

treatment related ulcer at 10 mg/kg/day and subepithelial inflammation at 5 mg/kg/day - and lesions in the liver (focal fibrosis and necrosis), all of which were considered very slight or slight to small. The no effect dose was 2 mg/kg/day in rats for the oral administration of metabolite L-755,190.

L-755,190 administration resulted in approximately a 40% decrease in PGE2 formation but no decrease in 6-keto PGF1 $\alpha$  formation. The decrease in both of the eicosanoids following administration of Ibuprofen was >99%.

## 9. Overall Summary

Studies were conducted both *in vitro* and *in vivo* to delineate [1] the selectivity of L-748,731 for inhibition of COX-2 compared to COX-1 and [2] efficacy of L-748,731 in inflammatory, pain, pyrexia, and arthritis animal models. Based on results from a battery of *in vitro studies* [results outlined in Summary of Pharmacology, p. —], L-748,731 was shown to be relatively specific for COX-2 vs. COX-1. L-748,731 did exhibit some COX-1 activity inhibition [e.g. human serum TXB<sub>2</sub> assay, kidney microsomal CO assay, CO activity of U-937 cell microsomes] in the assay systems evaluated. However, the concentrations required to achieve inhibition were high [ $\mu$ M] and significantly greater than the concentrations of indomethacin required [nM] to demonstrate inhibition within the same assay. In general, the potency for COX-2 inhibition was similar for L-748,731 and indomethacin and diclofenac.

It was also demonstrated by *in vitro* methods that L-748,731 exhibited little to no inhibitory activity against enzymes involved in leukotriene biosynthesis [e.g. lipooxygenase activity]. In addition, L-748,731 did not demonstrate activity at any other receptor or against any enzyme evaluated in the Panlabs Discovery Screen™. L-748,731 did not inhibit collagen induced platelet aggregation. However, in this system, indomethacin resulted in only a 28% decrease in this response.

L-748,731 demonstrated efficacy in the following *in vivo* animal models: [1] acute inflammatory model [carageenan-induced rat paw edema]; [2] pyrexia reversal in conscious rats and squirrel monkeys; [3] antinociception in an acute inflammation [carageenan-induced rat paw hyperalgesia] and chronic inflammation [CFA-induced hyperalgesia in rats]; and [4] adjuvant induced arthritis in rats. In general, the efficacy was comparable to indomethacin or diclofenac in the various models under the conditions tested. L-748,731 was less effective in the chronic inflammatory hyperalgesia model but more effective in the pyrexia reversal in conscious rats compared to indomethacin or diclofenac.

There are a number of differences in pharmacokinetic/pharmacodynamic parameters between animals and humans. Primarily these include differences in [1] route of elimination; [2] degree of enterohepatic recirculation; and [3] metabolism. Although for all species elimination of L-748,731 occurs almost entirely through metabolism, the route of excretion varies. In humans, 72% of the radioactivity of a 125-mg dose of radiolabeled drug is eliminated in the urine and 14% in the feces. In rats, however, approximately 58 – 76% of the radioactivity of a 5 mg/kg dose of radiolabeled L-748,731 was recovered in the bile and approximately 3 – 15% in the urine. In the dog, the drug was eliminated in the feces [approximately 76% based on radioactive elimination] and in the urine [approximately 17% based on radioactive elimination]. However, due to low bioavailability [e.g. approximately 40%], only 27% of drug was eliminated in the bile.

Rat studies also indicated that [1] enterohepatic recirculation played a role in the pharmacokinetics of both the parent compound and the primary metabolites [e.g. 5-OH furanone and its glucuronide] and [2] metabolic conversion of these metabolites to parent compound occurred in the lower GI tract beginning at the level of the cecum. Enterohepatic recirculation and reversible metabolism have essentially no role in the pharmacokinetics of L-748,731 in humans and minimal, if any, in dogs.

