

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

22-301

PHARMACOLOGY REVIEW(S)



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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER: 22-301
SERIAL NUMBER: 000
DATE RECEIVED BY CENTER: 01/03/08
PRODUCT: Encapsulated Mesalamine Granules
INTENDED CLINICAL POPULATION: Adult patients (18 years and older) with ulcerative colitis
SPONSOR: Salix Pharmaceuticals, Inc.
DOCUMENTS REVIEWED: Original NDA submission
REVIEW DIVISION: Division of Gastroenterology Products (HFD-180)
PHARM/TOX REVIEWER: Sushanta Chakder, Ph.D.
ACTING PHARM/TOX SUPERVISOR: Sushanta Chakder, Ph.D.
DIVISION DIRECTOR: Donna Griebel, M.D.
PROJECT MANAGER: Cristi Stark, M.S.

Date of review submission to Division File System (DFS): September 25, 2008

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability: From a nonclinical standpoint, the NDA application is approvable.

B. Recommendation for nonclinical studies: None.

C. Recommendations on labeling:

The sponsors' proposed labeling conforms to 21 CFR; 201.57. However, the following changes are recommended in the nonclinical sections of the proposed labeling.

8.1 Pregnancy

Proposed version:

F

b(4)

Recommended version:

8.1 Pregnancy

Pregnancy Category B. Reproduction studies with mesalamine have been performed in rats at oral doses up to 320 mg/kg/day (about 1.7 times the recommended human dose based on a body surface area comparison) and rabbits at doses up to 495 mg/kg/day (about 5.4 times the recommended human dose based on a body surface area comparison) and have revealed no evidence of impaired fertility or harm to the fetus due to mesalamine. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

1 Page(s) Withheld

 Trade Secret / Confidential (b4)

 ✓ Draft Labeling (b4)

 Draft Labeling (b5)

 Deliberative Process (b5)

Withheld Track Number: Pharm/Tox- 1

b(4)

Recommended version:

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

b(4)

Animal Toxicology and/or Pharmacology

Renal Toxicity

b(4)

Overdosage

Single oral doses of 800 mg/kg (about 2.2 times the recommended human dose, on the basis of body surface area) and 1800 mg/kg (about 9.7 times the recommended human dose, on the basis of body surface area) of mesalamine were lethal to mice and rats, respectively, and resulted in Gastrointestinal and renal toxicity.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings:

Oral mesalamine (5-aminosalicylic acid; 5-ASA) formulations have been used as the treatment for the induction and maintenance of remission of ulcerative colitis for many years. The sponsor submitted NDA 22-301 for encapsulated mesalamine granules pursuant to Section 505(b)(2) of the Federal Food, Drug and Cosmetics Act. A reference

was made to the toxicology data for mesalamine submitted under NDA 19-651 (Procter and Gamble Pharmaceuticals, Asacol) and NDA 21-252 (Axcen Scandipharm Inc., Canasa). Original study reports of the toxicology studies were also submitted.

Acute oral and intravenous toxicity studies with mesalamine were conducted in rats and mice. The clinical signs following oral and intravenous treatment in both rats and mice were similar, and included sedation, dyspnea, weight loss, coma (mice only) and ventral/curved body position. The kidney and the GI tract were the target organs of toxicity. Repeat dose toxicity studies were conducted in rats (including a 13-week and 26-week oral gavage and a 52-week dietary admixture) and dogs (including a 26-week and a 52-week oral toxicity study). The kidney was the target organ of toxicity in all studies in both rats and dogs. In the rat, a dose of 640 mg/kg (13 weeks) resulted in a number of deaths attributed to renal failure, urinary changes (polyurea, proteinurea and hematuria) and marked increases in serum LDH levels. Kidney weights were significantly increased and the histopathological changes comprised of renal lesions involving the papillary and cortical regions and characterized by areas of slight to extremely severe necrosis. A moderate papillary necrosis was also observed in male rats from the 13-week study at a lower dose of 160 mg/kg. In the 26-week rat study, papillary necrosis was observed at a dose of 320 mg/kg. Other renal changes observed in the 26-week rat study at a dose of 360 mg/kg included tubular degeneration of the kidney, tubular mineralization and urothelial hyperplasia. Increases in blood urea nitrogen, creatinine and globulin levels were observed at 360 mg/kg females in the same study. Papillary edema was observed in female rats at 170 mg/kg in the 26-week study, and minimal to slight tubular injury was observed in male rats receiving the drug for 13 weeks. In the 52-week oral toxicity study in rats, hyalinization of the tubular basement membrane and Bowman's capsule was observed at 100 and 320 mg/kg doses. Other treatment-related findings observed in rats included hemorrhagic changes in the gastric mucosa at 640 mg/kg (13-week study), mucosal/submucosal fibrosis of the stomach and inflammation of the urinary bladder at 360 mg/kg (26-week study), and ulceration of the stomach at 320 mg/kg (52-week study). In dogs, polyurea and an increased urinary excretion of gamma-glutamyltransferase and lactate dehydrogenase was observed at 120 mg/kg in the 26-week toxicity study. Papillary necrosis was observed at 60 mg/kg and higher doses in all three chronic toxicity studies in dogs. Chronic nephritis was observed in the 52-week oral toxicity study in dogs.

