CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

022519Orig1s000

PHARMACOLOGY REVIEW(S)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	22-519
Supporting document/s:	N/A
Applicant's letter date:	March 23, 2010
CDER stamp date:	March 23, 2010
Product:	DUEXIS (Ibuprofen/Famotidine)
Indication:	For the reduction of the risk of development of ibuprofen-associated, upper gastrointestinal ulcers.
Applicant:	Horizon Therapeutics , Inc.
Review Division:	Division of Gastroenterology product (HFD-180)
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Executive Summary

1.1 Recommendations

1.1.1 Approvability

From a nonclinical standpoint, approval of the NDA application is recommended.

1.1.2 Additional Non Clinical Recommendations

None

1.1.3 Labeling

The draft labeling of DUEXIS generally conforms to the format specified under 21CFR 201.57(c)(14) Requirements for PLR (Physician's Labeling Rule) Prescription Drug Labeling. However, the following changes should be incorporated.

8.1 Pregnancy

Sponsor's version:

(b) (4)

Evaluation: (b) (4)

Proposed version:

Pregnancy

Teratogenic Effects: Pregnancy Category C.
Animal reproduction studies have not been conducted with DUEXIS. Reproductive studies conducted with ibuprofen in rats and rabbits have not demonstrated evidence of developmental abnormalities. Reproductive studies with famotidine have been performed in rats and rabbits at oral doses of up to 2000 and 500 mg/kg/day, respectively (approximately 243 and 122 times the recommended human dose, respectively, based on body surface area), and have revealed no significant evidence of impaired fertility or harm to the fetus due to famotidine. While no direct fetotoxic effects have been observed, sporadic abortions occurring only in mothers displaying marked decreased food intake were seen in some rabbits at oral doses of 200 mg/kg/day (approximately 49 times the recommended human dose based on body surface area) or higher.

There are no adequate and well-controlled studies in pregnant women. Animal reproduction studies are not always predictive of human response. DUEXIS should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Nonteratogenic Effects

Sponsor's version:

Because of the known effects of NSAIDs on the fetal cardiovascular system (closure of ductus arteriosus), use during late pregnancy should be avoided.

Evaluation: No changes are recommended in this section.

8.2 Labor and Delivery

Sponsor's version:

In rat studies with NSAIDs, as with other drugs known to inhibit prostaglandin synthesis, an increased incidence of dystocia, delayed parturition, and decreased pup survival occurred. The effects of DUEXIS on labor and delivery in pregnant women are unknown.

Evaluation: No changes are recommended in this section.

8.3 Nursing Mothers

Sponsor's version:

(b) (4)

Evaluation: (b) (4)

Proposed version:

It is not known whether ibuprofen is excreted in human milk.

Famotidine is secreted into breast milk of lactating rats. Transient growth depression was observed in young rats suckling from mothers treated with maternotoxic doses of at least 300 times the usual human dose of famotidine. Famotidine is detectable in human milk. Because of the potential for serious adverse reactions in nursing infants from DUEXIS, a decision should be made whether to discontinue nursing or discontinue the drug, taking into account the importance of the drug to the mother.

13. NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Sponsor's version:

(b) (4)

(b) (4)

Evaluation: (b) (4)

Proposed version:
Studies to evaluate the potential effects of DUEXIS on carcinogenicity, mutagenicity, or impairment of fertility have not been conducted.

In a 106-week study in rats and a 92-week study in mice, famotidine was given at oral doses of up to 2000 mg/kg/day (approximately 122 and 243 times the recommended human dose, respectively, based on body surface area). There was no evidence of carcinogenic potential for famotidine.

Famotidine was negative in the microbial mutagenicity test (Ames test) using *Salmonella typhimurium* and *Escherichia coli* with or without rat liver enzyme activation at concentrations up to 10,000 µg/plate. In the *in vivo* mouse micronucleus test and a chromosomal aberration test with famotidine, no evidence of a mutagenic effect was observed.

In studies of famotidine in rats at oral doses of up to 2000 mg/kg/day (approximately 243 times the recommended human dose, based on body surface area) fertility and reproductive performance were not affected.

1.2 Brief Discussion of Nonclinical Findings

The sponsor did not provide any non-clinical study report under NDA 22-519. The following statement was made in the nonclinical section of the NDA. “HZT-501, a combination product of ibuprofen (a non-steroidal anti-inflammatory drug [NSAID]), and famotidine (a histamine type 2 [H_2] receptor antagonist), is being developed by Horizon

Therapeutics, Inc. for reduction of the risk of development of ibuprofen-associated, upper gastrointestinal (UGI) ulcers in patients who require use of ibuprofen. HZT-501 is a fixed-dose combination, immediate release, solid oral dosage form containing 800 mg ibuprofen and 26.6 mg famotidine. One tablet of HZT-501 is to be administered orally 3 times per day without regard to food. No new nonclinical pharmacology, pharmacokinetic, or toxicology studies have been performed by Horizon with ibuprofen or famotidine in support of their combined use in HZT-501 for reduction of the risk of development of ibuprofen-associated, UGI ulcers in patients who require use of ibuprofen."

This NDA is submitted under section 505(b) (2) of the Federal Food, Drug and Cosmetic Act and relies on studies that were not conducted by or for the applicant and for which this applicant does not have right of reference. Specifically, this NDA is supported by reference to the Agency's previous findings of safety and publically available information on the toxicology of ibuprofen and famotidine. In addition, the sponsor provided published literature to support the nonclinical safety of ibuprofen and famotidine. In addition, toxicology studies conducted by the innovators have established the safety of ibuprofen and famotidine.

Subchronic and chronic toxicity studies of ibuprofen were conducted in mice, rats, dogs, or monkeys. Overall, these studies indicated that the primary adverse effects of ibuprofen are gastrointestinal irritation and ulceration, and anemia.

Repeated dose toxicology studies of famotidine in rats and dogs showed that famotidine has minimal toxicological effects. The only noteworthy findings were slight weight loss in dogs and a reversible increase in incidence of eosinophilic cytoplasmic granularity of gastric chief cells in rats. Reproductive toxicity studies of famotidine were conducted in rats and rabbits at oral doses from 500 to 2000 mg/kg/day. These studies showed no significant evidence of impaired fertility or harm to the fetus. Changes in reproductive function occurred only at high doses and appeared related to the appetite suppressant properties.

Reproductive toxicity studies in rats have shown that ibuprofen has no teratogenic effects. However, ibuprofen inhibits prostaglandin biosynthesis. Prostaglandin plays an important role in ovulation and fetus implantation, and can cause direct effects on the fetus development in later stages of pregnancy. Studies in rats showed that NSAIDs and other drugs that inhibit prostaglandin synthesis can cause dystocia, delayed parturition, and decreased pup survival.

Famotidine was negative in the microbial mutagen test (Ames test). In the *in vivo* mouse micronucleus test and a chromosomal aberration test, no evidence of a mutagenic effect was observed. In a 106-week study in rats and a 92-week study in mice given oral doses of up to 2000 mg/kg/day, there was no evidence of carcinogenic potential for famotidine.

Carcinogenicity and mutagenicity information for Ibuprofen is not presented in the current labeling of ibuprofen.

Pharmacologic Activity:

Ibuprofen is a member of the propionic acid group of non-steroidal anti-inflammatory drugs (NSAIDs). The activity of ibuprofen results from its competitive, reversible, non-selective inhibition of primarily cyclooxygenase (COX)-1 and secondarily COX-2 enzymes, which leads to a reduction in the production of prostaglandins and other eicosanoids. Thus, the primary pharmacologic activity of ibuprofen is to act as an analgesic, anti-inflammatory, and antipyretic drug. With the same mechanism of action, the secondary pharmacodynamic effects of ibuprofen, includes the ability to produce upper gastrointestinal bleeding and ulceration.

Famotidine, a competitive inhibitor of histamine H_2-receptors, inhibits gastric acid secretion. Famotidine is thought to prevent non-steroidal anti-inflammatory drug (NSAID)-induced upper gastrointestinal ulceration due to reduction of gastric acid secretion. Inhibition of gastric acid secretion via concurrent administration of famotidine would reduce the incidence and severity of gastrointestinal ulceration caused by ibuprofen.

By combining the two products into a single oral dosage formulation, could reduce the incidence of ibuprofen-induced ulceration while maintaining ibuprofen's analgesic and anti-inflammatory efficacy.

2 Drug Information

2.1 **Drug:** DUEXIS (Ibuprofen and famotidine) Tablets

2.1.1 CAS Registry Number (Optional)

Ibuprofen: 15687-27-1
Famotidine: 76824-35-6

2.1.2 Generic Name

Ibuprofen
Famotidine

2.1.3 Code Name

HZT-501

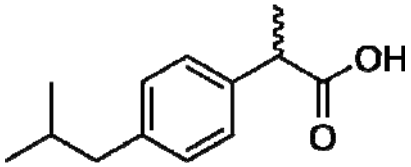
2.1.4 Chemical Name

Ibuprofen: (*RS*)-2-(4-(2-methylpropyl)phenyl) propanoic acid
Famotidine: 3-([2-(diaminomethyleneamino) thiazol- 4-yl] methylthio) - *N'*-sulfamoylpropanimidamide

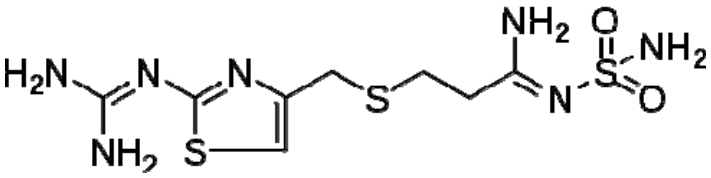
2.1.5 Molecular Formula/Molecular Weight

Ibuprofen: $C_{13}H_{18}O_2$/206.28
Famotidine: $C_8H_{15}N_7O_2S_3$/337.449

2.1.6 Structure



Ibuprofen



Famotidine

2.1.7 Pharmacologic class

Ibuprofen - Non-steroidal anti-inflammatory drug (NSAID)
Famotidine - Histamine type 2 (H_2) receptor antagonist.

2.2 Relevant IND/s, NDA/s, and DMF/s

NDA: 17-463: Motrin (Ibuprofen), McNeil Consumer Healthcare, PPC, Inc., PA.
NDA: 19-462: Pepcid (Famotidine), Merck research laboratories, PA.

2.3 Clinical Formulation

2.3.1 Drug Formulation

The HZT-501 (DUEXIS) drug product is an immediate release tablet containing 26.6 mg of famotidine (USP) and 800 mg of ibuprofen, USP. (b) (4)

(b) (4) The composition of drug product is shown in the sponsor's Table below.

Table 1: Components and Composition of the Drug Product

Component	Function	Amount per HZT-501 Tablet (mg)	% of HZT-501 Tablet (w/w)
(b) (4)			

NF = National Formulary; USP = United States Pharmacopoeia

(b) (4)

2.3.2 Comments on Novel Excipients

No novel excipients were used in the formulation of the drug product.