L-748,731 undergoes extensive hepatic metabolism in all three species. As the Sponsor notes, the metabolites are qualitatively similar in humans, rats, and dogs, but are quantitatively dissimilar. In humans, the main pathway is reduction of the parent to a *cis* and *trans*-dihydrohydroxy acid. To a much lesser

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extent, the glucuronide of the 5-hydroxy furanone [L-755,190] is formed. A total of six metabolites have been identified in man. In the rat, the primary metabolite is the glucuronide of the 5-hydroxy furanone metabolite that involves an oxidation pathway. This metabolite is the predominant metabolite found not only in the bile but in the urine as well. Small quantities of the 3', 4'-*trans*-dihydrodiol metabolite, parent compound, the sulfate conjugate of 4'-phenol derivative, and the dihydrohydroxy acid of L-748,731 were also identified in the urine of rats. Similar metabolites were observed in the urine of mice. The metabolism in the dog was more complex than in humans or rats with at least 14 identified but not all characterized. As in the rat, the glucuronide of the 5-hydroxy furanone metabolite was the most abundant metabolite in the bile but was a minor metabolite in urine. The *trans*-dihydro metabolite, which constituted 14-22% of the total radioactivity recovered in urine, was identified in the dog. No parent compound was found in either the urine or bile. The schematic below demonstrates the metabolism in these various species.



The quantitative differences in metabolism raises the issue of whether the toxicity of the various metabolites observed in humans have been adequately addressed in the nonclinical toxicology studies. The Sponsor has provided limited data on the activity of the metabolites. Based only on data from a single assay [e.g. *in vitro* human whole blood assay], the Sponsor concludes that the primary human metabolites are either inactive or only weakly active as COX-2 inhibitors [Vol. 1.92, Reference 99 - Memorandum]. Even if the metabolites have limited or no pharmacological activity, the possibility of metabolite-specific toxicities is not eliminated. The Sponsor indicated [Submission dated March 31, 1999] that assays to quantitate the various metabolites, with the exception of L-755,190, have not been developed. As a result plasma AUC data are not available. Consequently, comparison of the exposure to the major metabolite in humans [e.g. the *cis* and *trans*-dihydrohydroxy acid] to that in animals was extrapolated from the levels present in the urine. The Sponsor in submissions dated March 31, 1999 and April 13, 1999 provided the data in the table below.

Species	Percent of Dose [0-24 hour urine]			Amount mg/kg
	Rofecoxib Eq. [Total C-14]	Dose [mg/kg]	Total DHHA <sup>a</sup>	
Mouse	9.8	100	0.074	0.074
Rat	5	100	1.14	1.14
Dog	16.8	5	10.5	0.52
Human <sup>b</sup>	37.1	0.5	20.9	0.1

<sup>a</sup>DHHA=dihydrohydroxy acid of L-748,731

<sup>b</sup>dose of 0.5 mg/kg is equal to 25 mg/day for a 50-kg subject, this calculation was extrapolated from data following a total dose of 125 mg

Based on these data, the exposure in rats to the predominant human metabolite exceeded the exposure in humans in studies up to a 27-week duration. In the long term studies [e.g. 53 and 106 week studies], the exposure was approximately  $\leq 1X$  that observed in humans. These doses of parent compound were associated with significant GI toxicity in the rat. Exposure to this metabolite in the mouse in both 2-year bioassays was also approximately  $\leq 1X$  human exposure. Assuming linearity in exposure to the dihydrohydroxy acid metabolite over the range of doses used in the 53-week dog study, the exposure to this metabolite was greater than that observed in man. It should be noted, however, that the only stereoisomer observed in the urine of the dog was the *trans* form. In humans, both the *cis* and *trans* stereoisomers were detected. It would have been preferred that the Sponsor address the potential toxicity of *cis* and *trans*-DHHA in specifically designated nonclinical toxicology studies as they did for the toxicity of the 5-hydroxy furanone metabolite.

