day in the 13- and 27-week studies, respectively. [Dose range for the 13- and 27-week studies was 125–2000 mg/kg/day and 10-300 mg/kg/day, respectively.] Renal tubular basophilia was observed at ≥10 mg/kg/day in the 27-week study. The Sponsor proposed doses of 2, 5, and 8 mg/kg/day. The Executive Carcinogenicity Advisory Committee [ECAC] agreed that, based on intestinal ulceration, the high dose should be lower than 10 mg/kg/day. They also indicated that the data were insufficient to recommend a specific dose or accept the proposed doses. They recommended that the Sponsor [1] could "conduct an additional dose-ranging study at lower doses; or [2] if the sponsor [could] submit additional data to determine the drug's long term (at least 18 months) effect on survival, the committee could recommend a dose range for the carcinogenicity studies. If the sponsor chooses to provide sufficient survival data, the committee could accept the proposed protocol provided the data are acceptable." [ECAC reviewed this protocol on 11-7-95; comments faxed to the Sponsor on 11-21-95]. It does not appear that additional range finding studies were conducted prior to initiation of the carcinogenicity study. However, based on the presence of significant toxicity [e.g. MTD] and adequate survival, the doses are considered acceptable.

**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 daily for up to 5 days. For a 60-kg individual this represents a dose of 0.4 and 0.8 mg/kg/day. The AUC figures were obtained from Study TT #95-076-1: L-748,731: Twenty-Seven-Week Oral Toxicokinetic Study in Rats [Week 27]. 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 |
| 2 | 5[2.5]X** | 1[0.4]X | 2[<1]X | 1[<1]X |
| 5 | 12[6]X | 2 [1]X | 5[2]X | 3[1]X |
| 8 | 20[10]X | 3[2]X | 7[2.4]X | 5[2]X |

*Maximum human dose

**Multiples for the 50 mg dose are in brackets

**Statistical Analysis - Sponsor conducted analysis:** Treatment groups were compared to a single control value [the mean of values for Groups 1 and 2] for both mortality and tumor incidence. For evaluating the carcinogenic potential of L-748,731, the statistical method selected by the Sponsor incorporated the following factors:

- "Distinguishing between palpable and nonpalpable tumors
- Time to tumor detection
- Time to death without tumor
- Cause of death
- Permutation tests for tumor sites with few tumor bearing rats
- Proper assessment of results when many tumor sites are examined"

P-values were adjusted for multiplicity of tumor sites analyzed.

Mortality was assessed using the Mantel-Haenszel procedure.

**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; [2] combination of the following tumors for analysis: a. hepatocellular adenoma and adenocarcinoma: b. pancreatic islet adenoma and carcinoma. 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 in Peto et. al.[1980][1] and "the method of exact permutation trend test developed by the Division of Biometrics, FDA".

**Results**

**Clinical signs** – Clinical signs in all dose groups [apparently in premature decedents] included distended abdomens, urine staining, discharge around the eyes and/or nose, food remaining, and/or scant feces. Other signs were considered by the Sponsor to be unrelated to treatment. Clinical findings were not tabulated.

**Mortality** – According to the Sponsor, there was a statistically significant increase in mortality with increasing dose in both males and females. Statistical analysis by Dr. Taneja indicated that in males there was not a statistically significant trend. In females, survival analysis revealed a statistically significant increase in mortality [Cox test – p = 0.049 and Kruskal-Wallis – p = 0.0548]

| Dose Group | Total Mortality | | Drug-Related Mortality | | Mortality Unrelated to Drug | |
|---|---|---|---|---|---|---|
| | Female | Male | Female | Male | Female | Male |
| Vehicle 1 | 17[34%] | 17 [34%] | - | - | 17[34%] | 17 [34%] |
| Vehicle 2 | 19 [38%] | 16 [32%] | - | - | 19 [38%] | 16 [32%] |
| 2 mg/kg/day | 26 [52%] | 19 [38%] | 2 [4%] | - | 24 [48%] | 19 [38%] |
| 5 mg/kg/day | 21 [42%] | 21 [42%] | 8 [16%] | - | 13 [26%] | 21 [42%] |
| 8 mg/kg/day | 28 [56%]* | 24 [48%]* | 11 [22%] | 8 [16%] | 17 [34%] | 16 [32%] |

*indicates statistical significance.