Mesalamine was negative in the following genotoxicity assays: the Ames test, the *in vivo* mouse micronucleus assay, and the *in vivo* sister chromatid exchange assay in Chinese hamster bone marrow cells. Mesalamine was not genotoxic in any of.

Carcinogenicity studies with mesalamine were conducted in rats and mice following dietary administration of the drug. Mesalamine was not carcinogenic in rats at doses up to 480 mg/kg/day, or in mice at doses up to 2000 mg/kg/day.

Mesalamine had no effect on fertility or reproductive performance of male and female rats at oral doses up to 480 mg/kg/day. Mesalamine was not teratogenic in rats and rabbits at oral doses up to 480 mg/kg/day.

B. Pharmacologic activity

In vitro and *in vivo* studies indicate that the mechanism of the anti-inflammatory action of mesalamine is multi-faceted. It is believed to act by activating a class of nuclear receptors involved in the control of inflammation, cell proliferation, apoptosis and metabolic function, i.e., the gamma form of peroxisome proliferator-activated receptors. The receptors are expressed at high levels in colon epithelial cells. Mesalamine has effects on various inflammatory mediators including prostaglandins, interleukin-1 and thromboxane synthesis. Mesalamine also acts as an oxygen radical scavenger and blocks TNF- α -induced growth and critical signal transduction pathways.

C. Nonclinical safety issues relevant to clinical use:

In toxicology studies with mesalamine, kidney and the GI tract were the target organs of toxicity. In acute toxicity studies, oral doses of 800 and 1800 mg/kg were lethal in mice and rats, respectively, and the target organs were the kidney and the GI tract. Repeat dose toxicology studies in rats and dogs (13-week and 26-week oral toxicity studies in rats and 26-week and 52-week oral toxicity studies in dogs) have shown the kidney to be the major target organ of toxicity. Oral doses of 40 mg/kg/day produced minimal to slight tubular injury and doses of 160 mg/kg/day or higher in rats produced renal lesions including tubular degeneration, tubular mineralization, and papillary necrosis. Oral doses of 60 mg/kg/day or higher in dogs also produced renal lesions including tubular atrophy, interstitial cell infiltration, chronic nephritis, and papillary necrosis. Thus, encapsulated mesalamine granules have the potential for causing adverse effects on kidneys of patients taking the drug.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-301

Review number: 01

Sequence number/date/type of submission: 000/December 21, 2007/Original submission

Information to sponsor: Yes () No (X)

Sponsor and/or agent: Salix Pharmaceuticals, Inc.

Manufacturer for drug substance: **b(4)**

Reviewer name: Sushanta Chakder, Ph.D.

Division name: Division of Gastroenterology Products (DGP)

HFD #: 180

Review completion date: September 25, 2008

Drug:

Trade name: N/A

Generic name: Mesalamine

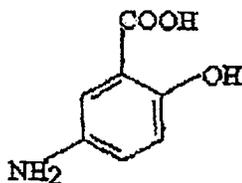
Code name: N/A

Chemical name: 5-Aminosalicylic acid; 5-Amino-2-hydroxybenzoic acid

CAS registry number: 61513-32-4

Molecular formula/molecular weight: 153.135

Structure:



Relevant INDs/NDAs/DMFs: IND 62, 113; 5-Aminosalicylic acid pellets, Salix Pharmaceuticals, Inc.

NDA 19-651; Asacol Delayed Release Tablets, Procter and Gamble, Inc.

Drug class: Antiinflammatory agent

Intended clinical population: Adult patients (18 years and older) with ulcerative colitis.

Clinical formulation: Each encapsulated mesalamine granule contains 375 mg mesalamine, and the following excipients:

Colloidal silicon dioxide NF — magnesium stearate NF —
microcrystalline cellulose NF — Simethicone USP []
— hypromellose USP []
[] talc — , titanium dioxide USP — triethyl citrate NF —]
[] aspartame NF — citric acid anhydrous USP —
[] providone USP — talc USP — titanium dioxide USP — , vanilla
flavoring — , hypromellose USP []

b(4)

Route of administration: Oral

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Data reliance: Except as specifically identified below, all data and information discussed below and necessary for approval of NDA 22-301 are owned by Salix Pharmaceuticals, Inc. or are data for which Salix has obtained a written right of reference. Any information or data necessary for approval of NDA 22-301 that Salix does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug’s approved labeling. Any data or information described or referenced below from a previously approved application that Salix does not own or from FDA reviews or summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 22-301.