2.3.3 Comments on Impurities/Degradants of Concern

Famotidine and (b) (4) did generate about (b) (4) when compared to control samples after being stored at 40 C/75% RH after 1 month. However, this level was not observed in samples stored at 50^0C for 2 weeks (b) (4) Additional impurities were observed in the combination of the Ibuprofen (b) (4) and (b) (4). The impurity was (b) (4) versus the control when stored at 1 month at 40 C/75% RH. Two

unidentified impurities were observed (b) (4) No change in impurities was observed in ibuprofen (b) (4) samples stored with (b) (4) at 50^0C for 1 month. Purity of HZT-501 was also conducted at point-in-time (beginning, middle and end) from 4 commercial batches. The HZT-501 showed impurities (b) (4) Thus, based on ICH Q3B(R2) guidance these impurities are within the acceptable ranges.

2.4 Proposed Clinical Population and Dosing Regimen

The proposed indication of the drug is to reduce the risk of development of ibuprofen-associated upper gastrointestinal (UGI) ulcers in patients who require using ibuprofen. HZT-501, a combination product of ibuprofen and famotidine, developed as a fixed dose combination (FDC), immediate release tablet, containing 800 mg of ibuprofen and 26.6 mg of famotidine. The proposed dosing regimen is one tablet three times a day without regard to food.

2.5 Regulatory Background

A pre-IND meeting was held on June 13, 2005, and the Agency agreed with the sponsor that new nonclinical studies would not needed to support the NDA application.

3 Studies Submitted

No nonclinical studies were submitted in this 505(b)(2) NDA.

3.1 Studies Reviewed

No New nonclinical studies with the combination of ibuprofen and famotidine or individual drugs were submitted. The sponsor submitted available published pharmacology, pharmacokinetics, and toxicology studies with ibuprofen and famotidine. The relevant published studies were reviewed.

3.2 Studies Not Reviewed

None

3.3 Previous Reviews Referenced

None.

4 Pharmacology

The sponsor submitted available published pharmacology studies on ibuprofen and famotidine, and relevant studies are reviewed in the following sections:

4.1 Primary Pharmacology

Mechanism of action of anti-inflammatory drugs. J. R. Vane and R. M. Botting. *Recent Advances in Prostaglandin, Thrombaxane, and Leukotriene Research*. Plenum Press, New York. 1998; 131-138.

Pharmacodynamic effects of anti-inflammatory drugs are mediated through inhibition of cyclooxygenase (COX), a mechanism that is common to all NSAIDs. COX inhibits prostaglandin G_2 and prostaglandin H_2. The inhibition is due to the irreversible acetylation of the COX. Prostaglandin H_2 is further metabolized by other enzymes to various prostaglandins, prostacyclin, and thromboxanes, collectively referred to as eicosanoids. There are at least two isoenzymes of COX. COX-l and COX-2 have similar molecular weights of about 70 kilodaltons. A number of factors control the levels of COX-2. These include cytokines, intracellular messengers, and substrates, which are normally very low in cells. COX-l performs a "housekeeping" function to synthesize prostaglandins, which regulate normal cell activity. The concentration of the enzyme largely remains stable, but small (2-4 folds) increases in expression can occur in response to stimulation with hormones or growth factors. Normally, little or no COX-2 is found in resting cells. However, its expression can dramatically be increased when cells are exposed to pro-inflammatory agents, like bacterial lipopolysaccharide, phorbol esters, cytokines, or growth factors. In most species, including man, COX-1 synthesizes prostaglandins in the stomach. Small quantities of COX-2, can also be expressed in the stomach of the rat. COX-2 is highly expressed in human and animal colon cancer cells, as well as in human colorectal adenocarcinomas.

Histamine, histamine H_2-receptor antagonists, gastric acid secretion and ulcers: an overview. K. Pattichis and L. Louca. *Drug Metabol. Drug Interact.,* 1995; 12:1-36.

The primary pharmacodynamic effect of famotidine is to inhibit gastric secretion, which is mediated through competitive inhibition of histamine H_2-receptors. Histamine is widely expressed in mast cells, gastric mucosa (particularly in the acid-secreting parietal cells), basophilic leucocytes, central nervous system (CNS), and in the bone marrow. There are three types of histamine receptors, H_1-, H_2- and H_3-receptors. Famotidine is highly specific and most potent H_2 receptor antagonist. It is thought that famotidine forms some sort of reversible association complex with the H_2 receptors. The H_2 antagonist ring (e.g. imidazole, furan, etc.) engages the receptor at the site that would otherwise bind the imidazole ring of histamine and the rest of the H_2-antagonist molecule contributes additional binding by interacting with some accessory region. In general, when compared to cimetidine or ranitidine, famotidine is unique. This is because it is a slowly reversible and competitive H_2 receptor antagonistic.

Studies on MK-208 a new, slowly dissociable H_2-receptor antagonist. R. G. Pendleton, M. L. Torchina, C. Chung, P. Cook, S. Wiese and B. V. Clineschmidt. *Arch. Int. Pharmacodyn.*1983; 266, *4-16.*

MK-208 (famotidine) is a highly potent histamine H_2-receptor antagonist in guinea-pig atria, acting via a unique binding mechanism. Unlike ranitidine, the onset of action of this compound was slow and its inhibitory action was difficult to remove from the tissues even after repeated washing. Preincubation of atria with ranitidine, however, protected the H_2-receptor from these prolonged inhibitory effects of MK-208. The compound did not alter the response of this tissue to isoproterenol or affect basal atrial rate under conditions where maximal H_2-receptor blockade was achieved. Famotidine was approximately 7-times more potent than ranitidine and 100-times more potent than cimetidine, on a molar basis for inhibiting acid secretion in dogs with chronic gastric fistulae that were stimulated by histamine, pentagastrin, or 2-deoxy-D-glucose. When single equivalent anti-secretory oral doses of famotidine and ranitidine were given to histamine-stimulated dogs, famotidine produced a 67% inhibition of secretion compared to a 2% inhibition by ranitidine at 24 hours after administration. The compound was also highly effective in inhibiting basal acid secretion in chronic gastric fistula rats.

4.2 Secondary Pharmacology

Effects of nonsteroidal anti-inflammatory drugs on ulcerogenesis and gastric secretion in pylorus-ligated rat. K. A. Wagner, J. Nandi, R. L. King and R. A. Levine. *Digestive Diseases and Sciences*, 1995; 40: 134-140.

This same mechanism of action that underlies ibuprofen's primary pharmacodynamic effects also underlies its secondary pharmacodynamic effects. Ibuprofen's secondary pharmacodynamic effects include increasing the incidence and severity of upper gastrointestinal bleeding and ulceration that was evaluated in a pylorus-ligated rat model. Oral administration of salicylate, aspirin, and indomethacin significantly increased ulcerogenesis in rats by 6- to 7-fold. However, ibuprofen causes ulcerogenesis only up to 4-fold. Aspirin with histamine significantly increased ulcerogenesis by 2.7-fold compared to histamine alone. Basal acid secretion was increased significantly after indomethacin, but not by other NSAIDs. In contrast, all NSAIDs, except indomethacin, significantly decreased histamine-stimulated acid secretion. Ranitidine pretreatment significantly decreased basal acid and pepsinogen secretion in all treatment groups by >85% and >40%, respectively, and ulcerations induced by salicylate, aspirin, and indomethacin were also inhibited by 90%, 60%, and 60%, respectively. The observed inhibition of prostaglandin E_2 generation by NSAIDs under basal secretory conditions appeared to correlate with the extent of ulcerogenesis.

Effect of a new potent H2-blocker, 3-[[[2-[(Diaminomethylene)amino]-4-thiazolyl]methyl]thiol-N2-sulfamoyl propionamidine (YM-11170), on gastric secretion, ulcer formation and weight of male accessory sex organs in rats. M. Takeda, T. Takagi, Y. Yashima, and H. Maeno. *Arzneimittelforschung,* 1982; 32: 734-737.

Famotidine has been demonstrated to inhibit the basal gastric secretion of both acid and pepsin in the pylorus-ligated rats. Famotidine showed much higher antisecretory effects (50 times more) than cimetidine when given by oral or i.v. routes. It has been

also seen that the development of gastric ulcer induced by indomethacin or acetylsalicylic acid in rats was significantly reduced by famotidine. However, famotidine did not inhibit the binding of testosterone to the androgen receptor in rat prostate cytosol in an *in vitro* study. Rats treated with exogenous testosterone, prostate or seminal vesicle weight was not decreased with oral administration of 50 mg/kg/day famotidine for 7 days. Similarly, in another study in castrated rats androgenized with testosterone, prostate, seminal vesicle, and body weight were not decreased with oral administration of 100 mg/kg/day (i.e., 50 mg/kg twice daily) famotidine for 7 days.

4.3 Safety Pharmacology

Absorption, distribution and toxicity of ibuprofen. S. *S.* Adams, R. G. Bough, E. E. Cliffe, B. Lessel and R. F. N. Mills. *Toxicology and applied pharmacology*, 1969; 15:310-330.

Ibuprofen (8 to 64 mg/kg) was administered as a single i.v. dose to anesthetized cats (i.v. pentobarbital sodium) to determine arterial blood pressure and respiration rate. Nonanesthetized dogs, on the other hand, were given a single intravenous dose of 1, 2, 4, 8, or 16 mg/kg ibuprofen at intervals of 8 to 39 minutes. The following observations were conducted in these dogs: heart rate, electrocardiogram (ECG), respiration, rectal temperature, and vascularity of the gingiva and conjunctiva.

Intravenous administration of ibuprofen had a transient hypotensive effect, in case of anesthetized cats. It showed a maximal effect at 10-20 sec after injection. The vasodepressor action began at a dose of 8 mg/kg and became progressively greater with increasing dose. Recovery was usually complete within 15 minutes after 64 mg/kg. The heart rate did not change significantly. Transient stimulation of respiration occurred with small doses, but with doses of 8 mg/kg and above respiration was depressed. In nonanesthetized dogs, no significant alterations in heart rate, ECG, peripheral vascularity, respiration, or rectal temperature were recorded. Behavior and general condition of the dogs remained normal, and no signs of toxicity were evident. These results suggest that ibuprofen has minor, and probably nonspecific, effects on the cardiovascular system.