The higher incidence of mortality in females compared to males was felt to be, at least in part, a function of higher exposure to drug in this gender. The first drug-related death occurred during Week 22 with the remainder occurring sporadically throughout the study.

[1] Peto, et. al. [1980]. Guidelines for simple sensitive significance tests for carcinogenic effects in long-term animal experiments. Long Term and Short Term Screening Assays for Carcinogens: A Critical Appraisal, International Agency for Research Against Cancer Monographs, Supplement 2, World Health Organization, Geneva. pp. 311-426.

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

| Dose Group | Weeks 1-52 | | Weeks 53-72 | | Weeks 73-89 | | Weeks 90-105/6 | |
|---|---|---|---|---|---|---|---|---|
| | Female | Male | Female | Male | Female | Male | Female | Male |
| Vehicle 1 | - | 4 | 3 | 4 | 7 | 3 | 7 | 6 |
| Vehicle 2 | 4 | 3 | 1 | 1 | 7 | 5 | 7 | 7 |
| 2 mg/kg/day | 3 | 3 | 2 | 2 | 8 | 5 | 13 | 9 |
| 5 mg/kg/day | 1 | 3 | 2 | 3 | 7 | 4 | 11 | 11 |
| 8 mg/kg/day | 6 | 3 | 3 | 3 | 8 | 7 | 11 | 11 |

GI distention was considered the cause of death in 1 female rat each at 2 and 5 mg/kg/day. This lesion was associated with erosive gastritis in one female. No additional GI lesions were described in the second female.

The most common causes of premature death which were considered unrelated to drug treatment included: [a] pituitary adenomas in males and females [35 and 40 rats, respectively]; [b] mammary gland adenocarcinomas, mammary gland fibroadenomas in females [10 rats each]; [c] undetermined in males [15 rats]; [d] intubation accidents in males [10 rats]; and [e] histiocytic sarcoma in females [5 rats]. Other causes of death were sporadic and/or were comparable across dose groups.

The incidence of mortality for this study was acceptable.

**Body weight** – Based on group means, there were no treatment-related differences in body weight for either males or females. There was weight loss in females [1/2, 6/10, 3/11 at 2, 5, and 8 mg/kg/day, respectively] and males [4/8] that died secondary to gastrointestinal toxicity

**Ophthalmic Examination** – No treatment related changes were reported. Tabulation of findings was not provided.

**Hematology** – Four samples were collected: 1 each - control male, 2 mg/kg/day female and 8 mg/kg/day male and female.

**Clinical Chemistry** - Not conducted.

**Organ Weights** – Not determined.

**Gross Necropsy** – Treated animals exhibited gastrointestinal ulceration and/or peritonitis.

**Histopathology** -

Non-neoplastic lesions - No statistical analysis was conducted on these data. The table below delineates histopathology findings that were considered treatment-related or potentially treatment-related, most notably ulceration of the pylorus, small and large intestine, as well as peritonitis. The incidence of GI ulceration/peritonitis was greater in females than in males. Findings, such as those observed in the lymph nodes and bone marrow, were considered to be secondary to the GI toxicity and peritonitis. Incidence of autolysis of the gastrointestinal tract was <12% with comparable incidences across treatment groups. The small intestine had undergone autolysis in all of the treated premature decedents and 1 control rat. Since this is a target organ, the presence of autolysis may have impacted interpretation.