Studies reviewed within this submission:

The sponsor submitted NDA 22-301 for encapsulated mesalamine granules pursuant to Section 505(b)(2) of the Federal Food, Drug and Cosmetics Act. The sponsor incorporated, by reference, mesalamine data concerning 6-month repeat dose rat, 12-month repeat dose dog and mouse and rat carcinogenicity studies (Procter and Gamble Pharmaceuticals, Asacol, NDA 19-651) and the mouse lymphoma assay (Axcan Scandipharm Inc., Canasa, NDA 21-252). The studies were reviewed under earlier submissions. NDA reviews, containing the above studies, as well as the study reports were submitted in this submission. In addition, a 4-week and a 26-week oral toxicity studies with — were submitted. These studies were reviewed.

b(4)

Studies not reviewed within this submission: None

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Mesalamine acts by activating a class of nuclear receptors involved in the control of inflammation, cell proliferation, apoptosis and metabolic function, i.e., the gamma form of peroxisome proliferator-activated receptors. The receptors are expressed at high levels in colon epithelial cells. Mesalamine has effects on various inflammatory mediators including prostaglandins, interleukin-1 and thromboxane synthesis. Mesalamine also acts as an oxygen radical scavenger and blocks TNF- α -induced growth and critical signal transduction pathways. Oral 5-ASA caused a dose-dependent inhibition of carrageenin-induced rat paw edema in rats. In an acetic acid-induced colitis model of rats, pretreatment of the animals with mesalamine (100 mg/kg) by the intra-rectal route caused a significant attenuation of the colonic myeloperoxidase activity and reduction of lesion score. Thus, mesalamine showed anti-inflammatory activity in animal models of inflammatory bowel disease.

2.6.2.2 Primary pharmacodynamics

Anti-inflammatory effect of 5-ASA on rat paw edema:

The anti-inflammatory effect of 5-ASA was examined in the carrageenin-induced rat paw edema model. 5-ASA was administered at 200 and 500 mg/kg doses and a 10 mg/kg dose of indomethacin was used as a positive control. The percentage of inhibition of the paw edema was measured.

Oral 5-ASA caused a dose-dependent inhibition of carrageenin-induced rat paw edema. At 200 and 500 mg/kg doses, it caused 9% and 22.8% inhibition of the edema; indomethacin, 10 mg/kg, caused 57.7% inhibition of the edema.

Published Studies:

3-Aminosalicylate: Oxidation by Activated Leucocytes and Protection of Cultured Cells from Oxidative Damage (Dull, et al., Biochem Pharmacol, 1987; 36: 2467-2472)

The mechanism of action of mesalamine in inflammatory bowel disease was studied by its ability to bind reactive hydrogen radicals and to protect cultured Chinese hamster ovary (CHO) cells from the lethal effects of superoxide radical and hydrogen peroxide.

Activated mononuclear cells and activated granulocytes, as well as hydroxyl radicals oxidized ^{14}C -mesalamine to a number of metabolites. Mesalamine (0.65 mM) protected the cultured CHO cells from the lethal effects of superoxide radicals and hydrogen peroxide. Superoxide dismutase had similar effects on the CHO cells. Thus, mesalamine protected the CHO cells from damage caused by free radicals, which may explain its mechanism of action in inflammatory bowel disease.

Mesalamine Blocks Tumor Necrosis Factor Growth Inhibition and Nuclear Factor $\kappa\beta$ Activation in Mouse Colonocytes (Kaiser, et al, Gastroenterology, 1999; 116: 602-609).

The effect of mesalamine on tumor necrosis factor- α (TNF- α)-initiated signaling events was studied in adult mouse colonic cells.

Mesalamine (20 mmol/L) blocked the proliferation of mouse colonic epithelial cells caused by the high dose of TNF- α (100 ng/mL) as well as epidermal growth factor (EGF)-mediated mitogenesis by TNF- α . Treatment of the cells with mesalamine completely blocked the TNF- α mediated activation of MAP kinase ERK/ERK2. TNF- α induced activation of NF $\kappa\beta$ by I κ -B α degradation and its nuclear translocation in mouse colonic epithelial cells were also blocked by mesalamine.

Effects of Mesalamine on the hsp 72 Stress Response in Rat IEC-18 Intestinal Epithelial Cells. (Burruss, et al, Gastroenterology, 1997; 113: 1474-1479).

The effect of mesalamine on the hsp 72 stress response was examined in IEC-18 intestinal epithelial cells (0.3, 1 or 3 nmol/L). The expression of hsp 72 in response to heat shock was examined in the presence and absence of mesalamine at both mRNA and protein levels.

Treatment of the IEC-18 cells with mesalamine (1 mmol/L) for 60 and 120 min caused a significant increase in the expression of hsp 72 stress protein in response to heat shock. Mesalamine also caused a dose-dependent increase in the expression of hsp 72 mRNA in response to heat shock. Thus, treatment with mesalamine caused augmentation of the thermal induction of the intestinal epithelial hsp 72 expression and this effect was accompanied by increased cellular protection against oxidant injury.

Anti-Oxidant Properties of 5-Aminosalicylic Acid (Pearson, et al., Free Radical Biology and Medicine, 1996; 21: 367-373)

The anti-oxidant properties of mesalamine were studied in the guinea pig intestinal brush border membrane preparation. Oxidation was induced from within the membrane by 2,2' azobis (2-amidinopropane AMVN) hydrochloride (AAPH).

AMVN-induced lipid peroxidation within the microvillus membrane was dose-dependently inhibited by mesalamine with an IC₅₀ value of 1.5 μ M. Mesalamine was more effective than tocopherols and ascorbate as an anti-oxidant in this study. Mesalamine was also very effective in inhibiting lipid peroxidation on the outer surface of the membrane vesicles.