Following iv administration of L-748,731 at 2 mg/kg in the rat, maximum mean concentration was achieved in most tissues at 5 minute. With the exception of the small intestine, large intestine, stomach [including contents and wash]; liver; and kidneys, tissue levels by 24 hours were  $\leq 1 \mu\text{g}$  equivalents/g of tissue. The tissue:plasma ratio was generally  $\leq 1$ . This was not the case for the adrenals, kidneys, liver, and small intestine. Following an oral dose of L748,731 to rats, the elimination profile from the brain and the CSF was similar to plasma elimination. The brain:plasma ratio was generally 0.4. The level of drug in the brain at 24 hours was  $9.6 \text{ ng} \pm 1.9 \text{ ng/g}$  tissue. With respect to GI distribution in the rat following a single oral dose [5 mg/kg], the amount of radioactivity recovered from the tissues and luminal contents was approximately 60-70%, with the majority of the radioactivity associated with luminal contents. Radioactivity tended to be highest in the jejunum and ileum, and increased in the cecum and large intestine with time. At the early time points [e.g. 1 hour], the majority of the radioactivity was associated with parent compound in the jejunum and ileum. However, at 6 hours post dosing the 5-OH furanone metabolite and its glucuronide accounted for the entire radioactivity. At 6 hours in the cecum, the majority of the radioactivity was attributed to parent compound, supporting the Sponsor's hypothesis that the cecum is the site for the conversion of metabolite to parent compound.

In general, exposure, based on AUC in dogs, rats, and mice, was not dose proportional, although a linearity was observed over restricted dose ranges. The exposures on Day 1 were similar to those observed following repeat dosing.

The repeat dose toxicity and the reproductive toxicity studies identified several potential target organs that included [1] the gastrointestinal tract; [2] kidney; [3] immune system; [4] liver; and [5] female reproductive system. GI toxicity was observed in three species: the rat, mouse, and dog. In the rat and mouse, small and large intestinal ulceration/perforation and a secondary peritonitis characterized the GI toxicity. The Sponsor indicated that the most common site for intestinal ulceration was the jejunum and cecum, although ulcers were also observed in the colon, duodenum, ileum, and/or stomach. Mortality was associated with this lesion in both species. The incidence of GI toxicity tended to be greater in female rats and male mice and tended to occur at lower doses. Exposure to drug was greater at comparable doses in the gender that exhibited the higher incidence of GI toxicity for the respective species. The NOAEL for GI ulceration/perforation in rats following drug administration for 52 weeks was determined to be 1 mg/kg/day. The NOAEL for GI ulceration/perforation in mice following drug administration for 104 weeks was determined to be 5 mg/kg/day. Both doses are  $\leq 1X$  human exposure based on AUC for a 25 or 50 mg/day dose. The NOAEL based on GI toxicity was higher in studies of shorter duration for both species

indicating that this toxicity is not only dependent on dose but also on the duration of exposure to L-748,731. The signs of GI toxicity in the dog were not as severe. The NOAEL for GI toxicity, for both a 14-week study and 53-week study, was 10 mg/kg/day. This represents a multiple of the human exposure based on AUC for a 25[50] mg/day dose of approximately 3[1]. In the 14-week study, histopathological changes at 50 mg/kg/day (13[5] X human AUC at 25[50] mg/day) included intestinal ulceration, which occurred over Peyer's patches, in 1/4 males and females, and reddening of the gastric mucosa in 1/4 females. Clinical signs [e.g. bloody stools] but no GI histopathological changes were observed at the high dose [30 mg/kg/day] in the 53-week study. It should be noted that based on the limited and mild toxicity observed in the 53-week dog study, it was felt that the MTD was not reached.

The following parameters were altered in both rats and dogs in the studies in which GI ulceration was observed and were considered consistent with changes secondary to GI toxicity [e.g. ulceration and potential blood loss]. These changes included [1] decrease in RBC indices; [2] increase in neutrophils; [3] increase in platelets; and [4] increase in BUN without a concomitant increase in creatinine. Mice also exhibited similar changes in RBC indices and neutrophils. The following effects were also considered secondary to GI toxicity and occurred in the mouse and/or rat: [1] clinical signs; [2] decreases in albumin and total protein; [3] extramedullary hematopoiesis in the spleen and/or liver; [4] increased bone marrow erythropoiesis and/or granulopoiesis; [5] hepatic subcapsular necrosis; [6] lymph node histiocytosis and/or reactive hyperplasia; [7] splenic and lymph node lymphocytic necrosis; [8] thymic atrophy/involution; [9] adrenal cortical hypertrophy; and [10] peritonitis. Weight loss and decreased food consumption was observed in the 14-week dog study. Changes in body weight and food consumption in rodents were inconsistent.