| Lesion | Dose [mg/kg/day] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | FEMALE | | | | | MALE | | | | |
| | 0 | 0 | 2 | 5 | 8 | 0 | 0 | 2 | 5 | 8 |
| Stomach | [50]* | [50] | [50] | [50] | [50] | [50] | [50] | [50] | [50] | [50] |
| Fundus, erosion | 1 | 1 | 2 | 2 | 3 | - | 1 | 6 | 1 | 5 |
| Nonglandular mucosa ulcer | - | 2 | 1 | - | - | - | 1 | 2 | - | 1 |
| Pylorus erosion/ulcer | 4 | - | 3 | 7 | 5 | - | 5 | 1 | 4 | 4 |
| Small intestine | [50] | [50] | [50] | [50] | [50] | [50] | [50] | [50] | [50] | [50] |
| Erosion/ulcer/perforated ulcer | - | - | 2 | 10 | 21 | 1 | - | - | - | 15 |
| Muscularis, focal hyperplasia | - | 1 | - | 2 | 3 | - | - | - | - | - |
| Chronic focal inflammation | - | 1 | 1 | 4 | - | - | 1 | 2 | 9 | 2 |
| Large intestine | [50] | [50] | [50] | [50] | [50] | [50] | [50] | [50] | [50] | [50] |
| Cecum edema | - | - | - | - | - | - | - | 1 | - | 2 |
| Cecum ulcer | - | - | 4 | 4 | 4 | - | - | - | - | 2 |
| Liver | [50] | [50] | [50] | [50] | [50] | [50] | [50] | [50] | [50] | [50] |
| Diffuse vacuolation | 3 | 3 | 2 | 4 | 2 | 4 | 7 | 8 | 11 | 6 |
| Focal necrosis | 1 | - | 3 | 1 | 3 | 4 | 7 | 5 | 4 | 14 |
| Kupffer cell diffuse proliferation | 1 | 1 | - | 1 | 2 | - | - | 1 | - | 5 |
| Peritoneum | - | [2] | [3] | [13] | [18] | [2] | [2] | [3] | [2] | [10] |
| Chronic peritonitis/ fibrinopurulent peritonitis | - | - | 2 | 9 | 18 | - | - | - | - | 9 |
| Lymph node | [50] | [50] | [50] | [50] | [50] | [50] | [50] | [50] | [50] | [50] |
| Lymphoid hyperplasia | 11 | 9 | 17 | 22 | 30 | 22 | 22 | 22 | 29 | 31 |
| Reticuloendothelial hyperplasia | 1 | 1 | 4 | 5 | - | 2 | 7 | 6 | 6 | 3 |
| Lymphadenitits | - | - | 2 | 2 | 3 | - | - | 3 | 1 | 3 |
| Lymphangiectasis | - | - | 3 | 12 | 12 | 7 | 8 | 15 | 16 | 24 |
| Bone marrow | [50] | [50] | [50] | [50] | [50] | [50] | [50] | [50] | [50] | [50] |
| Myeloid hyperplasia | 14 | 18 | 18 | 26 | 33 | 13 | 23 | 26 | 24 | 36 |

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

The other non-neoplastic histopathological lesions observed were either sporadic or comparable in incidence and severity across all groups, and were consistent with changes associated with age.

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 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Female | | | | | Male | | | | |
| | 0 | 0 | 2 | 5 | 8 | 0 | 0 | 2 | 5 | 8 |
| Liver | [50]* | [50] | [50] | [50] | [50] | [50] | [50] | [50] | [50] | [50] |
| **Neoplastic lesions** | | | | | | | | | | |
| Hepatocellular adenoma | - | - | - | 2 | - | 1 | 4 | 3 | 2 | 2 |
| Hepatocellular carcinoma | 1 | 1 | - | 1 | - | 2 | 2 | 3 | 5 | 2 |
| **Non-neoplastic lesion** | | | | | | | | | | |
| Hepatocyte, focal basophilic cellular alteration | 24 | 22 | 17 | 21 | 21 | 23 | 15 | 16 | 25 | 15 |
| Hepatocyte, focal clear cellular alteration | 2 | 2 | - | 3 | 1 | 7 | 5 | 5 | - | 2 |
| Hepatocyte, focal eosinophilic cellular alteration | 16 | 22 | 16 | 20 | 20 | 21 | 24 | 22 | 21 | 18 |
| Focal necrosis | 1 | - | 3 | 1 | 3 | 4 | 7 | 5 | 4 | 14 |