Famotidine does not induce long QT syndrome: experimental evidence from *in vitro* and *in vivo* test systems. A. Sugiyama, Y. Satoh, A. Takaharaa, Y. Nakamura, M. Shimizu-Sasamatac, S. Sato, K. Miyata and K. Hashimoto. *European Journal of Pharmacology*, 2003; 466: 137-146

Famotidine at a dose up to 10^{5} M did not inhibit HERG K^{+} current expressed in human embryonic kidney 293 (HEK293) cells or affect any of the action potential parameters of guinea pig papillary muscles. Intravenous doses of 0.3, 3, or 10 mg/kg famotidine did not affect the repolarization process in the halothane-anesthetized dogs. Doses of 3 or 10 mg/kg exerted positive chronotropic, inotropic and dromotropic effects without affecting the mean blood pressure in dogs. Intravenous doses of 1 or 10 mg/kg

famotidine did not induce torsades de pointes or prolonged QT interval in the canine chronic atrioventricular conduction block model. These results suggest that famotidine possesses no cardiovascular effects at 0.3 mg/kg, while it may exert cardiostimulatory actions by increasing the intracellular ca^+ concentration.

Cardiovascular and bronchial actions of famotidine in anesthetized dogs. K. Miyata, T. Kamato, A. Fujihara and M. Takeda,M. 1990. *Arzneimittelforschung.* 40: 1234-1238.

The effects of famotidine on cardiovascular and bronchial functions were investigated in anesthetized dogs. Famotidine did not affect heart rate, blood pressure, left ventricular pressure, maximum dLVP/dt, cardiac output, or coronary blood flow at intravenous doses of 1 to 30 mg/kg in anesthetized dogs. Famotidine did not produce any remarkable change in the ECG at doses up to 30 mg/kg in anesthetized dogs. The only exception was of a transient increase or decrease in the T-wave amplitude in the ECG at a dose of 30 mg/kg. No hemodynamic changes were observed after famotidine administration to anesthetized dogs whose cardiac function was depressed by propranolol (1 mg/kg I.V.).

5 Pharmacokinetics/ADME/Toxicokinetics

The sponsor did not conduct any pharmacokinetic studies with DUEXIS. However, the sponsor submitted published reports on pharmacokinetics of ibuprofen and famotidine.

5.1 PK/ADME

Absorption:

Absorption, distribution and toxicity of ibuprofen. S. S. Adams, R. G. Bough, E. E. Cliffe, B. Lessel and R. N. F. Mills. 1969; *Toxicol. Appl. Pharmacol.*, 15: 310-330.

In this study, absorption of ibuprofen was determined in rats, rabbits and dogs. Rats, rabbits and dogs were given a single dose of 20, 60 and 8 mg/kg ^{14}C-labeled ibuprofen, respectively by oral gavage. Blood samples were collected at 10, 20, 45, 90, 180 and 360 min post-dose, and plasma was analyzed for radioactivity. Absorption readily occurred in all three species. In rats, the maximal concentration was obtained at 20 min and was followed by rapid decline to a very low level by 6 hours after dosing. Absorption was slower in rabbits. Even with a dose three times greater than in rabbits, the maximal concentration of ^{14}C in plasma was lower in rats. The rate of disappearance of ^{14}C from plasma was also less rapid than in the rat. Analysis of blood from a single rabbit showed that most of the radioactivity at the peak time of 1.5 hours came from ibuprofen. In dogs, the rise and fall in the plasma concentration of radioactivity followed a pattern similar to that in rabbits, though the maximal concentration relative to dose was much higher. Mean ± SE C_{max} was 26 ± 5 µg/g wet weight (n=4 samples) and occurred at 90 minutes post-dose, followed by a decline in radioactivity by 6 hours post-dose. The

concentrations of radioactivity in plasma obtained from rats, rabbits, and dogs at various times after single oral doses are presented in the Table below.

In another study, the absorption of a single dose of ibuprofen in rats was evaluated. The pylorus or intestine was surgically ligated. One hour after ligation of the pylorus, 5 Wistar rats were given a single dose of 20 mg/kg ^{14}C-labelled ibuprofen in 0.5% methylcellulose by oral intubation. In another group of 5 rats, the small intestine was ligated at the pyloric and ileocecal junctions, followed by intraduodenal administration of 20 mg/kg ^{14}C-labelled ibuprofen. At 3, 22, 45, and 90 minutes after dosing, the stomach and its contents or the ligated intestine and its contents, plus blood samples, were analyzed for radioactivity. At 3 minutes after oral dosing, 73% of the radioactivity was recovered from the stomach and its contents, and no radioactivity was detected in plasma. Absorption in the intestine of intraduodenally administered ibuprofen was rapid; at 3 minutes post-dose, only 40% of the radioactivity remained at the administration site and the maximum concentration was achieved in plasma.

In summary, the above studies showed that absorption and distribution in plasma were rapid in the rat, rabbits and dogs, after single oral dose of ^{14}C-ibuprofen. Time to maximum concentration (T_{max}) occurred at 20 minutes in rats and at 90 min in rabbits and dogs after dosing. In rats, the site of absorption of ibuprofen was faster and more extensive from the intestine, than from the stomach.

Distribution:

Absorption, distribution and toxicity of ibuprofen. S. S. Adams, R. G. Bough, E. E. Cliffe, B. Lessel and R. N. F. Mills. 1969; *Toxicol. Appl. Pharmacol.* 15: 310-330.

The tissue distribution of ibuprofen was evaluated following single oral dose of ibuprofen in rats, and repeated doses of ibuprofen in rats and dogs. In this study, female Wistar rats were administered ^{14}C-labeled ibuprofen at a daily dose of 40 mg/kg/day by oral gavage (i.e., 20 mg/kg twice daily) for 1, 7, or 28 days. Blood and tissue samples were collected 17 hours after the first dose, 17 hours after the last dose on day 7 (i.e., after 13 doses), and 17, 42, 84, and 180 hours after the last dose on day 28 (i.e., after 55

doses). Plasma and tissue samples were analyzed for radioactivity. Similarly, male and female beagle dogs were given daily doses of 16 mg/kg/day ^{14}C-labeled ibuprofen in gelatin capsule (8 mg/kg twice daily) for 14 days. Blood and tissue samples were collected from 1 male dog 17 hours after the last dose on day 13, and from the remaining 3 dogs 17 hours after the last dose on day 14. Plasma and tissue samples were analyzed for radioactivity.

Radioactivity was detected in the thyroid (18.4 µg/g Wet Weight) of rats seventeen hours after the first dose. After repeated dosing, radioactivity accumulated in the fat, thyroid, ovaries, and adrenals, and to a lesser extent in the skin. Radioactivity was negligible in other tissues examined. The radioactivity in all tissues was greater after 28 days of dosing than after 7 days of dosing. Data are presented in the Table below.

The result in dogs showed that the concentrations of radioactivity in the tissues examined, were similar to, or did not exceed the concentration of radioactivity in the plasma. The exception was that, there was extremely high concentration in the bile. Minimal amounts of radioactivity were found in brain tissue as well. Data is presented in the Table below.

In summary, ibuprofen and its metabolites accumulated in the thyroid of rats, after single oral administration. In addition, the accumulation was also in the adrenals, ovaries, thyroid, fat, and skin of rats following repeated oral administration. In case of dogs, the presence of enterohepatic circulation was indicated by the very high ibuprofen concentration in the bile.

The metabolism of ibuprofen. R. F. Mills, S. S. Adams, E. E. Cliffe, W. Dickinson and J. S. Nicholson. 1973; *Xenobiotica*. 3: 589-598.

The objective of this study was to evaluate the protein binding of ibuprofen in rat, dog, baboon, and human plasma. Protein binding was measured by equilibrium dialysis at 37^0C.

The final concentration of ibuprofen in plasma was 20 µg/ml, the percentages bound in plasma were 96% in rats, 99% in dogs, 99% in human, and 95% in baboon. The data were consistent with binding of ibuprofen to a single binding site in the plasma albumin, the association constants being: rat, 4x 10^4 L/mole; dog, 2 x 10^5 L/mole; man, 1 x 10^6 L/mole; baboon, 2 x 10^4 L/mole. At very high plasma concentrations (> 140 pg/ml), evidence was obtained for a second weaker binding site in each species, but the significance of this was not determined.

In summary, ibuprofen was strongly bound to plasma proteins *in vitro* with percentages bound of 96% in rat plasma, 99% in dog plasma, 95% in baboon plasma, and 99% in human plasma at an ibuprofen concentration of 20 µg/mL.

Metabolism:

Absorption, distribution and toxicity of ibuprofen. S. S. Adams, R. G. Bough, E. E. Cliffe, B. Lessel and R. N. F. Mills. 1969; *Toxicol. Appl. Pharmacol.* 15: 310-330.

In this study, metabolism of ibuprofen was evaluated after a single oral administration of ibuprofen in rats, rabbits and dogs. Rats, rabbits and dogs were administered a single dose of 20, 60 and 8 mg/kg ^{14}C-labeled ibuprofen, respectively by oral gavage. The metabolites were determined in the plasma.

The result showed that in rats, most of the radioactivity in the plasma was in the form of unchanged ibuprofen, with the remainder of radioactivity present as OH-ibuprofen, and in smaller amounts, as COOH-ibuprofen and metabolite C (unidentified). In rabbits, the most prevalent metabolite was COOH-ibuprofen. Other metabolites included OH-ibuprofen, as well as 2 other unknown metabolites were also detected. However, no metabolites were detected in dogs after a single oral dose of ^{14}C-labelled ibuprofen.

The metabolism of ibuprofen. R. F. Mills, S. S. Adams, E. E. Cliffe, ,W. Dickinson and J. S. Nicholson. 1973; *Xenobiotica*. 3: 589-598.

The objective of this study was to evaluate the plasma concentrations and metabolism following repeated daily oral administration of ibuprofen to rats, dogs and baboons. Rats, dogs and baboons were dosed orally twice daily for 14 days at 0900 and1600 h. The doses in rats were of 20 mg/kg/day, and for dogs and baboons, was 16 mg/kg/day (8 mg/kg b.i.d.). Radioactive ibuprofen was used for rats and dogs and non-radioactive ibuprofen was used for baboons. The metabolites were determined in the plasma by HPLC and non-radioactive ibuprofen. Its metabolites were measured in plasma by gas-liquid chromatography.

The major metabolite in rat was OH-ibuprofen, following repeated oral administration of ^{14}C-ibuprofen for 14 days. COOH-ibuprofen, and an unknown metabolite was also present in the plasma. However, they were in lower concentrations. Similarly, in baboons, the most prevalent metabolites were OH-ibuprofen and COOH-ibuprofen, which were present in the plasma at lower concentrations, than the parent compound. Interestingly, in dogs, there were no metabolites detected, even after repeated oral administration of ^{14}C-ibuprofen for 14 days.

A major role for cytochrome P450TB (CYP2C Subfamily) in the actions of non-steroidal anti-inflammatory drugs. T. D. Leemann, C. Transon, P. Bonnabry and P. Dayer, 1993; *Drugs Exp Clin Res.* 19: 189-195.