The apparent difference in sensitivity to GI toxicity between rodents and dogs may, in part, be due to differences in the pharmacokinetics of L-748,731 in these species. In the dog, although 76% of a radiolabeled 5 mg/kg dose was eliminated in the feces, bioavailability was approximately 30%. Consequently, of the absorbed dose, approximately 26% of the total dose was eliminated in the bile. In the rat, bioavailability was essentially quantitative and approximately 60-80% of the total radioactivity was eliminated in the bile. In addition, enterohepatic recirculation and reversible oxidative metabolism also increased the exposure to L-748,731 in the rat. In humans, 72% and 14% of a 125-mg dose radiolabeled drug was excreted in the urine and feces, respectively.

Based on data generated by the Sponsor, there appears to be considerable variability in the levels of COX-1 distribution in the gastrointestinal tract of the various species evaluated. The rank order of COX-1 levels throughout the GI tract was generally humans > monkey > dog > rat. The dog tended to have higher levels than humans in the stomach antrum/pylorus. The Sponsor suggests that there "appears to be an inverse relationship between levels of PGHS-1 protein observed and the sites within the gastrointestinal tract where ulceration is commonly observed". In the 14-week dog study, immunohistochemical staining for distribution of COX-1 and COX-2 in the GI tract revealed comparable staining between control dogs and dogs treated with  $\leq 50$  mg/kg/day with the exception of the ulcerated areas. There was a decrease in COX-1 staining in the areas of the ulcerated jejunum. This suggests that at higher doses, L-748,731 may be less selective, e.g. exhibits COX-1 inhibiting activity.

Several studies were conducted in which the effect of non-selective NSAIDs [e.g. indomethacin, diclofenac] on GI COX activity and mucosal integrity was compared to the effect of L-748,731 on these parameters. Administration of either ibuprofen [100 mg/kg X 15 days] and L-748,731 [100 mg/kg X 15 days] to rats indicated that L-748,731 resulted in minimal change in COX activity and PG levels in the intestinal tract [e.g. up to only 20-30%]. Ibuprofen, however, resulted in a decrease in COX activity and PG levels by approximately 60-95%. Studies conducted to assess the integrity of the GI tract [e.g. fecal and urinary  $^{51}\text{Cr}$  excretion] showed no statistically significant increase in fecal  $^{51}\text{Cr}$  excretion in either rats or squirrel monkeys following administration of L-748,731 at doses up to 100 mg/kg/day for 4-5 days. There was a statistically significant increase in urinary  $^{51}\text{Cr}$  excretion in male rats and a statistically nonsignificant increase in female rats administered L-748,731 at 200 mg/kg/day for 5 days.  $^{51}\text{Cr}$  excretion [both urinary and fecal] was significantly increased in these studies following administration of indomethacin or diclofenac [1-3- mg/kg BID X 3-5 days]. As noted above, the development of gastrointestinal lesions in rats following administration of L-748,731 is both dose and time-dependent. It is

probable, therefore, that had these parameters been measured under different experimental conditions [e.g. following chronic dose administration], there would have been a statistically significant increase in <sup>51</sup>Cr-excretion.

The findings in these studies in which the GI toxicity of nonselective COX inhibitors was compared to L-748,731 are consistent with reports in the literature indicating that the COX-2 inhibitors demonstrate less GI toxicity, especially gastric toxicity, than non-specific COX inhibitors.<sup>1,2</sup> However, significant GI erosion/ulceration/perforation was observed in the rat following chronic administration of L-748,731 at exposure levels based on AUC that were  $\leq 1X$  human exposure. As noted above, the apparent increased sensitivity for GI toxicity in the rat compared to the dog may, in part, be a function of pharmacokinetic/pharmacodynamic considerations. It should be noted, however, that COX-2 appears to play a role in the elimination of pathogens and repair of mucosal injury in the GI tract.<sup>3</sup> Therefore, inhibition of COX-2 may delay healing of GI lesions or potentiate ulceration in the GI tract.