Action of Phenolic Derivatives (Acetaminophen, Salicylate and 5-Aminosalicylate) as Inhibitors of Membrane Lipid Peroxidation and as Peroxyl Radical Scavengers (Dinis, et al, Acta Biochem Biophys, 1994; 315: 161-169)

In this study, the effects of acetaminophen, salicylate and 5-aminosalicylate (5-ASA) on lipid peroxidation were examined *in vitro*. The anti-oxidant properties were determined with diphenylhydrazine (DPPH) from the changes in absorbance at 517 nm. Lipid peroxidation in rabbit muscle sarcoplasmic reticulum (SR) membrane was measured by gas chromatography.

Among the three compounds tested mesalamine was the most effective in causing a reduction in DPPH. Lipid peroxidation in the SR was dose-dependently inhibited by mesalamine. Mesalamine was also found to be a strong peroxyl radical scavenger in rabbit SR membranes. Thus, the anti-inflammatory actions of mesalamine may be due to its capability of protecting the colonic mucosa from free-radical induced damage.

Primary Colonic Epithelial Cell Culture of the Rabbit Producing Prostaglandins (Hata, et al., Prostaglandins, 1993; 45: 129-141).

The effects of mesalamine and indomethacin on prostaglandin production were examined in cultured colonic epithelial cells from Japanese white rabbits.

Rabbit colonic epithelial cells in primary culture produced PGE₂ and treatment of the cells with mesalamine caused a dose-dependent reduction of the prostaglandin production. Indomethacin had a similar inhibitory effect on PGE₂ production in cultured epithelial cells. The data suggest that mesalamine may exert its anti-inflammatory action by inhibiting the production of prostaglandins.

Effect of Aminophenols (5-ASA and 4-ASA) on Colonic Interleukin-1 Generation (Rachmilewitz, et al., Gut, 1992; 33: 929-932).

The effects of mesalamine and 4-ASA on nitrobenzene sulfonic acid-induced colitis in rats and IL-1 generation in cultured biopsy samples from active ulcerative colitis patients were studied.

Treatment with mesalamine (50 mg/kg for 3 and 1 weeks) and 4-ASA (50 mg/kg for 3 weeks) caused significant reduction of colonic IL-1 generation (65.4% decrease) and the extent and severity of inflammation in the rat model of colitis. Mesalamine also caused a significant decrease in the tissue IL-1 content (62%) and its release into the culture medium from the cultured biopsy specimen (48% of control).

Anti-Inflammatory Effects of Various Drugs on Acetic Acid Induced Ulcerative Colitis in the Rat (Fitzpatrick, et al., Agents and Actions, 1990; 30: 393-402).

The effects of sulfasalazine, mesalamine and other anti-inflammatory agents on acetic acid-induced colitis were studied in male Sprague Dawley (SD) rats. Colonic mucosal myeloperoxidase activity was measured in mucosal samplings by measuring the changes in absorbance.

Oral administration of sulfasalazine (400 mg/kg) prior to induction of colitis caused a significant reduction of the inflammatory response and mucosal myeloperoxidase activity ($ED_{50} > 400$ mg/kg p.o). Pretreatment of the animals with mesalamine (100 mg/kg) by the intra-rectal route also caused a significant attenuation of the colonic myeloperoxidase activity and reduction of lesion score.

Actions of Sulfasalazine and 5-Aminosalicylic Acid as Reactive Oxygen Scavengers in the Suppression of Bile Acid-Induced Increases in Colonic Epithelial Cell Loss and Proliferative Activity (Craven, et al., Gastroenterology, 1987; 92: 1998-2008).

In this study, the mechanism of mucosal injury by sulfasalazine and mesalamine was examined in a rat model in which the colonic epithelial cell loss and subsequent increases in epithelial proliferative activity were induced by intracolonic administration of sodium deoxycholate (DOC).

Intraluminal administration of DOC caused a 6-fold increase in luminal DNA content compared with that in animals treated with saline. Sulfasalazine and mesalamine both completely prevented the DOC-induced increases in luminal DNA content. Sulfasalazine and mesalamine also blocked xanthine-xanthine oxidase-induced loss of DNA and subsequent proliferative response in the colonic epithelium. DOC-induced increase in ^3H -Thymidine incorporation into rat colonic luminal DNA was blocked by sulfasalazine and mesalamine. Increased superoxide production by bile acid was also abolished by mesalamine.

Participation of Thromboxane and Other Eicosanoid Synthesis in the Course of Experimental Inflammatory Colitis (Vilaseca, et al, Gastroenterology, 1990; 98: 269-277).

The role of inflammatory eicosanoids on trinitrobenzene sulfonic acid (TNBS)-induced colonic inflammation and the effects of prednisone and mesalamine (10% enema) on the inflammation and eicosanoid levels were examined in male SD rats.

Three days after intracolonic injection of different doses of TNBS, there were significant increases in prostaglandin E₂, 6-keto prostaglandin F_{1 α} , thromboxane B₂ and leukotriene B₄ release as compared to control. Mesalamine caused a reduction of the increase in PGE₂ and leukotriene B₄; the levels of other eicosanoids also declined, except thromboxane B₂ which continued to increase during the chronic stage of

inflammation. Treatment with mesalamine enema also improved the TNBS-induced morphological damage, assessed by macroscopic and histological evaluation.