| Lesion | Dose/mg/kg/day | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Female | | | | | Male | | | | |
| | 0 | 0 | 2 | 5 | 8 | 0 | 0 | 2 | 5 | 8 |
| Pancreas | [50] | [50] | [50] | [50] | [50] | [50] | [50] | [50] | [50] | [50] |
| **Neoplastic lesion** | | | | | | | | | | |
| Islet adenoma | 1 | 4 | - | - | 2 | 5 | 3 | 9 | 6 | 9 |
| Islet carcinoma | - | - | - | - | - | 1 | 2 | - | - | 1 |
| Acinus adenoma | - | - | - | - | - | - | - | - | - | 2 |
| **Non-neoplastic lesion** | | | | | | | | | | |
| Islet fibrosis | 2 | 3 | - | - | - | 24 | 25 | 13 | 15 | 12 |
| Chronic focal inflammation | 1 | 5 | 1 | 1 | 2 | 3 | 4 | 2 | 3 | 6 |
| Islet hyperplasia | 1 | - | - | - | - | - | - | 4 | - | 1 |
| Adrenal | [50] | [50] | [50] | [50] | [50] | [50] | [50] | [50] | [50] | [50] |
| **Neoplastic lesion** | | | | | | | | | | |
| Adrenal cortex adenoma | 2 | 1 | 2 | - | 2 | - | - | - | - | 2 |
| Adrenal cortex carcinoma | - | 1 | 1 | - | - | - | - | 1 | - | - |
| Benign pheochromocytoma | - | - | 2 | 3 | 1 | 2 | 3 | 4 | 2 | 3 |
| Malignant pheochromocytoma | - | - | - | - | - | 1 | - | - | 2 | 1 |
| **Non-neoplastic lesion** | | | | | | | | | | |
| Cortex, diffuse hyperplasia | - | 3 | - | 1 | 1 | - | - | - | - | 1 |
| Cortex, focal hyperplasia | 4 | 3 | 2 | 3 | 3 | 8 | 5 | 2 | 6 | 8 |
| Cortex, focal hypertrophy | 14 | 20 | 15 | 12 | 15 | 14 | 11 | 11 | 10 | 11 |
| Medulla, focal hyperplasia | 1 | 3 | 2 | 4 | 2 | 8 | 5 | 2 | 6 | 8 |
| Pituitary | [49] | [50] | [50] | [50] | [50] | [50] | [50] | [50] | [50] | [50] |
| **Neoplastic lesion** | | | | | | | | | | |
| Adenoma | 41 | 37 | 40 | 37 | 29 | 28 | 29 | 30 | 25 | 28 |
| **Non-neoplastic lesion** | | | | | | | | | | |
| Focal hyperplasia | 2 | 4 | 1 | 2 | 1 | 4 | 7 | 5 | 7 | 8 |
| Focal hypertrophy | - | - | - | - | 1 | 1 | 2 | 5 | 5 | 3 |
| Thyroid | [50] | [50] | [50] | [50] | [50] | [50] | [50] | [50] | [50] | [50] |
| **Neoplastic lesion** | | | | | | | | | | |
| Parafollicular cell adenoma | 3 | 2 | 7 | 4 | 3 | 4 | 9 | 9 | 8 | 8 |
| Parafollicular cell carcinoma | 1 | 1 | 1 | - | - | 1 | - | - | 1 | - |
| **Non-neoplastic lesion** | | | | | | | | | | |
| Parafollicular cell, diffuse hyperplasia | 4 | 8 | 7 | 6 | 5 | 7 | 12 | 7 | 8 | 12 |
| Parafollicular cell, focal hyperplasia | 5 | 2 | 4 | 6 | 3 | 5 | 3 | 3 | 3 | 3 |
| Skin | | | | | | | | | | |
| Fibroma | 2 | - | 1 | - | - | 6 | 1 | 3 | - | 2 |
| Fibrosarcoma | - | - | - | - | - | 2 | 1 | 1 | 1 | 2 |
| Mammary gland | [48] | [48] | [47] | [48] | [48] | - | - | - | - | - |
| **Neoplastic lesion** | | | | | | | | | | |
| Adenocarcinoma | 7 | 14 | 11 | 9 | 3 | - | - | - | - | - |
| Adenoma | 2 | - | 3 | - | 2 | 1 | - | - | - | 1 |
| Carcinosarcoma | 1 | 1 | - | - | - | - | - | - | - | - |
| Fibroadenoma | 13 | 14 | 17 | 22 | 11 | - | - | - | - | - |
| **Non-neoplastic lesion** | | | | | | | | | | |
| Focal hyperplasia | 1 | 1 | 2 | 3 | - | | | | | |
| Brain | [50] | [50] | [50] | [50] | [50] | [50] | [50] | [50] | [50] | [50] |
| Malignant glioma | - | - | 2 | - | 3 | - | 1 | - | 1 | 2 |
| Benign granular cell tumor | - | 1 | 1 | - | - | 2 | 2 | 1 | - | 1 |
| Spinal cord | [50] | [50] | [50] | [50] | [50] | [50] | [50] | [50] | [50] | [50] |
| Malignant glioma | 1 | - | - | - | - | 1 | - | 1 | - | - |
| Primary site undetermined | [50] | [50] | [50] | [50] | [50] | [50] | [50] | [50] | [50] | [50] |
| Histiocytic sarcoma | 4 | 1 | 1 | 1 | 1 | 1 | 1 | - | - | - |

The other non-neoplastic and neoplastic histopathological lesions were either sporadic or comparable in incidence and severity across all groups, and/or were consistent with changes associated with age.