The objective of this *in vitro* study was to evaluate the metabolism of ibuprofen and other non-steroidal anti-inflammatory drugs (NSAIDs) in human liver microsomes. Human liver microsomes were used to study the selective inhibition by known substrates or inhibitors of cytochrome P450 monooxygenases, specifically mephenytoin (substrate of P450MP, CYP2C subfamily), tolbutamide (substrate of P450TB, CYP2C subfamily), sulfaphenazole (a potent selective inhibitor of P450TB), dextromethorphan (substrate of P450DB1, CYP2D6, responsible for the debrisoquine type polymorphism), and midazolam (substrate of P450NF, CYP3A subfamily). Sulfaphenazole inhibited ibuprofen, diclofenac, and mefenamic acid clearance from the incubation medium at low concentrations compatible with its known K_i.

In summary, a single cytochrome P450 monooxygenase (CYP2C9) plays a key role in the oxidation by human liver of ibuprofen.

Famotidine: A notable lack of drug interactions. T.J. Humphries, 1987; *Scand. J Gastroenterol.* Suppl. 134:55-60.

The main aim of this study was to investigate the effects *in vitro* inhibition of famotidine on cytochrome P450. The effects of famotidine co-administration were evaluated in the *in vitro* studies of aminopyrine and diazepam demethylase activity, disturbances of P450 spectra, and metabolism of specific substrates such as deethylation of 7-ethoxycoumarin and demethylation of benzphetamine. Famotidine, added at a concentration of 0.5 mmol, caused minimal inhibition of both enzymes (aminopyrine N-demethylase and diazepam N-demethylase). A 10-mmol concentration of famotidine decreased aminopyrine N-demethylase activity by 40% and diazepam N-demethylase activity by 55%. Additional *in vitro* spectral and kinetic studies of the potential interaction of famotidine with the cytochrome P450 system, using cimetidine and ranitidine as active controls, were performed. In these studies, drug-induced disturbances of the P450 spectra and the inhibition of substrate metabolism were explored. Only cimetidine showed a pronounced different spectrum, indicative of a relatively strong ligand interaction with the ferriheme protein. No disturbances were noted with famotidine or ranitidine. Famotidine, cimetidine, and ranitidine were examined for their effect on the cytochrome P450-catalyzed *0*-deethylation of 7-ethoxycoumarin and demethylation of benzphetamine. Cimetidine caused a substantial concentration-dependent inhibition of both substrates, whereas little or no inhibition was shown for ranitidine and famotidine. Thus, in conclusion, above study showed that famotidine had negligible interaction with the cytochrome P450.

Inhibitory effects of H_2-receptor antagonists on cytochrome P450 in male ICR mice. D. H. Kim, E. J. Kim, S. S. Han, J. K. Roh, T. C. Jeong and J. H. Park. 1995; *Hum. Exp. Toxicol*. 14: 623-629.

The main objective of this study was to examine the effects of H2-receptor antagonists including famotidine on cytochrome P450 *in vitro*. To determine *in vitro* effects on selective P450 isozymes, selective P450 inducers were used to enrich specific types of isozymes in liver microsomes. They are 3-methylcholanthrene (MC) for P4501A1/2, Phenobarbital (PB) for P4502B1/2, ethanol (Et0H) for P4502E1 and dexamethasone

(DEX) for P4503A1. Liver microsomes were prepared from male ICR mice that were pre-treated with either one of MC (25 mg/kg, i.p. for 3 days) in olive oil, PB (80 mg/kg, i.p. for 3 days) in saline, EtOH (5 ml/kg , p.o., for 3 days) or DEX (50 mg/kg, i.p. for 3 days) in olive oil. Animals were starved for 16h prior to sacrifice. Ethoxyresorufin-*O*-deethylase, pentoxyresorufin-*O*-dealkylase, p-nitrophenol hydroxylase, and erythromycin *N*-demethylase activity were determined in the presence of 0.1 to 500 µM famotidine (approximately 0.03 to 169 µg/mL) in the respective liver microsome preparations. The result showed that famotidine did not inhibit P450A1/2, P450B1/2, P4502E1, and P4503A1 in an *in vitro* study.

Excretion:

The metabolism of ibuprofen. R. F. Mills, S. S. Adams, E. E. Cliffe, W. Dickinson and J. S. Nicholson. 1973; *Xenobiotica*. 3: 589-598.

The objective of this study was to evaluate the urinary excretion of ibuprofen after oral administration to rats, dogs and baboons; and biliary excretion by rats and dogs after single intravenous administration of ibuprofen. Two Wistar rats and two beagle dogs and two baboons were administered with ^{14}C-labelled ibuprofen at oral dose of 40, 16 and 8 mg/kg/day. Rats and dogs were dosed for 5 days whereas baboons were given single dose. Urine samples were collected for 24 hours. The collected samples were assayed for total radioactivity for ibuprofen and its metabolites by thin layer chromatography.

In order to study the biliary excretion of ibuprofen in rats and dogs, a single intravenous dose of ^{14}C-labelled ibuprofen was administered to Wistar rats (20 mg/kg) and Beagle dogs (8 mg/kg) in a 0.9% sodium chloride solution. A control group of rats and dogs underwent a sham-operation and were treated with ibuprofen. Blood was collected from rats and dogs at 0.08, 0.33, 0.75, 1.5, and 2.5 and 3 hours post-dose. Bile was collected for up to 3 hours post-dose. Blood and bile were assayed for total radioactivity (parent compound and metabolites) by liquid scintillation counting.

The above study showed that in rats, 42% and 18% of the dose was excreted in the urine as OH- and COOH-ibuprofen, respectively. Only 6% of the dose was excreted as conjugated ibuprofen. In dogs, more than 50% of the dose was excreted in the urine, primarily in conjugated forms. Seven percent (7%) of the dose was excreted in the urine as conjugated ibuprofen, and 23% and 13% of the dose was excreted as conjugated OH-and COOH-ibuprofen, respectively. In baboons, no ibuprofen was excreted in the urine. Only 3% and 5% of the dose was excreted as unconjugated and conjugated OH-ibuprofen, respectively, and 2% and 4% of the dose was excreted as unconjugated and conjugated COOH-ibuprofen, respectively.

The biliary excretion study showed that rats excreted about 28% and dogs excreted about 25% of an intravenous dose of radioactive ibuprofen in the bile in 3 h. In each species, one quarter to one-third of the biliary radioactivity was due to ibuprofen and its two metabolites. Small amounts of glucuronides of ibuprofen and its metabolites were found in dog bile, but not in rat bile. No sulphates were found in either rat or dog bile.

The remaining radioactivity was converted to ibuprofen and its metabolites by refluxing with 3 M HCl for 4 h indicating it was in conjugated form. Result is presented in the Table below.

In conclusion, a total of 60% and 36% ibuprofen was excreted in the urine in the form of OH- and COOH-ibuprofen by rats and dogs, respectively. Similarly, 6-7% ibuprofen was excreted in the urine as conjugated forms. In baboons, only a small percentage of the total ibuprofen dose was excreted in the urine as metabolites (OH- and COOH-ibuprofen). However, no parent drug was detected in the urine of the baboons. The biliary excretion of ibuprofen was 28% in rats and 25% in dogs after single i.v. administration.

Urinary excretion kinetics of famotidine in rats. J. H. Lin, L. E. Los, E. H. Ulm and D. E. Duggan. 1987; *Drug Metab Dispos*. 15: 212-216.

In order to evaluate the urinary excretion of famotidine in rats, male Sprague-Dawley rats were cannulated in the right jugular vein for blood sampling and left carotid artery for famotidine administration. Rats received a single intra-arterial administration of famotidine at a dose of 0.3, 3, 10 and 30 mg/kg. ^{14}C-Inulin was given by intra-arterial injection at a fixed dose level of 6 mg/kg. Blood samples were collected at appropriate time (exact time not indicated) and urine was collected for the duration of blood sampling and then at 24h. The concentration of famotidine in plasma and urine were determined by high performance liquid chromatography and radioactivity of inulin was determined by liquid scintillation method. The plasma protein binding of famotidine was determined by equilibrium dialysis.

The experiment showed that the consistency in glomerular filtration rate (GFR) over a wide range of famotidine concentration indicated that the drug had no significant effect on renal function. Renal clearance of famotidine remained reasonably constant in the concentration range of 0.2-4.6 µg/ml. Urinary recovery of unchanged drug decreased from approximately 70% to 30% of the dose as the concentration increased, indicating that renal excretion became quantitatively less important as the concentration of the drug in plasma increased.

Saturable urinary excretion kinetics of famotidine in the dog. S. P. Boom, S. Hoet and F. G. Russel. 1997; *J Pharm. Pharmacol.* 49: 288-292.

The objective of this study was to evaluate urinary excretion following intravenous administration of famotidine to dogs. Male beagle dogs were anaesthetized and both cephalic veins were cannulated for blood sampling and drug administration. A constant infusion of 5% mannitol and 0.5% inulin was administered to obtain a sufficiently high urine flow and for determination of the glomerular filtration rate (GFR). Famotidine was administered intravenously either for a short time or as a continuous infusion. Blood samples (7 mL) were taken at regular intervals. Urine was collected quantitatively by use of a double-walled urinary catheter by rinsing the bladder at the end of each collection interval with 10 mL 0.9% NaCl. Famotidine was analyzed by high-performance liquid chromatography (HPLC). The concentration of famotidine in plasma and urine was determined by comparing the peak area ratio of famotidine/internal standard (procaine) with a calibration curve.

This study suggests that recovery of famotidine in the urine ranged from 52% to 74% of the dose following intravenous administration of famotidine to male dogs. The renal clearance of famotidine in the dog appears to be saturated at relatively high plasma concentrations (i.e., above therapeutic levels of 0.1 µg/mL). Data is presented in the Table below.

5.2 Toxicokinetics

None

6 General Toxicology

No general (single- or repeated-dose) toxicology study reports were submitted. However, the sponsor has submitted a summary of general toxicology studies from selected published articles. Selected articles are reviewed and summarized below.

6.1 Single-Dose Toxicity

Absorption, distribution and toxicity of ibuprofen. S. S. Adams, R. G. Bough, E. E. Cliffe, B. Lessel and R. N. F. Mills. 1969; *Toxicol. Appl. Pharmacol.* 15: 310-330.

The main objective of this study was to investigate the single dose toxicity of ibuprofen in mice, rats and dogs. Ten male mice received single dose of ibuprofen orally (80 mg/kg) or by intraperitoneally (320 mg/kg). Similarly, 10 male rats were administered 1600 and 1300 mg/kg ibuprofen orally or subcutaneously. Gross reactions were observed and mortalities were recorded over 14 days. Male and female dogs were administered ibuprofen orally at a dose of 20, 50 and 125 mg/kg and were fed immediately afterwards. Hematologic examination and biochemical analyses of blood and urine were performed before, and at various times after dosing. Fecal samples were tested daily for occult blood. Some dogs were killed 24 hours after dosing and the remainder after 7 days. Major organs were examined grossly for any pathological changes.