Histopathological lesions in the kidney were also observed in all three species: mouse, rat, and dog. Renal tubular basophilia was observed in rats administered L-748,731 at doses of  $\geq 10$  mg/kg/day (6[2]X human exposure at 25[50] mg/day based on AUC) for 26 weeks and at  $\geq 100$  mg/kg/day (20[7]X human exposure at 25[50] mg/day based on AUC) for 13 weeks. This lesion was reversible within 14 days following cessation of L-748,731 at 300 mg/kg/day for 14 weeks and was more common in males than females. Tubular basophilia was also observed in 1-2 mice at  $\geq 30$  mg/kg/day (2[<1] X human exposure at 25[50] mg/day based on AUC). Treatment-related tubular basophilia, however, was not described in rats and mice in studies conducted for  $\geq 53$  weeks. In rats, this may, in part, be a function of dose. Renal papillary necrosis, which has been associated with the administration of non-specific COX inhibitors, was sporadically observed in all 3 species.

Prostaglandins are involved in renal physiology [e.g. salt and water homeostasis and glomerular hemodynamics<sup>4</sup>] and inhibition of the enzymes involved in their synthesis can lead to nephrotoxicity and toxicities associated with disturbances in salt and water homeostasis [e.g. edema]. The major isoform that has been associated with prostaglandin synthesis is COX-1.<sup>5</sup> L-748,731 administration resulted in a decrease in urinary PGE<sub>2</sub>, urine volume, and Na excretion in dogs suggesting that this drug does inhibit renal eicosanoid synthesis. In conscious male dogs, this decrease was transitory with baseline values observed after 3 hours. The plasma level of L-748,731 at which changes in Na excretion and urine volume were observed was  $>1.0$   $\mu$ M. In volume-depleted, normotensive male Wistar rats L-748,731 administration [10 mg/kg of p.o.] resulted in a decrease in glomerular filtration rate, renal blood flow, sodium excretion, and urine volume. The magnitude of the perturbations in both dogs and rats was generally less than that observed with a non-selective COX inhibitor [e.g. indomethacin, diclofenac]. Based on reported differences in the distribution of COX-2 in the rat and dog [e.g. primarily in the macula densa, thick ascending loops, a few papillary interstitial cells] compared to humans [e.g. glomerular podocytes, small arteries, arterioles, and veins]<sup>6</sup>, differences in toxicity between these species would be anticipated. However, disturbances in salt and/or water homeostasis, characterized by edema and hypertension, were observed in the human clinical trials.

Histopathological findings also suggested that the immune system was a target organ in both the dog and the rat administered L-748,731. Cervical lymph node granulomas were observed at doses  $\geq 100$

<sup>1</sup> Vane, J.R., Bakhle, Y.S., Botting, R.M. [1998] Cyclooxygenases 1 and 2. *Annu. Rev. Pharmacol. Toxicol.* 38:97-120.

<sup>2</sup> Hirata, T. et al. [1997]. Cyclooxygenase isozymes in mucosal ulcerogenic and functional responses following barrier disruption in rat stomachs. *Br. J. Pharmacol.* 122:447-454.

<sup>3</sup> Furst, D.F. [1998]. Perspectives on the Cyclooxygenase-2/Cyclooxygenase-1 Hypothesis. *J. Clin. Rheumatology.* 4[5]:S40-S48.

<sup>4</sup> Dubois, et al. [1998]. Cyclooxygenase in biology and disease. *FASEB.* 12:1063-1073.

<sup>5</sup> Khan, K.N.M., et al. [1998]. Interspecies differences in renal localization of cyclooxygenase isoforms: Implications in nonsteroidal antiinflammatory drug-related nephrotoxicity. *Toxicol. Pathol.* 26[5]:612-620.

<sup>6</sup> Khan, K.N.M., et al., *ibid.*

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mg/kg/day (20[7]X human exposure at 25[50] mg/day based on AUC) in rats. This lesion was reversible within 27 weeks following cessation of drug. Although, this lesion occurred at exposures greater than those anticipated in humans, it is known that functional disturbances in the immune system can precede the induction of histopathological changes.<sup>7</sup> Immunohistochemical staining indicated that there was an increase in COX-2 positive macrophages in the cervical lymph nodes of treated rats compared to control rats. The Sponsor speculates that this increase in COX-2 macrophages may be either due to loss of PGE<sub>2</sub> inhibitory effects or drug-induced activation. Cellulitis was observed in male dogs at 50 mg/kg/day X 13 weeks. That the immune system had the ability to respond normally, at least to certain stimuli, is indicated by changes associated with GI ulceration/perforation and peritonitis. These changes included an increase in neutrophils in rats, mice, and dogs and an increase in bone marrow granulopoiesis and lymph node histiocytosis and/or reactive hyperplasia in mice and/or rats. The potential immune suppression/enhancement associated with COX-2 inhibitors, or more specifically with L-748,731, has not been clearly defined.