Mechanism of action: The exact mechanism of action of mesalamine is not clear. However, *in vitro* and *in vivo* studies indicate that the mechanism of action is multifaceted. Mesalamine is believed to act by activating a class of nuclear receptors involved in the control of inflammation, cell proliferation, apoptosis and metabolic function, i.e., the gamma form of peroxisome proliferator-activated receptors. The receptors are expressed at high levels in the colonic epithelial cells. Mesalamine has effects on various inflammatory mediators including prostaglandins, interleukin-1 and thromboxane synthesis. Mesalamine also acts as an oxygen radical scavenger and blocks TNF- α -induced growth and critical signal transduction pathways.

Drug activity related to proposed indication: Mesalamine is an anti-inflammatory agent that acts locally in the GI tract. The anti-inflammatory activity of mesalamine has been shown in *in vitro* and *in vivo* pharmacology studies.

2.6.2.3 Secondary pharmacodynamics

Antispasmodic Activity of 5-ASA:

The antispasmodic activity of 5-ASA was examined on isolated rat uterus. Isotonic contractions of the uterine strips were measured using a force displacement transducer. The effects of 5-ASA on spontaneous contractions and 5-hydroxytryptamine-induced contractions were recorded.

5-ASA, at concentrations of 10^{-4} and 10^{-3} g/ml, had no effects on spontaneous contractions or 5-hydroxytryptamine-induced contractions of rat uterine muscles.

5-Aminosalicylic acid had no effects on phenylquinone-induced writhing in male mice at an oral dose of 500 mg/kg. It had no effects on metazolol- or electroshock-induced convulsions in male NMRI mice at an oral dose of 500 mg/kg. It had no antipyretic effects in male Wistar rats at a dose of 500 mg/kg. Mesalamine had no local anesthetic effects in rabbits, when applied as a 1% solution in the eyes of the animals.

2.6.2.4 Safety pharmacology

Neurological effects:

Effects on Spontaneous Motor Activity:

The effects of mesalamine (500 mg/kg p.o.) on spontaneous motor activity were evaluated in 2 groups of 5 male NMRI mice. Motor activity was monitored at 0.5, 1.5 and 2.5 hr post-dose.

Mesalamine, at a dose of 500 mg/kg, had no effect on spontaneous motor activity in mice.

Effects of 5-Aminosalicylic Acid on Hexobarbitone Sleeping Time.

The effects of 5-ASA (mesalamine, 500 mg/kg) on hexobarbitone (70 mg/kg, i.p.) sleeping time were examined in 2 groups of 5 female NMRI mice. Hexobarbitone was administered 30 min after mesalamine or vehicle.

Mesalamine had no effect on the hexobarbitone sleeping time in female NMRI mice at an oral dose of 500 mg/kg.

Mesalamine had no effects on tremorine-induced tremor and body temperature in mice at an oral dose of 500 mg/kg.

Cardiovascular effects:

The effects of 5-ASA on blood pressure, heart rate and the respiratory rate were examined in anesthetized female rabbits following i.v. administration of 1.0 and 10.0 mg/kg doses.

5-ASA, at i.v. doses of 1.0 and 10.0 mg/kg, had no effects on the heart rate and blood pressure of anesthetized rabbits.

Pulmonary effects:

The effects of 5-ASA on the respiratory rate were examined in anesthetized female rabbits following i.v. administration of 1.0 and 10.0 mg/kg doses.

5-ASA, at i.v. doses of 1.0 and 10.0 mg/kg doses had no effects on the respiration rate of anesthetized rabbits.

Renal effects:

The effects of 5-ASA on urine volume and electrolyte excretion (for 24 hours) were examined in mice after oral (gavage) administration of 200 and 600 mg/kg doses of the drug.

At 200 mg/kg, 5-ASA had no effects on the urine flow, specific gravity and electrolyte excretion. At the 600 mg/kg dose, there was an increase in Na^+ (0-6 hr; 795%) and protein (0-24 hr) excretion and a decrease in K^+ (0-24 hr; 69%) excretion. The volume of urine was also increased at this dose. At 600 mg/kg, increased erythrocytes and epithelial cells were observed in the urinary sediment. Thus, the 600 mg/kg dose may have caused functional disorders in the kidneys of mice.

Other:

5-ASA was also examined for its analgesic and antipyretic effects in mice following oral administration of a 500 mg/kg dose. It was examined for its local anesthetic effect following administration in the eye of rabbits.

It had no significant analgesic or antipyretic effects in mice. It had no local anesthetic effect in the rabbit's eye.

2.6.2.5 Pharmacodynamic drug interactions

N/A

2.6.3 PHARMACOLOGY TABULATED SUMMARY

N/A

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

The sponsor did not provide any Pharmacokinetics/Toxicokinetics study report under the current submission

2.6.4.1 Brief summary: Pharmacokinetic parameters for mesalamine were measured in mice, rats and dogs exposed to the drug during toxicology studies. In a 13-week oral toxicity study in rats, plasma concentrations of mesalamine and its metabolite, N-acetyl-5-ASA were measured, and the data indicated a distinct accumulation, particularly in the males. At week 5, plasma mesalamine levels in male/female animals were 0.64/0.37, 6.05/4.23, 97.8/92.7 $\mu\text{g/ml}$ at 40, 120 and 640 mg/kg/day, respectively, and at week 13, the values were 7.05/<0.03, 95.2/8.0, 2421.4/136.6 $\mu\text{g/ml}$, respectively.