The result showed that the LD_{50} values for ibuprofen were 800 mg/kg orally and 320 mg/kg intraperitoneally in the mouse and 1600 mg/kg orally and 1300 mg/kg subcutaneously in the rat. Clinical signs included prostration in both species. Death occurred within 3 days from perforated gastric ulcers in mice and intestinal ulceration in rats, irrespective of the route of administration. The clinical signs observed in dogs were emesis, scouring, albuminuria, fecal blood loss and erosions in the gastric antrum and pylorus at 125 mg/kg. However, dogs at 20 and 50 mg/kg dose group did not exhibit any clinical signs.

In conclusion, ibuprofen in lethal doses was ulcerogenic in both rodents and nonrodents.

6.2 Repeat-Dose Toxicity

Ibuprofen-induced gastrointestinal changes. J. Dudkiewicz 1981. *Acta Physiol Pol.* 32: 693-701.

30 Days rat study:
The main objective of this study was to determine the toxicity of ibuprofen in rats after 30 days of oral administration. Groups of seven male Wistar rats were administered ibuprofen orally (0, 15.7, 47, or 157 mg/kg/day) for 30 days. Food intake and body weight were measured daily. Biochemical determinations of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT), alkaline phosphatase,

and bilirubin were also measured. Rats were sacrificed after 30 days of dosing to measure organ weights and histopathological changes in the gastrointestinal tract.

Clinical signs included diarrhea and thirst at a dose of 157 mg/kg/day. Rats showed dose-dependent reduction in body weight. Significant decrease in weight was observed at 157 mg/kg/day during the first week of dosing. However, food consumption was unaffected.

On day 7, ALT and AST values at 157 mg/kg/day were twice as high as they were in the controls. On day 30, ALT and AST values were still elevated compared to control values, alkaline phosphatase values were increased compared to control values, and bilirubin values were similar to controls. There were no treatment-related changes in ALT, AST, alkaline phosphatase, or bilirubin at 15.7 and 47 mg/kg/day dose groups.

There were no treatment-related changes in organ weight. Histological findings included slight inflammatory infiltrations, and numerous spasmodic nodes in the muscle fibers of the stomach and intestine. The most pronounced of these effects were seen at the high dose of 157 mg/kg/day.

Absorption, distribution and toxicity of ibuprofen. S. S. Adams, R. G. Bough, E. E. Cliffe, B. Lessel and R. N. F. Mills. 1969; *Toxicol. Appl. Pharmacol.* 15: 310-330.

(a) 13-week oral toxicity study in rats:
Groups of 10 male and 10 female Wistar rats were administered a daily oral dose of 0, (0.4% cellosize), 20, 60, 180 and 540 mg/kg/day ibuprofen for 13 weeks. Hematological examinations were performed at 4, 8, and 12 weeks. Half of the rats in each group were sacrificed at 24 hours after the last dose, and the remaining animals were sacrificed after a 3-week recovery period.

Due to high mortality and moribund condition, all animals at 540 mg/kg/day were sacrificed by day 4. Necropsy findings included intestinal ulceration with peritonitis in all animals, and slight renal tubular dilatation in some animals.

Males at 180 mg/kg/day showed enlarged kidneys, spleen, and testes. Whereas, females in all three doses (20, 60 and 180 and 540 mg/kg/day) showed enlarged kidneys, the extent of enlargement was dose dependent. In addition, enlargement of the liver and ovaries occurred in females at 180 mg/kg/day, and of spleen and ovaries at 60 mg/kg/day. However, histopathological examination revealed no abnormalities of those organs. Three weeks after withdrawal of treatment, the organ to body weight ratios returned to normal.

Rats receiving 180 mg/kg/day ibuprofen showed anemia from week 4 until at the end of the study. A few rats in this dose group showed intestinal lesions. These effects were not seen at the lower doses.

In conclusion, up to 60 mg/kg/day ibuprofen was well tolerated in 13-week toxicology study in rats.

(b) 26-week oral toxicity study in rats:

Groups of 10 male and 10 female Wistar rats were administered a daily oral dose of 0, (0.4% cellosize), 7.5, 20, 60 and 180 mg/kg/day ibuprofen for 26 weeks. The following parameters were recorded; rats were weighed 3 times per week, hemoglobin concentration was determined colorimetrically and erythrocyte and leukocyte counts were made with an electronic recorder. Blood was collected by heart puncture for measuring plasma glutamic pyruvic transaminase (GPT) activity. The weights of the adrenals, brain, heart, kidneys, liver, lungs, ovaries or testes, spleen, thyroid, and uterus or seminal vesicles and prostate were recorded, and sections of these organs together with pancreas, stomach, small intestine, mesenteric lymph node, bladder, salivary gland, skeletal muscle, eyes, and femoral bone marrow, were stained with hematoxylin and eosin for histologic study.

There was no difference in body weight except for males on 180 mg/kg/day which had significantly lower body weight than the controls. One male on 180 mg/kg/day group died due to intestinal lesion. The hematological study showed that both males and females at 180 mg/kg/day were anemic with low erythrocyte counts, hemoglobin concentrations, and hematocrits. However, the leukocyte counts were not affected significantly. Result is presented in the Table below.

Organ to body weight ratios of male and females at 180 mg/kg/day showed enlarged liver, kidney, and spleen. The combined seminal vesicle and prostate weight reduced in males, and the uterine weight increased in females. Males exhibited a slight increase of the thyroid glands weight at all doses (20, 60 and 180 mg/kg/day). Result is presented in the Table below.

Histological examination showed no significant changes. However, intestinal ulcers were observed in 1 male and 3 females at 180 mg/kg/day.

In conclusion, an oral dose up to 60 mg/kg/day of ibuprofen was well tolerated in a 26-week oral toxicity study in rats.

(c) 30 days oral toxicity study in dogs:

The main objective of this study was to evaluate the toxicity of ibuprofen in dogs after 30 days of oral administration. In this study, 16 beagle dogs weighing 6-9 kg were randomly divided into 4 groups. Animals received 0, 4, 8 or 16 mg/kg/day of ibuprofen or placebo in divided doses immediately before food for 30 days.

The study showed that no gross or clinical signs of toxicity at all doses. However, postmortem examination revealed gastric ulcers or erosions, usually in the antrum or pylorus, less often in the fundic or cardiac regions, and intestinal inflammation at 8 and 16 mg/kg/day. Dogs at 4 mg/kg/day dose group did not show any pathological changes.

In conclusion, medium and high dose group (16 mg/kg/day) dogs showed gastric ulceration and intestinal inflammation.

(d) 26-week oral toxicity study in dogs:

The main objective of this study was to evaluate the toxicity of daily oral administration of ibuprofen for 26 weeks in dogs. Groups of 2 male and 2 female beagle dogs weighing 5-7 kg were dosed with 2, 4 and 16 mg/kg/day ibuprofen or placebo in divided doses for 26 weeks. The following parameters were conducted: dogs were weighed once weekly. Blood samples were analyzed before and 3, 6, 12, 18, and 25 weeks after the dosing. Biochemical determinations on blood or plasma included urea, sugar and bilirubin,

glutamic-pyruvic and glutamic-oxaloacetic transaminase, and sodium and potassium. Urine samples were collected to determine the following: color, pH, protein, bilirubin, and sugar and blood. Periodically feces were collected to test for occult blood. Liver function was assessed by measuring sulfobromophthalein retention in the weeks 13 and 26 post-dosing. At the end of the study, the dogs were euthanized with intravenous pentobarbital sodium, and certain organs (not listed) were weighed. Samples of these organs and of various other tissues were removed for histologic examination.

Female dogs at 16 mg/kg/day dose group showed gross signs of gastrointestinal disturbance in week 8 of dosing, characterized by frequent vomiting, diarrhea with occasional passage of fresh blood, and loss of weight. These dogs were anorexic for two weeks, and became drowsy and weak. The appetite improved later and was completely recovered. Male dogs, however, did not show any clinical signs and were normal throughout the dosing period. Occult blood was detected irregularly in fecal samples obtained from all male and female dogs from day 8 onward. The hematologic and blood biochemical values did not alter significantly, except that the females had a transient leukocytosis. No abnormal constituents were found in the urine. Liver function test was normal. At autopsy, organ weights were normal and, pathologic changes were confined to ulcerative lesions in the gastrointestinal tract. There were no adverse reactions or gastrointestinal damage at doses 2 and 4 mg/kg/day.

In summary, gastrointestinal toxicity was more prominent in female dogs at 16 mg/kg/day.

Famotidine: summary of preclinical safety assessment. J. D. Burek, J. A. Majka, and D. L. Bokelman. 1985; *Digestion.* 32 Suppl 1:7-14.

(a) 13-Week, 6-Month, and 1-Year Oral Toxicity Studies in Rats:

The main aim of this study was to evaluate the toxicity of famotidine in rats after 13 weeks, 6 months, and 1-year oral administration. The study showed that rats receiving an oral dose of famotidine 4000 mg/kg/day (2000 mg/kg/ b.i.d) for 13 weeks had no notable toxicological findings. Similarly, rats administered famotidine orally for 6 months at doses up to 1000 mg/kg/day had only minimal changes (increased urine osmolarity). Likewise, rats receiving an oral dose of famotidine up to 2000 mg/kg/day for 1 year had no changes of toxicological significance.

In all of these studies, a dose- and time-dependent cytoplasmic granularity was observed in gastric chief cells compared to controls, in rats. Minimal evidence of eosinophilic cytoplasmic granularity was seen after 3 months, when given orally at dose levels of 4000 mg/kg/day. In case of doses given orally for 6 months at levels of 50 to 1000 mg/kg/day, the incidence was not dose dependent. In 2 other studies, in which doses between 20 and 2000 mg/kg/day were administered for 1 year, there was a dose-related increase in the incidence and extent of eosinophilic granularity at doses of 200 mg/kg/day and higher. The appearance of eosinophilic cytoplasmic granularity in control rats was also time-dependent. There was little evidence of the change in animal studies

of 3 months, a minimal incidence (about 10%) after 6 months of treatment, and up to 50% incidence in control after 1 year of study.

In conclusion, oral administration of famotidine up to 2000 mg/kg/day in rats for a period of 1 year was well tolerated. Dose- and time-dependent eosinophilic cytoplasmic granularity in gastric chief cells was noticed in treatment and control groups.

(b) 30-day, 13-Week and 1-Year Oral Toxicity Studies in dogs:

The main aim of this study was to evaluate the toxicity of famotidine in dogs after 30 days, 13 weeks, and 1-year oral administration. Details regarding the methods were not reported in the article by the authors.

The study showed that high doses (dose not indicated) of famotidine administered orally were well tolerated in beagle dogs. However, minimal changes like slight weight loss, slight increase in serum albumin, and reduced serum globulin were noticed in dogs that received famotidine at a dose level of 1000 mg/kg/day for 13 weeks. Slight weight loss was also noted in dogs that received up to 4000 mg/kg/day of famotidine for 30 days, and for 1 year at a dose level of 500 mg/kg/day.

7 Genetic Toxicology

No genetic toxicology studies were submitted. However, the sponsor has summarized genotoxicity findings from the published literatures and publically available documents.