Mild increases in ALT, AST, were observed in both rats [≥27 week studies] and mice [14-week study] and suggested hepatic effects. These changes did not correlate with any histopathological findings and were not always consistent. In addition, an adaptive response, characterized by centrilobular hepatocellular hypertrophy and an increase in liver weight, was noted in female rats administered 300 mg/kg/day [14-week study].

There are a number of reports which have shown that COX-2 plays a role in ovarian function and pregnancy.<sup>8,9</sup> Administration of L-748,731, which has been shown to readily cross the placenta in rats and rabbits, resulted in a number of perturbations in female reproduction. In addition, studies have shown that placental transfer of parent compound was high in both rats [90-100%] and rabbits [40-70%], so fetal exposure is significant. In both the 14-week and 53-week study in dogs, there was a decrease in ovary weights. In the 14-week study, a decrease of 13-28% was observed at ≥10 mg/kg/day. In the 53-week study, there was a decrease of 20, 12, and 26% at 3, 10, and 30 mg/kg/day. Administration of L-748,731 to rats resulted in a partial inhibition of ovulation. A NOAEL was not determined but was <10 mg/kg/day which represents 8[3]X human exposure based on AUC at 25[50] mg/day. Effects on fertility, fecundity, and gestation length were variable. There were no treatment-related effects on estrous cyclicity or time to mating. A decrease in embryofetal survival, characterized by an increase in resorptions, peri/post implantation losses and dead fetuses/female, was observed in both rats and rabbits. In rats, the data suggested that the magnitude of the dose at which the decreased embryofetal survival was observed was in part of function of timing of dosing with respect to stage of pregnancy. The NOAEL for this finding occurred at doses that were approximately 2[<1]X the human exposure based AUC at 25[50] mg/day in both rats and rabbits. Reversibility of compromised embryofetal survival was demonstrated in rats following a 14-day recovery period. Maternotoxicity [e.g. GI toxicity/peritonitis] NOAEL was 3-10 mg/kg/day.

There was no clear evidence of teratogenic effects observed in rabbits and in rats up to a dose of 50 mg/kg/day. There was a statistically nonsignificant increase in the incidence of vertebral malformations. The Sponsor states [submission dated April 19, 199] that "the spectrum of [vertebral] malformations is different for each fetus and when broken down by incidence, according to region and description, ...all values are within MARTA historical control range reference". Based on these considerations, the evidence is not conclusive with respect to a drug-related effect.

Structure activity relationship evaluation by the Office of Testing and Research [Review dated April 30, 1999] indicated that based on results from four teratogenicity database modules, L-748,731 was "not predicted to be a trans-species mammalian teratogen, and [was] evaluated as inactive in the MCASE QSAR Rodent Teratogenicity Test"

<sup>7</sup> Schurrman, H.-J., et al. [1994]. Histopathology of the immune system as a tool to assess immunotoxicity. *Toxicology*. 86:187-212.

<sup>8</sup> Dubois, R.N., et al. [1998] Cyclooxygenase in biology and disease. *FASEB*. 12:1063-1073.

<sup>9</sup> Furst, D.E. [1998] Perspective on the Cyclooxygenase-2/Cyclooxygenase-1 Hypothesis. *J Clin Rheumatol*. 5:S40-S48.