2.6.4.2 Methods of Analysis

The concentrations of mesalamine and N-acetyl mesalamine were measured using a reverse phase HPLC method.

2.6.4.3 Absorption

N/A

2.6.4.4 Distribution

No studies on distribution of mesalamine have been submitted.

2.6.4.5 Metabolism

N-Acetylmесalamine has been identified as the main metabolite in the plasma from mice, rats and dogs following treatment with mesalamine.

2.6.4.6 Excretion

No studies have been submitted.

2.6.4.7 Pharmacokinetic drug interactions

N/A

2.6.4.8 Other Pharmacokinetic Studies

[] The Excretion and Tissue Distribution of Radioactivity after Oral Administration to Rats (Study # 86-RGC1/562)

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[] was administered to rats at single oral doses of 55-75 mg dry polymer substance (corresponding to 9.3 – 12.7 μ Ci) per animal. Fecal excretion of the radioactivity, and plasma and tissue concentrations were monitored for up to 14 days.

b(4)

A mean of 97.2% (range, 93.6 – 101.7%) of the administered radioactivity was recovered in the feces within 7 days. The majority of the radioactivity was recovered during the first 3 days. The sponsor stated that the radioactivity in blood, liver, kidney, spleen, mesenteric lymph nodes and small and large intestine at Days 1, 3, 7 and 14 after administration of a single radioactive dose of [] were not significantly different from those in tissues from the corresponding control animals. Only trace quantities of radioactivity (0.00092%) were excreted in the urine. Most of the radioactivity in the urine was detected in samples collected within 48 to 72 hours after dosing.

b(4)

The results suggest that following oral administration of 14 [] to rats, there is very small absorption of the radioactivity, and most of the administered radioactivity is excreted in the feces. There was no evidence of retention of the radioactivity by any tissues.

b(4)

2.6.4.9 Discussion and Conclusions

Following oral administration to mice, rats and dogs, mesalamine is primarily metabolized to N-acetyl-5-ASA. There was a distinct accumulation of mesalamine and its metabolite in rats following repeated administration. As dog was a poor acetylator, very little N-acetyl-5-ASA was formed in this species. Rats were more efficient acetylator, and the plasma concentrations in rats were higher than that in dogs.

2.6.4.10 Tables and figures to include comparative TK summary

N/A

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

N/A

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

Acute oral and intravenous toxicity studies with mesalamine were conducted in rats and mice. The clinical signs following oral and intravenous treatment in both rats and mice were similar, and included sedation, dyspnea, weight loss, coma (mice only) and ventral/curved body position. The kidney and the GI tract were the target organs of toxicity. Repeat dose toxicity studies were conducted in rats (including 13-week and 26-week oral gavage and a 52-week dietary admixture) and dogs (including a 26-week and a 52-week oral toxicity study). The kidney was the target organ of toxicity in all studies in both rats and dogs. In the rat, a dose of 640 mg/kg (13 weeks) resulted in a number of deaths attributed to renal failure, urinary changes (polyurea, proteinurea and hematuria) and marked increases in serum LDH levels. Kidney weights were significantly increased and the histopathological changes comprised of renal lesions involving the papillary and cortical regions and characterized by areas of slight to extremely severe necrosis. A moderate papillary necrosis was also observed in male rats from the 13-week study at a lower-dose of 160 mg/kg. In the 26-week rat study, papillary necrosis was observed at a dose of 320 mg/kg. Other renal changes observed in the 26-week rat study at a dose of 360 mg/kg included tubular degeneration of the kidney, tubular mineralization and urothelial hyperplasia. Increases in blood urea nitrogen, creatinine and globulin levels were observed at 360 mg/kg females in the same study. Papillary edema was observed in female rats at 170 mg/kg in the 26-week study, and minimal to slight tubular injury was observed in males receiving the drug for 13 weeks. In the 52-week oral toxicity study in rats, hyalinization of the tubular basement membrane and Bowman's capsule was observed at 100 and 320 mg/kg doses. Other treatment-related findings observed in rats included hemorrhagic changes in the gastric mucosa at 640 mg/kg (13-week study), mucosal/submucosal fibrosis of the stomach and inflammation of the urinary bladder at 360 mg/kg (26-week study), and ulceration of the stomach at 320 mg/kg (52-week study).

In dogs, polyurea and an increased urinary excretion of gamma-glutamyltransferase and lactate dehydrogenase was observed at 120 mg/kg in the 26-week toxicity study. Papillary necrosis was observed at 60 mg/kg and higher doses in all three chronic toxicity studies in dogs. Chronic nephritis was observed in the 52-week oral toxicity study in dogs.

2.6.6.2 Single-dose toxicity

Acute Toxicity Studies:

Acute toxicity studies with 5-ASA were conducted in mice and rats after single oral and i.v. administration. In mice, oral doses of 0, 800, 1500, 2800 and 5000 mg/kg, and i.v. doses of 1000, 1800 and 3000 mg/kg were used. In rats, oral doses of 0, 900, 1800, 3900 and 8000 mg/kg were used. For the acute i.v. toxicity study in rats, a single dose of 2000 mg/kg was used. The animals were observed daily for 14 days, after which they were sacrificed and complete necropsies performed.