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Mutagenicity testing of selected analgesics in Ames *Salmonella* strains. J. W. Oldham, R. F. Preston, and J. D. Paulson. 1986; *J Appl. Toxicol*. 6: 237-243.

The main aim of this study was to evaluate the mutagenic potential of ibuprofen in the Ames *Salmonella* plate incorporation assay. The mutagenicity of ibuprofen was tested in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538, using the standard plate incorporation assay without metabolic activation and in the presence of metabolic activation of a rat, hamster or mouse liver post-mitochondrial supernatant (S-9, Aroclor 1254-induced). The plates were incubated with ibuprofen at concentration levels of 0, 1, 10, 100, 500, 750, or 1000 µg/plate. As a negative control, DMSO was used, whereas, as positive controls Dexon, 2-anthramine, 9-aminoacridine HCl, and sodium azide were used.

Ibuprofen was not mutagenic in *S. typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100 at concentrations up to 1000 µg/plate, with or without metabolic activation.

Comparative mutagenic and genotoxic effects of three propionic acid derivatives ibuprofen, ketoprofen and naproxen. B. Philipose, R. Singh, K. A. Khan and A. K. Giri. 1997; *Mutat. Res.* 393: 123-131.

The aim of this study was to evaluate the mutagenic potential of ibuprofen in a bacterial reverse mutation test. The mutagenicity of ibuprofen was tested in *Salmonella typhimuriurn* strains TA97a, TA100 and TA102 using the standard plate incorporation method, without metabolic activation, and with metabolic activation by rat S-9. Ibuprofen was dissolved in dimethyl sulphoxide (DMSO) and different concentrations (1, 10, 100, 1000, 5000 and 10000 µg/plate) and tested for mutagenicity. The plates were incubated at 37^0C for 48 h. Dimethyl sulfoxide was used as the solvent and the negative control. Positive controls were 4-nitro-*O*-phenylenediamine, 2-aminofluorene, methylmethane sulphonate, and sodium azide. After 48 h of incubation, the revertant colonies on all the plates were counted.

Ibuprofen was not mutagenic in *S. typhimurium* strains TA97a, TA100, and TA102 at concentrations up to 5000 µg/plate, with or without metabolic activation.

Famotidine: summary of preclinical safety assessment. J. D. Burek, J. A. Majka, and D. L. Bokelman. 1985; *Digestion.* 32 Suppl 1:7-14.

Famotidine was tested in a reverse-mutation test (Ames test) using *Salmonella typhimuriurn* and *Escherichia coli,* with and without metabolic activation. No mutagenic potential was seen. A similar study showed that famotidine/sodium nitrite reaction mixture and C-nitroso derivatives of famotidine are also negative to Ames test. Likewise, famotidine and its C-nitroso derivative showed negative for DNA-damaging in the Rec-Assay using *Bacillus subtilis* H17 and M45 strains.

7.2 *In Vitro* Chromosomal Aberration Assays in Mammalian Cells

Famotidine: summary of preclinical safety assessment. J. D. Burek, J. A. Majka, and D. L. Bokelman. 1985; *Digestion.* 32 Suppl 1:7-14.

Famotidine: an appraisal of its mode of action and safety. R. G. Berlin, B. V. Clineschmidt and J. A. Majka. 1986; *Am. J Med*. 81: 8-12.

Details regarding chromosomal aberration assays in mammalian cells were not reported in the article provided by the sponsor. However, the authors summarized that famotidine did not induce mutations in Chinese hamster lung fibroblasts, Chinese hamster ovary cells with or without metabolic activation. No mutagenic effect was seen in a chromosomal aberration test in mice.

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

None

7.4 Other Genetic Toxicity Studies

None

8 Carcinogenicity

Some aspects of the pharmacology, metabolism, and toxicology of ibuprofen. I. Pharmacology and metabolism. S. S. Adams, R. G. Bough, E. E. Cliffe, W. Dickinson, B. Lessel, K. F. McCullough, R.F. Mills, J. S. Nicholson, and G. A. Williams. 1970; *Rheumatol. Phys. Med. Suppl* 10: 9-26.

The aim of this study is to investigate the potential carcinogenic activity of ibuprofen in mice and rats after 80 weeks and 2 years of oral administration, respectively.

Groups of 50 male and 50 female mice were given daily doses of 0 or 300 mg/kg/day ibuprofen for 43 weeks (the route of administration was not provided). Because of high mortality from intestinal ulceration and perforation in males, the dose was decreased to 100 mg/kg/day for the remainder of the 80-week study. In rats, groups of 30 males and 30 females were given daily oral (diet) doses of 0 or 180 mg/kg/day ibuprofen for 56 weeks. Because of high mortality, the dose was decreased to 60 mg/kg/day for the remainder of the 2-year study.

The mice study showed that by 43 weeks, 18 males and 9 females had died at 300 mg/kg/day compared to 2 males and 8 females in the control group. After the ibuprofen dose was decreased to 100 mg/kg/day, similar numbers of deaths occurred in the control and treated groups; 33 males and 30 females died in the control group compared to 26 males and 17 females in the treated group during weeks 43 to 80. Six treated males, 15 control males, 24 treated females, and 12 control females survived until the scheduled sacrifice. The incidence of tumors among mice surviving for longer than 43 weeks did not differ between the dosed and control groups. The incidence of hepatomas and liver haemangiomas, and the latent period for the development of these tumors, were similar for both control and treated groups. Tumors in mice surviving for longer than 43 weeks are shown in the Table below.

Study in rats showed that by 56 weeks, 8 males and 9 females died at 180 mg/kg/day compared to no deaths in the control group. After the ibuprofen dose was decreased to 60 mg/kg/day, similar numbers of deaths occurred in the control and treated groups; 14 males and 7 females died in the control group compared to 12 males and 10 females in the treated group during weeks 56 to 104. Ten treated males, 16 control males, 11 treated females, and 23 control females survived until the scheduled sacrifice. Gastrointestinal ulceration was observed more frequently in the females treated with ibuprofen.

The incidence of tumors in rats surviving for at least 56 weeks is shown in the Table below. The proportion of rats with tumors was similar between the treated and control groups. The results were similar to those obtained in mice. There were few liver tumors or lymphomas. Majority of the benign tumors were in the uterus or were thyroid adenomas.

In conclusion, these two studies show that ibuprofen does not induce tumors of the liver or of any other organ. Furthermore, despite prolonged treatment, no drug-induced lesions were seen in the livers of either mice or rats. However, the mouse study was of only 80 weeks duration, and only one dosage level was used in this study. In the rat study, there was only one dosage level studied, and the number of animals (30/sex) were lower than that used in standard carcinogenicity studies. In addition, both studies are non-GLP studies.

Famotidine: summary of preclinical safety assessment. J. D. Burek, J. A. Majka, and D. L. Bokelman. 1985; *Digestion.* 32 Suppl 1:7-14.

CD-1 mice and CD rats were used in the carcinogenicity studies. Both mice and rats received famotidine orally at dose levels of 20, 200, or 2000 mg/kg/day for 92 and 105 weeks respectively. The female mice showed slightly increased incidence of distention of gastric glands at 2000 mg/kg/day. In case of rats, there was a slightly higher mortality in females at 2000 mg/kg/day dose group compared with controls. In addition, slight distention of gastric glands was present in females in the highest dosage group, and an increased incidence of eosinophilic cytoplasmic granularity was seen in rats in the 200 and 2000 mg/kg/day dose groups compared to controls. No evidence of a carcinogenic effect was seen in either species.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Fertility and general reproductive performance study of famotidine (YM-11170) in rats by oral administration. T. Uchida, M. Fujiwara, Y. Odani and Y. Shiobara. 1983; *Pharmacometrics.* 26: 551-564.

The main objective of this study was to investigate the effects of famotidine in fertility and general reproductive performance in rats.

Groups of 24 male and 48 female CD:CRJ(SD) rats were given a daily oral gavage dose of 0 (0.5% methylcellulose), 100, 500, or 2000 mg/kg/day famotidine at a dose volume of 5 mL/kg. Males were dosed for 12 weeks before mating, and during the mating period. Females were dosed from 14 days before mating to day 21 postpartum. The following evaluations were conducted: clinical signs and mortality, body weight and food consumption (weekly prior to and during mating; on gestation days 0, 3, 8, 13, 17, and 20; and on days 0, 4, 7, 10, 14, 17, and 22 during lactation). Males were sacrificed after mating. One-fourth of the females were sacrificed on gestation day 13, and one-half of the females were sacrificed on gestation day 20. Various reproductive parameters were evaluated in these animals (e.g., number of corpora lutea, implantation scars, number of live pups and dead embryos), and the offspring that were obtained on gestation day 20 were examined for gross, visceral, and skeletal defects.

The remaining one-fourth of the females was allowed to deliver naturally and was sacrificed on day 22 postpartum. The development and reproductive capacity of the F_1 generation was determined, and the early growth of the F_2 generation was observed. F_1 dams and F_2 pups were sacrificed on day 7 postpartum and examined macroscopically.

The study showed that no notable changes in behavior and clinical signs were found in parent animals (F_0). Body weights and food consumption of males (F_0) and body weights of females (F_0) showed no abnormality, but food consumption of females (F_0) showed a slight and transient reduction in the treating period before mating at the doses of 500 and 2000 mg/kg. There was no significant difference between the treated and control groups in mating performances and fertilities of both sexes. Litter size, fetal loss, and body weights of fetuses were not affected by famotidine. No external, visceral, or skeletal defects caused by treatment were observed in fetuses. No abnormalities were observed in delivery and rearing of pups. The body weights of newborns showed no difference at birth among the groups. However, during the period from day 10 to day 28 postpartum, there was a slight and transient depression of body weights at 2000 mg/kg dose group of male offspring and at 500 and 2000 mg/kg dose groups of female offspring. There were no effects of famotidine on the external development, behavior and reproductive performance of the offspring (F_1), and no abnormality in the pups (F_2) was observed.

In conclusion, oral administration of up to 2000 mg/kg/day famotidine in rats had no effect on fertility, general reproductive performance, or the rate of teratogenicity. There were no abnormalities in the F_1 generation (other than a slight body weight decrease) or in the F_2 generation.

9.2 Embryonic Fetal Development

Absorption, distribution and toxicity of ibuprofen. S. S. Adams, R. G. Bough, E. E. Cliffe, B. Lessel and R. N. F. Mills. 1969; *Toxicol. Appl. Pharmacol.* 15: 310-330.

Groups of pregnant female Wistar rats received oral ibuprofen at 0, 7.5, 20, 60 and 180 mg/kg/day doses from day 1 to day 20 of gestation. On day 21 of gestation, the pregnant rats were sacrificed and uterine contents were examined macroscopically. The fetuses were examined for external, visceral, and skeletal abnormalities. An additional group of pregnant rats received 7.5 and 20 mg/kg/day of ibuprofen throughout pregnancy until parturition, and the pups were examined 3 weeks after delivery.