In the rabbits, there was an increase in the litter and fetal incidence of incomplete ossification at  $\geq 25$  mg/kg/day. This dose is  $<1X$  human exposure at 25 mg/day based on AUC. Incomplete ossification was observed in the sternbra, metacarpus, and talus/calcaneus. The Sponsor considers the increased incidence of incomplete ossification to be of "minimal toxicological significance since the average number of sacrocaudal vertebrae, an indicator of overall fetal ossification, were similar across all groups". There was a minor increase in the incidence of incomplete ossification in the rats across the dose groups when compared to control values. It should be noted that in rabbits, there was an increase in several parameters associated with bone metabolism [e.g. vertebral malformations, incomplete ossification] and that prostaglandins appear to play a role in bone metabolism. That role has not been well defined.<sup>10</sup>

Increased pup mortality and postnatal toxicity [e.g. decrease in pup weights] was also seen in rats. This increase in postnatal survival [stillborn and dead pups] was observed beginning postnatal day 0. Significant narrowing of the ductus arteriosus [approximately 50-60%] probably contributed to these deaths. The effects on the ductus arteriosus were observed at doses that were  $\leq 1X$  the human exposure based on AUC at 25[50] mg/day. In addition, a cross-fostering study [TT #95-730-0] indicated that this postnatal mortality was related to both gestational and lactational exposure of the pups to L-748,731. The conclusion that pup mortality is impacted by lactational exposure to L-748,731 is further supported by the fact that the drug is readily excreted in the milk in rats. At a rat maternal dose of 1 and 10 mg/kg, the mean milk:plasma ratio was 0.52 and 1.27, respectively.

Plasma toxicokinetics in pregnant vs. non-pregnant rats were similar, the pregnant rats appeared to have increased sensitivity to the GI toxicity induced by MK-0966 compared to non-pregnant rats.

There were no effects on male fertility [mating performance, fertility indices, embryonic/fetal survival, sperm count and motility, testicular/epididymal organ weights, histopathology] at doses up to 100 mg/kg/day.

L-748,731 was negative in the following battery of assays conducted to assess the mutagenic and clastogenic potential of the drug: [1] bacterial mutagenicity assay; [2] chromosomal aberrations in Chinese hamster ovary (CHO) cells; [3] V-79 mammalian cell mutagenesis assay; [4] alkaline elution/rat hepatocyte assay; [5] alkaline elution/rat liver DNA damage assay in male and female rats; and [6] chromosomal aberrations in mouse bone marrow in male and female mice. This battery includes those assays recommended in the ICH guidelines. The Genetic Toxicology Committee concurred with the Reviewer's assessment, e.g. that the data generated in these assays "provided convincing evidence that MK-0966 has been shown to be non-genotoxic under the conditions tested".

The full Carcinogenicity Assessment Committee, which met on April 8, 1999, discussed the carcinogenic potential of L748-731 based on results of 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 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. In general, for both the rat study and low dose mouse study, it was agreed that the doses used were adequate. The exposure in male and female rats at the highest dose evaluated was approximately 5[2]X and 7[2]X that in humans, based on AUC, receiving a dose of 25[50] mg/day. 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[2] based on AUC at a human dose of 25[50] mg/day.

<sup>10</sup> Dubois, R.N., et. al. *Ibid.*

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. 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-specie rodent carcinogenic" based on 8 rodent carcinogenicity database modules and was negative in the MASE QSAR Rodent Carcinogenicity.

## 10. Recommendations:

### 10.1 Internal Comments:

The majority of the toxicities observed in the nonclinical studies were, in general, consistent with toxicities observed with other COX-2 inhibitors and nonspecific NSAIDs. The data should be interpreted with an understanding of the differences in metabolism. The primary target organs identified included [1] gastrointestinal tract; [2] kidney; [3] female reproduction; and [4] immune system. Mild increases in ALT and AST were observed in rats and mice suggesting hepatic effects. These changes did not correlate with histopathological changes. Gastrointestinal toxicity was characterized by intestinal ulceration/perforation in the mouse, rat, and dog. This toxicity was accompanied by peritonitis and mortality in the rat and mouse. Gastric erosions/ulceration occurred, but the frequency was sporadic and less than that observed with nonspecific NSAIDs [e.g. indomethacin]. Renal toxicity in rats and mice was characterized by renal tubular basophilia that was shown to be reversible in the rat. Papillary necrosis was sporadically observed in the rat, mouse, and dog. Effects on renal physiology were also observed in rats and dogs. In dogs, administration of L-748,731 resulted in a decrease in urinary PGE<sub>2</sub>, urine volume, and Na excretion. In volume-depleted, normotensive rats, there was a decrease in GFR, renal blood flow, Na excretion, and urine volume. Perturbations in female reproductive function included partial inhibition of ovulation, decrease in embryofetal survival [e.g. increases in resorptions, peri/post implantation losses, and dead fetuses/female], increased postnatal mortality following both gestational and lactational exposure of pups, and delayed ossification.