The oral minimal lethal dose (MLD) in mice and rats were 800 and 1800 mg/kg, respectively, for both males and females. The i.v. MLDs in mice were 3000 mg/kg for males and 1000 mg/kg for females. The i.v. MLD in rats is not known, as 20% of the animals died at the 2000 mg/kg dose, used in the study. Clinical signs observed after administration of a single oral or i.v. dose of mesalamine to rats and mice included sedation, dyspnea, abdominal position, tremor, somnolence and coma (mice only). Gross pathological examinations showed that the kidney and the GI tract were the target organs of toxicity. The effects on the kidney included discoloration of the renal cortex and papillae and papillary necrosis. Gastrointestinal effects included reddening of mucous membranes, gastrectasis, vascular congestion and dark intestinal contents.

2.6.6.3 Repeat-dose toxicity

Study title: 28-Day Palatability Study with Salofalk (5-aminosalicylic acid)

Key study findings: In the 28-day oral palatability study in rats, 5-ASA was well-tolerated at doses up to 900 mg/kg/day.

Study no.: 026008

Conducting laboratory and location: [

b(4)

Date of study initiation: October 03, 1983.

Methods

Doses: 0, 100, 300 and 900 mg/kg/day
Species/strain: Rats (Wistar KFM-Han)
Number/sex/group or time point (main study): 5 animals/sex/group
Route, formulation, volume, and infusion rate: The drug was administered as a dietary admixture.
Age: Approximately 4 weeks
Weight: 70 – 89 g, males; 51 – 68 g, females.

The animals were observed twice daily for mortality and clinical signs. Body weight and food consumption were measured weekly. At the end of the dosing period, all animals were sacrificed and necropsies performed.

Results:

There were no mortalities in any group, and no treatment-related clinical signs were observed. No differences in food consumption were observed between the control and treatment groups. No treatment related gross pathological changes were observed.

Thus, in the 28-day oral palatability study with 5-ASA in rats, 100, 300 and 300 mg/kg/day doses were used. The 900 mg/kg/day dose was well-tolerated. However, hematology, clinical chemistry, urinalysis and histopathology examinations were not conducted.

Study title: A 13-Week Oral Toxicity Study with 5-ASA in Rats

Key study findings: In the 13-week oral toxicity study with 5-ASA in Wistar rats, groups of animals were administered the drug at oral doses of 40, 160 and 640 mg/kg/day doses. The target organs of toxicity were the kidney, GI tract, heart and the hematopoietic system. The no effect dose was not identified.

Study no.: 017447

Volume #, and page #: vol 2, page 4.

Conducting laboratory and location:

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Date of study initiation: March 7, 1983

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity: Salofalk (5-aminosalicylic acid); Lot number not specified.

Methods

Doses: 0, 40, 160 and 640 mg/kg/day.
Species/strain: Wistar KFM-Han rats

Number/sex/group or time point (main study): 25 animals/sex/group

Route, formulation, volume, and infusion rate: The drug was administered by oral gavage at a dosing volume of 10 ml/kg (as a suspension in 2% carboxymethylcellulose).

Satellite groups used for toxicokinetics or recovery: 5 animals/sex/group were used for a 4-week recovery period.

Age: 8 weeks

Weight: 184 – 219 g, males; 147 – 181 g, females.

Observations and times: The animals were observed twice daily for clinical signs and mortality. Body weights and food consumption were measured weekly. Water consumption was measured daily. Ophthalmologic examinations were conducted prior to dosing and in Weeks 4, 13 and 17. Blood samples for hematology and clinical chemistry, and urine samples for urinalysis examinations were collected in Weeks 5, 9 and 13 from the main study groups and at the end of the recovery period from the recovery animals. At the end of the treatment or recovery periods, the animals were sacrificed and complete necropsies performed. Histopathology of the all organs was conducted from the control and high dose animals. From the low and mid dose animals, kidneys, stomach and liver were examined microscopically.

For toxicokinetic analyses, blood samples were withdrawn from 5 animals/sex/ at Week 5 and 13.

Results

There were mortalities at the 640 mg/kg/day dose. Four males and 4 females (out of 25) died during the study period. There was a marked accumulation of 5-ASA and its metabolite, acetyl-5-ASA following 13 weeks repeated administration in rats. Polyurea with a lower specific gravity of urine and proteinuria were observed in the high dose animals in Weeks 5, 9 and 13. Renal toxicity was observed in all treatment groups. Renal lesions consisted of papillary necrosis and multifocal tubular injury. The renal lesions were still present at the end of the 4-week recovery period. Focal hemorrhagic erosions of the gastric mucosa, myocardial necrosis and erythropoietic depression in the bone marrow were observed in animals receiving the 640 mg/kg/day dose. The target organs of toxicity were the kidney, GI tract, heart and the hematopoietic system. The no effect dose was not identified.

Determination of the plasma levels of 5-ASA and N-Ac-5-ASA showed that there is accumulation of both the parent drug and the metabolite during the 13-week of repeated dosing. The plasma concentrations for 5-ASA and N-Ac-5-ASA at week 5 and week 13 are shown in the Table below.