In rabbits, groups of pregnant female New Zealand white rabbits were given a daily oral dose of 0, 7.5, 20 and 60 mg/kg/day of ibuprofen from day 1 to 29 of gestation. All females were killed on day 30 of pregnancy, and their uterine contents were examined. The number of live, dead, and resorbed fetuses, and the number of corpora lutea were recorded. All live fetuses were weighed and examined for external and visceral abnormalities. The brain, eyes, gonads, kidneys, liver, and lungs from some rabbits were examined histologically. A histologic study was conducted on adrenal, kidney, liver, lung, ovary, spleen, thyroid, and any grossly damaged organs of the mothers.

In rats, no GI lesions were observed in those on 7.5 mg/kg/day dose or in the controls. However, females at 20, 60 and 180 mg/kg/day dose groups showed GI lesions. The severity of the lesion was dose-dependent. Females on the high dose (120 mg/kg/day) had a diminished rate of growth. No treatment-related effects on number of live and dead fetuses, live litter size, fetal body weight, or implantation index were noted. For those animals allowed to deliver, there were no treatment-related effects on viability index or weaning weight. Result is presented in the Table below.

Various external and visceral malformations were observed with low incidence in both ibuprofen-treated and control groups. None of the fetuses from 180 mg/kg/day dose group was malformed, and microscopic examination of organs revealed no histologic abnormalities. The various skeletal abnormalities (shortened ribs, fused ribs, sternebrae underdevelopment etc.) that were noted were within the accepted range of normal variation for Wistar rats.

Embryonic fetal development studies in rabbits showed reduction in body weight and stomach ulcers in females at 60 mg/kg/day dose group. Some had pneumonia and a mild degree of focal hepatitis. Similar, but less profound, findings were noted at 20 mg/kg/day dose group. Growth was normal at 7.5 mg/kg/day, but some animals at this

dose had gastric ulcers or erosions. Minimal gastric damage was noted in 2 of 23 controls. Two females receiving 60 mg/kg/day gave birth prematurely to normal young on days 26 and 28 of pregnancy. There were no significant differences between treated animals and controls in the number of dead fetuses and resorption. At 60 mg/kg/day there were fewer live fetuses per litter, reduction in the ratio of implants to corpora lutea, which suggests that the decrease in live litter size was due to interruption of early pregnancy. Litter size was not affected at doses of 7.5 and 20 mg/kg/day. There were no treatment-related changes in fetal weight. Result is presented in the Table below.

External, visceral, and skeletal abnormalities occurred sporadically in all treated and control groups. There were no dose-dependent skeletal malformations. However, 4 fetuses in 1 litter from the 60 mg/kg/day group had multiple malformations characteristic of cyclopia.

In summary, both pregnant rats and rabbits studies showed that ibuprofen has no embryotoxic or teratogenic activity even when administered in ulcerogenic doses.

Developmental toxicity evaluation of ibuprofen and tolmetin administered in triple daily doses to Wistar CRL:(WI)WUBR rats. F. Burdan. 2004; *Birth Defects Res.* 71: 321-330.

The aim of this study was to investigate the effect of oral administration of ibuprofen on embryo fetal development in rats. Groups of 20 pregnant female Wistar rats were administered ibuprofen intragastrically (0, 8.5, 85, and 200 mg/kg/dose) three times daily with 8 h apart (total dose: 25.5, 255, or 600 mg/kg/day, respectively) in a volume of l0 mL/kg from day 8 through 21 of gestation. Clinical signs, mortality, and body weight were evaluated daily. On gestation Day 21, animals were sacrificed followed by necropsy and histopathological examination of the stomach, small and large intestine, and liver were conducted. Blood samples were collected for measurement of ALT, AST, total protein, and urea. Reproductive parameters were determined (e.g., dead and live fetuses, resorption, number of total implantations and corpora lutea, and fetal sex,

weight, and length), and fetuses were examined for external, visceral, and skeletal abnormalities. The proximal femoral epiphyses were separated, and the ultrastructure was evaluated.

High incidence of deaths and serious clinical signs were observed in the high dose (600 mg/kg/day) group compared to other groups. A single case of abortion was noted in the group exposed to ibuprofen in doses of 255 mg/kg/day. The dam died on the same day. The decrease in body weight gain was noted in groups at 255 and 600 mg/kg/day dose groups. Gastrointestinal toxicity was observed in all dose groups. Histopathological examination showed single, or frequently multiple, gastric and intestinal injuries. Lesions were noted in either the forestomach or the glandular stomach. Intestinal lesions were located mostly in the ileum and cecum. At 600 mg/kg/day, profound lesions were noted, and the perforation rate of intestine was high. The erosions in all examined organs were accompanied by sparse acute inflammatory infiltrate, with ulcerations, abundant inflammatory infiltration, and extensive granulation tissue formation. Hepatic lesions were usually seen together with severe gastrointestinal injury especially perforations. Blood chemistry showed significant decrease in urea and protein level, alanine and aspartate aminotransferase activity level in the high dose (600 mg/kg/day) group.

Maternal reproductive parameters were not statistically affected by ibuprofen. However, postimplantation loss was over 4- and 8-times higher in groups exposed to the highest doses of ibuprofen. In these groups, the fetal weight and length were significantly decreased. There was a statistically significant increase in the number of fetuses with external variations in the groups exposed to the highest dose of ibuprofen. However, the percentage of fetuses with external variation per litter was increased only in the middle dose group of ibuprofen. Result is presented in the Table below.

A significant increase in skeletal variations was noticed at the high dose group (600 mg/kg/day). They are reduction of cranial, sternebrae, metacarpal, and metatarsal bone ossification and asymmetry of vertebral bodies and other sternebral variations were the most frequently observed. However, no gross statistically significant anomalies were observed in soft tissue examination.

In conclusion, dose-dependent mortality, gastrointestinal toxicity, and significant changes in serum chemistry were observed in rats given daily oral ibuprofen on gestation days 8 to 21 or for 14 days, respectively. There was a significantly greater incidence of intrauterine growth retardation and developmental variations at the high dose of 600 mg/kg/day.

Teratological study of famotidine (YM-11170) administered orally to rats. M. Shibata, K. Kawano and Y. Shiobara. 1983; *Pharmacometrics.* 26: 489-497.

The objective of this study was to assess the effects of oral administration of famotidine on embryo fetal development in rats. Groups of 30 to 34 gravid female Sprague-Dawley rats were administered a daily oral dose of 0, 100, 500, or 2000 mg/kg/day famotidine at a dose volume of 5 mL/kg from days 7 to 17 of gestation. Animals were evaluated for clinical signs and mortality, body weight and food consumption. Approximately two-thirds of the dams were sacrificed on gestation day 20 and various reproductive parameters were evaluated (e.g., number of corpora lutea, implantations, number of live and dead fetuses), and live fetuses were weighed (body weight and placenta weight), sexed, and examined for external, visceral, and skeletal abnormalities. The remaining dams were allowed to deliver naturally and were observed through day 21 after parturition, at which time they were sacrificed and necropsied. The naturally born pups (F_1 generation) were observed for the number of live and dead pups, sex, weight, and external abnormalities at birth and during development. At weaning (21 days), one male and one female pup from each dam were evaluated further for reproductive function,

and behavioral function, and the remaining pups were sacrificed, necropsied, and examined for skeletal abnormalities.

The result showed that the body weight and food intake of dams in 2000 mg/kg/day showed transient depressions in the early period of treatment. These dams showed lower body weight during lactating period, but food intake was the same as the control group after parturition. In the examination of fetuses, famotidine did not show teratogenicity, mortality or other embryo/fetal toxicities. In addition, famotidine had no influence on growth and physical, behavioral, or functional development as well as reproductive capacities in newborns (F_1).

In conclusion, there was no teratogenicity, mortality, or embryo fetal toxicity with oral doses up to 2000 mg/kg/day famotidine in rats.

Teratology study of famotidine (YM-11170) in rabbits by oral administration. T. Uchida, T. Katayama, Y. Odani and Y. Shiobara. 1983; *Pharmacometrics.* 26: 565-571.

The main aim of this study was to assess the effects of oral administration of famotidine on embryo fetal development in rabbits. Famotidine was administered orally to groups of 15 pregnant female New Zealand white rabbits at doses of 0, 30, 200 and 500 mg/kg/day, during the period of organogenesis from gestation day 6 to 18. The following evaluations were conducted: clinical signs and mortality (daily), and body weight and food consumption (gestation days 0, 6, 7, 8, 10, 12, 15, 18, 19, 20, 23, 26, and 29). All dams were sacrificed on gestation day 29, and thoracoabdominal organs were examined macroscopically. Reproductive parameters were evaluated (e.g., number of corpora lutea, implantation scars, number of live and dead fetuses). Live fetuses were weighed (body weight and placenta weight), sexed, and examined for external, visceral, and skeletal abnormalities.

In the 30 and 200 mg/kg/day dose groups, there was no effect of the drug on the appearance or behavior of the dams. In the 500 mg/kg/day dose group, food intake and body weight gain were suppressed and abortion was observed in 3 out of 13 rabbits. Abortion was considered to be caused by the decreased food consumption. Five animals in this group did not take any food from the middle or later stage of administration to the end of gestation. There were no deaths in each dose group. Macroscopic examination revealed fatty metamorphosis of liver caused by starvation in several animals of the 500 mg/kg/day dose group. In the examination at caesarean section, no difference was observed between the control and treated groups in the number of implantation, dead fetuses or live fetuses, sex ratio, and placental weight. Although there was no significant difference in the group mean values of the weight of live fetuses, the body weight of fetuses from dams showing a very marked decrease in food intake. Fetuses with external, visceral and skeletal abnormalities caused by the drug administration were not observed in any group. The number of sacrocaudal vertebrae decreased slightly, and a delay in ossification was seen in the 500 mg/kg/day dose group.

In conclusion, in an embryo fetal development study, with oral administration of famotidine to pregnant rabbits during organogenesis, the maximum non-toxic dose was 200 mg/kg/day. There was no teratogenicity.

9.3 Prenatal and Postnatal Development

Peri- and postnatal study of famotidine (YM-11170) administered orally to rats. M. Shibata, T. Yoshinaga and Y. Shiobara. 1983; *Pharmacometrics,* 26: 543-549.