Based on the pharmacology of L-748,731 [e.g. anti-inflammatory, inhibition of the production of a PGE<sub>2</sub> a known immunosuppressant] it is anticipated that this drug will impact immune function, suppression and/or enhancement. In male dogs, there was an increase in the incidence of cellulitis compared to controls. In addition, cervical granulomas were observed in rats. Dermatitis at a low incidence was observed in dogs administered other COX-2 inhibitors. Concern was expressed that potential immunosuppressive activity could have contributed to some of the tumor findings in the 2-year bioassays in rats and mice [see meeting minutes dated April 18, 1999 of the Carcinogenicity Assessment Committee]. There was no consensus regarding this point. The Sponsor will be asked to address the potential immunotoxicity of L-748,731 [see External Recommendations].

It is recognized that COX-2 is induced during a number of physiological and pathological conditions and that the role of COX-2 in these various processes has not been fully delineated. The consequences of selective inhibition of COX-2 may be either potentially beneficial or deleterious depending on the process involved. Therefore, it is difficult to define at-risk patient populations.

THIS SECTION  
WAS  
DETERMINED  
NOT  
TO BE  
RELEASABLE

3 pages

N-21,042  
Merck & Co.

Vioxx  
FINAL

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discontinue nursing or to discontinue the drug, taking into account the importance of the drug to the mother.

[The multiple of the human exposure was extrapolated from rat AUC data from Study TT #95:721-0: L-748,731: Oral Fertility in Female Rats with a Recovery Period.]

Reviewer's Signature:

/S/  
Susan D. Wilson, D.V.M., Ph.D.

5-7-99

Date

Team Leader Concurrence:

/S/  
Andrea Weir, Ph.D., D.A.B.T.

7 May 99

Date

CC: list:

cc:Original  
HFD-550:Division Files  
HFD-550/Pharm/SDWilson/AWeir  
HFD-550/CSO/ Scook  
HFD-550/MO/LVillalba/Mmordechai/JHyde

APPEARS THIS WAY  
ON ORIGINAL

Appendix:

Draft Date:

Addendum:

**ADDENDUM TO PHARMACOLOGY/TOXICOLOGY REVIEW - NDA #21-042**  
**[MAY 7, 1999]: VIOXX; MERCK & CO., INC.**

**TO:** Sandy Cook  
**FROM:** Susan Wilson  
**THROUGH:** Andrea Weir  
**DATE:** May 24, 1999  
**RE:** Labeling Addendum

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In the label line 519 reads that the multiple of human exposure was "5- and 2-fold". This should have read "4- and 1-fold". Since these figures are similar, it was felt that it was not necessary to change the label to read "4- and 1-fold".

**Reviewer's Signature:**

*/S/*

Susan D. Wilson, DVM, PhD

*5-24-99*

Date

**Team Leader Concurrence:**

*/S/*

Andrea B. Weir, PhD, DABT

*24 May 99*

Date

CC: Original  
Div Files  
CSO:SCook  
MO:LVillalba/MAverbuch  
Dep.Div.Dir:JHyde

APPEARS THIS WAY  
ON ORIGINAL



**Reviewer's Signature:**

/S/

Susan D. Wilson, DVM, PhD

5-24-99

Date

**Team Leader Concurrence:**

/S/

Andrea B. Weir, PhD, DABT

May 24, 1999

Date

APPEARS THIS WAY  
ON ORIGINAL

42  
52

**ADDENDUM TO PHARMACOLOGY/TOXICOLOGY REVIEW - NDA #21-047**  
**[MAY 7, 1999]: VIOXX; MERCK & CO., INC.**

**TO:** Sandy Cook  
**FROM:** Susan Wilson  
**THROUGH:** Andrea Weir  
**DATE:** May 24, 1999  
**RE:** Labeling Addendum

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**Reviewer's Signature:**

/S/

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Susan D. Wilson, DVM, PhD

5-24-99

Date

**Team Leader Concurrence:**

/S/

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Andrea B. Weir, PhD, DABT

24 May 99

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