Prior to adjustment for multiplicity of tests, there was a significant increase in pancreatic islet [p=0.028] and acinar [p=0.032] adenomas in males and brain malignant glioma [p=0.041] in females.

Following adjustment for multiplicity of tests, there was no statistically significant evidence of a dose-dependent increase in tumor bearing rats. This is in agreement with the statistical analysis conducted by Dr. Taneja.

The incidence of pancreatic islet adenoma in males in this study was 10%, 6%, 18%, 12%, and 18% at 0, 0, 2, 5, and 8 mg/kg/day, respectively. This effect lacked a dose response. The incidence range of pancreatic islet adenoma in historical controls [1993-1995: Appendix 2, p. 14; Submission dated April 1, 1999] from the Sponsor's lab for diet optimized animals was 2-24% [overall incidence of 71/759 (9.3%)]. In addition, combining pancreatic islet adenoma and carcinoma did not result in a statistically significant positive linear trend. The incidence range of pancreatic acinar adenoma in this study was 4% at 8 mg/kg/day. The incidence of pancreatic acinar adenoma in historical controls [1993-1995: Appendix 2, p. 14; Submission dated April 1, 1999] from the Sponsor's lab for diet optimized animals was 0-4% [overall incidence of 3/759 (0.4%)]. Therefore, the difference in the incidence of these two tumors is of questionable biological significance.

The incidence of brain malignant glioma in females in this study was 0%, 0%, 4%, 0%, and 6% at 0, 0, 2, 5, 8 mg/kg/day, respectively. The occurrence of this neoplastic lesion did not demonstrate a dose-dependent relationship. In addition, there was no apparent increase in tumorigenesis in other tissues of similar embryological origin [e.g. sarcomas]. There was no increase in the incidence of preneoplastic lesions. The incidence range of brain malignant glioma in females in historical controls [1993-1995: Appendix 2, p. 14; Submission dated April 1, 1999] from the Sponsor's lab for diet optimized animals was 0-2.6% [overall incidence of 2/759 (0.3%)]. Analysis by Dr. Taneja of brain and spinal malignant glioma combined yielded a p value = 0.0914.

In addition, Dr. Taneja's analysis indicated that there was no statistically significant positive linear trend observed following tumor combination of hepatocellular adenoma and carcinoma.

**Reviewer's Comments – Study Design and Presentation**

1. Based on the presence of significant toxicity/mortality, the MTD was reached. Survival to the end of study was adequate. Therefore, the doses used in this study are considered acceptable.
2. It is recommended that hematology, serum chemistry, and urinalysis be conducted prior to and 3, 6, 12, and 18 months post-dosing [draft Redbook II]. These evaluations were not included in this study.

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

1. Administration of L748,731 for approximately 106 weeks did not demonstrate a statistically significant trend for an increase in any tumor type for either males or females. **Reviewer's Comments** – Following adjustment for multiplicity of tests, no significant increase was observed n any tumor type. This is in agreement with Dr. Taneja's analyses. The statistically significant increase in pancreatic acinar and islet adenomas in males and the brain malignant glioma in females, which was observed prior to adjustment for multiplicity of tests, is of questionable biological significance for the reasons stated previously in this review. 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. In both sexes, there was an increase in mortality with increasing dose secondary to GI toxicity and peritonitis. **Reviewer's Comment** – Although there was an increase in mortality in the high dose of males and females, statistical analysis by Dr. Taneja indicated that there was a significant increase in females only.

3. Animals from all treatment groups exhibited drug induced GI toxicity, including small and large intestine ulceration and inflammation. There were a number of other changes that were considered

secondary to the GI lesions including lymph node lymphoid hyperplasia, lymphadenitits, lymphangiectasis; and bone marrow increased myeloid hyperplasia.

**Reviewer's comment-** In general, the Reviewer concurs with the Sponsor. The increased incidence and severity of GI toxicity observed in females may reflect an increase in exposure as indicated by the toxicokinetic data. As noted above, the animals were fed a diet restricted by an estimated 30%. It was anticipated that this would have reduced not only mortality but also the incidence of several spontaneous tumors, e.g. pituitary and mammary gland tumors.[2] However, the incidence of the pituitary tumors was comparable to that observed in control group rats fed *ad libitum* based on historical controls [1989-1993: Appendix 2, p. 22; Submission dated April 1, 1999] from the Sponsor's lab.