The aim of this study was to assess the effects of oral administration of famotidine on peri- and post-natal development in rats. Famotidine was administered orally to groups of 20-25 pregnant female Sprague-Dawley rats at doses of 0 (0.5% methylcellulose), 100, 500, and 2000 mg/kg/day during the period of organogenesis from gestation day 15 to postnatal day 21. Following parameters were observed in all groups like general, delivery, and nursing conditions. Body weight and food consumption were measured on gestation days 0, 7, 10, 14, 15, 16, 17, 18, and 20, and on days 0, 4, 7, 10, 14, 17, and 22 after parturition. All dams were allowed to deliver naturally and wean their young. Dams were sacrificed and necropsied after weaning. The newborns (F_1 generation) were observed for the number of live and dead pups, sex, weight at parturition and on days 4, 7, 10, 14, 17, and 22, external abnormalities at birth and during development, growth differentiation, and reflex function. At 22 days after parturition, 1 male and 1 female pup from each dam were evaluated further for reproductive function, and 1 male and 1 female pup from each of 10 dams were evaluated for behavioral function (e.g., open field, revolving wheel, rotarod test, T-maze test). The remaining pups were sacrificed, necropsied, and examined for skeletal abnormalities. The pups that have been weaned for the purpose of reproductive performance testing were weighed on a weekly basis, and mating between non-siblings took place 11 weeks after birth, reproductive functions were evaluated, and the pups were allowed to deliver naturally. Observations continued until 7 days after birth of the F_2 generation, at which time all F_1 dams and males used for mating and all F_2 nurslings were sacrificed and necropsied.

There were no abnormalities in general conditions, delivery, or nursing conditions in the dams in any dose groups. Dams on high dose group (2000mg/kg) had lower body weight once they entered the administration period; there was no difference in body weight during postnatal period. Food consumption was reduced in both the 500 and 2000 mg/kg/day dose groups, during the prenatal administration period. There was improvement after parturition. The autopsy examination at the time of weaning showed no abnormalities macroscopically. However, lower heart weight was observed in the 500 and 2000 mg/kg/day dose groups.

All pregnant dams gave birth to live offspring, and there were no abnormal values in the numbers of pups, stillborns, and weights of the live pups. In addition, no external abnormalities were found among the live pups. There were no treatment-related changes in survival rate during lactation period, number of fatalities during the peri-natal period, and the weaning rate. Pups at 500 and 2000 mg/kg/day dose groups showed reduced body weight following 10 days after birth, and a significant difference from the

control group was noted between the age of 5 and 9 weeks. Autopsies of F_1 pups at the time of weaning showed 4, 1, 2 and 1 cases of enlarged pylem in controls, 100, 500, and 2000 mg/kg/day dose groups, respectively. In addition, one pup showed unilateral poor development of the orchis in 500 mg/kg/day dose group and one mild case of diaphragmatic hernia was observed in 2000 mg/kg/day dose group. No treatment related changes in organ weight and skeletal abnormalities were observed. No abnormalities were found in growth differentiation, pupillary reflex, or behavioral function tests.

Reproductive performance test of F_1 generation (at the age of 11 weeks) showed one case of unsuccessful mating out of 25 cases in the 500 mg/kg group and 2 cases out 20 cases in the 2000 mg/kg dose group. In addition, there were 2 cases of infertility in each group, including the control group. There were no variations in terms of delivery rate, number of pups, and number of stillborns. None of the pups (F_2) showed signs of external abnormalities. The weights of the newborns were decreased in the 2000 mg/kg/day group, and the pups in the 500 and 2000 mg/kg/day groups had decreased body weight at 7 days of age. Although the 2000 mg/kg/day group had a slightly higher number of mortalities during the peri-natal period, there were no group differences in survival rate in the first 7 days after parturition. Necropsy did not reveal any abnormalities.

In conclusion, a transient depression of body weight gain and food intake was observed in dams after initiation of the treatment in case of higher dosages of famotidine. No abnormalities were observed in delivery and nursing of pups. Newborns showed a slight depression of body weight gains after birth, but there were no abnormalities in their physical and functional development and reproductive capacities.

10 Special Toxicology Studies

No special toxicology studies were submitted.

11 Integrated Summary and Safety Evaluation

In the current submission, the sponsor is seeking approval of a fixed-dose combination (FDC) product of ibuprofen and famotidine (HZT-501) for the reduction of development of ibuprofen-associated upper gastrointestinal ulcers in patients, who require the use of ibuprofen. HZT-501 is an immediate release tablet formulation containing 800 mg of ibuprofen and 26.6 mg of famotidine. The recommended oral dose is one tablet, three times a day.

Ibuprofen is an NSAID, which acts as an anti-inflammatory, analgesic, and antipyretic agent. These pharmacodynamic effects are mediated through inhibition of COX pathway. There are two isoforms of COX (COX-1 and COX-2) with similar molecular weights of about 70 kilodaltons. COX-1 is constitutively expressed, i.e., it is present in most normal cells and tissues under physiological conditions. COX-2 is not normally

found in resting cells. COX-2 is expressed in the stomach, and is activated by inflammatory stimuli, such as the release of the cytokine interleukin-1 that induces the synthesis of COX-2 in cells such as macrophages, resulting in the reduction of prostaglandins secretion. The prostaglandins together with proteases and other inflammatory mediators such as reactive oxygen radicals results in inflammation, which ultimately can cause gastric toxicity (i.e., ulceration) and nephrotoxicity (i.e., salt and water retention).

Famotidine, a competitive inhibitor of histamine H_2-receptors, inhibits gastric acid secretion. The pharmacologic rationale for the ibuprofen-famotidine combination drug product, is that famotidine is thought to prevent NSAID-induced upper gastrointestinal ulceration due to reduction of gastric acid secretion. Ibuprofen can cause gastrointestinal bleeding and the development of gastric and duodenal ulcers due to reduced secretion of gastric mucous secondary to inhibition of prostaglandin synthesis. By combining the two products into a single oral dosage formulation, co-administration of famotidine with ibuprofen could reduce the incidence of ibuprofen-induced ulceration while maintaining ibuprofen's analgesic and anti-inflammatory efficacy.

The pharmacological and toxicological properties of ibuprofen and famotidine have been studied extensively by the respective innovators. Absorption and distribution of ibuprofen from plasma were rapid in the rat, rabbits and dogs after single oral dose. In rats, the distribution of ibuprofen was found in adrenals, ovaries, thyroid, fat and skin; whereas in dogs high concentration of ibuprofen was found in the bile. Ibuprofen was strongly bound to plasma proteins. Cytochrome P450 (CYP2C9) plays a key role in the oxidation of ibuprofen by human liver. Famotidine, on the other hand, does not interact with cytochrome P450. The major route of excretion of ibuprofen and famotidine is urine.

Subchronic and chronic toxicity studies of ibuprofen were conducted in mice, rats, dogs, or monkeys. Overall, these studies indicated that the primary adverse effects of ibuprofen are gastrointestinal irritation and ulceration, and anemia. In 1, 4, 13 and 26 weeks oral toxicity studies in dogs, and 13, 26 and 52 weeks toxicity studies in rats, emesis, scouring, albuminuria, fecal blood loss, and erosions in the gastric antrum and pylorus were noted. These studies indicated that the gastrointestinal region was the common target organ of toxicity for the ibuprofen.

Repeated dose toxicity studies of famotidine were conducted up to 1 year in rats and dogs. Overall, these studies showed that famotidine has minimal toxicological effects. The only noteworthy findings were slight weight loss in dogs and a reversible increase in incidence of eosinophilic cytoplasmic granularity of gastric chief cells in rats after oral administration. There was no overt clinical reaction and serious toxicity in rats and dogs treated orally with famotidine up to 2000 mg/kg/day twice daily for 4 to 13 weeks.

Ibuprofen was not mutagenic in the bacterial reverse mutation test and human lymphocytes assay. Similarly, famotidine was negative in the microbial mutagenicity test (Ames test). However, ibuprofen was positive in mice chromosomal aberration assay.

No evidence of mutagenic effect of famotidine was observed in *in vivo* studies in mice, with a micronucleus test and a chromosomal aberration test.

In published non-GLP studies, the carcinogenic potential of ibuprofen was evaluated in mice (80-week study) and rats (104-week study). In the mouse study, single dose of ibuprofen (300 mg/kg/day) was given for up to 43 weeks. Due to mortality, the dose was reduced to 100 mg/kg/day for the remainder of the 80-week study. Similarly, in rats the initial dose of ibuprofen (180 mg/kg/day for 56 weeks) was reduced to 60 mg/kg/day for the remainder of the 104-week study. These two studies showed that ibuprofen does not have carcinogenic potential. However, these are not valid studies because the duration was not long enough, a single dose level was used or inadequate number of animals were used.

The carcinogenic potential of famotidine was evaluated in a 105-week study in rats and a 92-week study in mice given oral doses of 0, 20, 200 and 2000 mg/kg/day. No evidence of a carcinogenic effect of famotidine was seen in either species.

Reproductive toxicity studies in rats and rabbits have shown that ibuprofen has no teratogenic effects. However, ibuprofen inhibits prostaglandin biosynthesis. Prostaglandins play an important role in ovulation, implantation, and has direct effects on the fetus in later stage of pregnancy. Reproductive toxicity of NSAIDs in rats showed dystocia, delayed parturition, and decreased pup survival. Reproductive studies of famotidine were conducted in rats and rabbits showed no impaired fertility or harm to the fetus. Famotidine does not cause any teratogenic affects in rats.

Each tablet of DUEXIS contains 800 mg of ibuprofen and 26.6 mg of famotidine. The dose of ibuprofen and famotidine as FDC are within the recommended dose.

In conclusion, both ibuprofen and famotidine have a long history of use in humans. Ibuprofen was approved for marketing in 1974. This is one of the most widely used drugs in humans for its analgesic, anti-inflammatory and anti-pyretic activities. Famotidine was approved in 1986 for the treatment of duodenal ulcer and GERD by inhibiting gastric secretion. The safety of the individual components in the FDC product has been well documented in humans. There is no potential for drug interaction between ibuprofen and famotidine based on available information. Based on the existing safety data (in humans and animals) of the individual components of DUEXIS, there are no safety concerns for the use of the combination for the proposed indication at the proposed doses. The available nonclinical safety data appear to support the approvability of the proposed FDC of ibuprofen and famotidine for the proposed indication.

12 Appendix/Attachments: None

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/s/

DINESH C GAUTAM
12/06/2010

SUSHANTA K CHAKDER
12/07/2010

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 22-519 **Applicant: Horizon Pharma Inc.** **Stamp Date: 4/29/2010**

Drug Name: HZT-501 **NDA Type: 505(b)(2)**

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	Yes		NDA is 505(b)(2) application for HZT-501 (Famotidine and Ibuprofen). No new Pharmacology/Toxicology studies were submitted. Summaries of available data on famotidine and ibuprofen, and published literatures were submitted.
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	Yes		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	Yes		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	Yes		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	Yes		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	Yes		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	Yes		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?			N/A

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	Yes		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	Yes		
11	Has the applicant addressed any abuse potential issues in the submission?		No	N/A
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?		No	

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? Yes

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

None

Dinesh Gautam, Ph.D

Reviewing Pharmacologist Date

Sushanta Chakder, Ph.D

Team Leader/Supervisor Date

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22519	ORIG-1	HORIZON PHARMA INC	HZT-501

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04/29/2010

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