## 6.2 CARCINOGENCITY – MOUSE STUDIES

### 6.2.1. L-748,731: One Hundred-Six-Week Oral Carcinogenicity Study in Mice: [Vol. 1.36; p. E-705, Vol. 1.37; p. E-760, and Vol. 1.38; p. 1132]

**Study Identification:** TT#96-603-0;-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. 14, 1996 - Feb. 18, 1998
**Formulation:** Test Article - L-748,731; Vehicle - 0.5% aqueous methylcellulose
**Lot No.** L-748,731-000R027; [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, 19, 26, 39, 52, 56, 65, 78, 91, 96, and 104 with results, according to the Sponsor, within acceptable limits
**Final Report (X)** July 23, 1998
**GLP and Quality Assurance Statements: Yes (X)**
**Objective:** "To evaluate the carcinogenic potential of L-748,731 [MK-0966] when administered chronically for approximately 106 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 = 730-735<br>M = 728-730 | M | 50 | -Crl:CD-1® [IBR] BR albino mice -<br>-37 days at start of study,<br>-F - 17.3 to 26.6 g; M - 22.2 to 32.2 g<br>-housed 2-3/box |
| Group 2 - Vehicle | | | | | F | 50 | |
| Group 3 - L-748,731 | 5 | | | | | | |
| Group 4 - L-748,731 | 10 | | | | | | |
| Group 5 - L-748,731 | 20 | | | | | | |
| Group 6 - L-748,731 | 30 | | | | | | |

*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 study



[2] Semler, David E. in *Animal Models in Toxicology*, [Gad and Chengalis, Eds.], Marcel Dekker, Inc., New York: 1992;p. 21.

| Parameter Evaluated | Time Point(s) |
|---|---|
| **Clinical observations/mortality**<br><br>**-Palpated for masses** | Daily,<br><br>q4wks beginning Week 26 |
| **Body weight** | pretest, 1X/week for Week 1, then generally 2X/week through Week 13, then weekly thereafter |
| **Ophthalmic Examination** - indirect, slit lamp biomicroscopy as needed, per protocol | pretest, Week 52, Weeks 95-96 |
| **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 animals – 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
**this represents the complete list of tissues evaluated

**Dose Selection** - Dose selection was based on the maximum tolerated dose [MTD] with intestinal ulceration/perforation and peritonitis the dose limiting toxicities. Studies cited by the Sponsor to support the dose selection were: [1] TT#95-610-0: L-748,731: Fourteen-Week Oral Range-Finding Study in Mice; [2] TT#617-0: L-748,731: Single Dose Oral Toxicokinetic Study in Mice; and [3] TT#95-611-0: L-748,731: Five-Week Oral Toxicokinetic Study in Mice. In the 14-week study, GI ulceration was noted at ≥ 30 mg/kg/day and mortality occurred at ≥100 mg/kg/day. It should be noted that [1] the protocol was not provided to the Division for review and [2] the carcinogenicity study was initiated prior to the meeting of the ECAC. The following statements reflect recommendations by the ECAC, which met on April 2, 1996: "Based on the data provided, the committee recommended increasing the HD to 60 mg/kg and possibly dropping the 20 mg/kg group. The recommended dose range, if the sponsor is committed to 4 dose groups, would be 5, 10, 30, and 60 mg/kg. The committee further recommended that if the 60 mg/kg dose produced excessive toxicity, the HD could be dropped or lowered." [Fax to the Sponsor dated 8-16-96]. However, based on the excessive mortality observed in this study, the MTD was achieved for L-748,731.

**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, where available. 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 | Exposure [XMHD*] | | | |
|---|---|---|---|---|
| [mg/kg/day] | Body mass | Surface Area | AUC | |
| | | | Female | Male |
| 5 | 12[6]X | 1[0.5]X | NP | NP |
| 10 | 25[12]X | 2[1]X | NP | NP |
| 20 | 50[25]X | 4[2]X | NP | NP |
| 30 | 75[37]X | 7[3]X | 2[<1]X | 5[2]X |

*Maximum human dose
NP – not provided