		5-AS ug/ml	AC-5-AS ug/ml
At 5 weeks			
Males	group 2	0.64	1.93
	group 3	6.05	7.90
	group 4	97.8	19.35
Females	group 2	0.37	1.23
	group 3	4.23	6.25
	group 4	92.7	20.43
At 13 weeks			
Males	group 2	7.05	12.4
	group 3	95.2	16.1
	group 4	2421.4	616.8
Females	group 2	<0.03	5.7
	group 3	8.0	25.2
	group 4	136.6	323.4

**Combined 52-Week Chronic Toxicity and 127-Week Oncogenicity (Feeding)
Study with 5-ASA in Rats (Study #036628; iE**

b(4)

In this combined chronic toxicity/oncogenicity study with 5-ASA in Wistar (KFM-Han, SPF) rats, four groups of animals received 0, 50, 100 and 320 mg/kg/day doses of the drug as a dietary mixture for up to 127 weeks. The achieved average nominal intakes of the drug were 0, 50.2, 98.3 and 308.0 mg/kg/day for males and 0, 49.8, 96.9 and 311.9 mg/kg/day for females.

In the toxicology portion of the study, there were no deaths in any group. Suppression of body weight gains was observed in females receiving the 320 mg/kg/day dose (about 6%). Degenerative changes in the kidneys were observed at 100 and 320 mg/kg/day doses. There were higher incidences of ulceration of the gastric mucosa and atrophy of the testes and/or seminal vesicles for males of the 320 mg/kg/day group. The target organs of toxicity were the kidney, stomach and male reproductive organs. The no effect dose was 50 mg/kg/day.

In the carcinogenicity portion of the study, the animals were sacrificed after treatment with 0, 50, 100 and 320 mg/kg/day doses of 5-ASA for 127-weeks. Although, the mortality was high at the end of the treatment period it was less than 50% at Week-104. There was no increase in the tumor incidences in male and female rats receiving 5-

ASA at doses up to 320 mg/kg/day for 127 weeks.

Study title: A 6-Month Oral Toxicity Study with Salofolk (5-ASA) in Beagle Dogs

Key study findings: In the 6-month oral toxicity study with 5-ASA in beagle dogs, the drug was administered at oral doses of 40, 80 and 120 mg/kg/day. The kidney was the target organ of toxicity, and the no adverse effect level (NOAEL) was 40 mg/kg/day.

Study no.: 017458

Volume #, and page #: Vol 4, Page 1.

Conducting laboratory and location:]

b(4)

Date of study initiation: April 26, 1983

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity: Salofolk (5- Aminosalicyclic acid), Lot # AMS 3; purity, 99.8%

Methods

Doses: 0, 40, 80 and 120 mg/kg/day

Species/strain: Beagle dogs

Number/sex/group or time point (main study): 4 animals/sex/group

Route, formulation, volume, and infusion rate: 5-ASA was administered to the animals as oral capsules.

Satellite groups used for toxicokinetics or recovery: None.

Age: 17 to 26 weeks

Weight: males, 5.3 to 9.0 kg; females, 4.6 to 6.7 kg.

Observations and times:

The animals were observed at least twice daily for clinical signs and mortality. Food consumption was recorded daily. The body weight of each animal was recorded daily. All animals were tested for hearing impairment and ophthalmologic changes. Samples for standard hematology, clinical chemistry and urinalysis determinations were collected from all animals at pretest, and at 1, 3 and 6 months of treatment. A complete necropsy was performed on all dogs at the end of the treatment period. Histopathologic examinations of all organs/tissues were conducted on all animals.

Results

There were no deaths in any group. No treatment-related clinical signs or changes in body weight were observed in any group. No effects on hearing or ophthalmological

abnormalities were observed in any group. No treatment-related changes in hematology and clinical chemistry parameters were observed.

Polyurea was observed in high dose males at 1 and 6 months of the treatment, and at the mid dose at 6-month treatment period. Slight to moderate renal papillary necrosis and minimal to moderate tubular injury were observed at 80 and 120 mg/kg/day doses. The papillary necrosis occurred unilaterally, and was located at the tip of the renal papilla. The tubular injury was characterized by multi-focal tubular atrophy that affected mainly the proximal convoluted portions of the nephrons. Tubular atrophy was characterized by thickened basement membranes, increased cytoplasmic basophilia, flattened epithelial lining and occasionally intracytoplasmic deposits of brownish pigment. The kidney was the target organ of toxicity, and the no adverse effect level was 40 mg/kg/day.

2.6.6.4 Genetic toxicology

The genotoxic potential of 5-ASA was examined in the Ames test, the *in vivo* mouse micronucleus assay, and the *in vivo* sister chromatid exchange assay in Chinese hamster bone marrow cells. The studies were reviewed in earlier submissions and are summarized below.

Bacterial Reverse Mutation (Ames) Test with 5-ASA (Project # 014670,

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The Ames test was conducted by the plate incorporation method using *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 or the *E. coli* strain WP2uvrA. The test was performed with and without activation with rat liver microsomal fraction. The concentrations of 5-ASA used were 1.58, 5, 15.8, 50, 158, 500, 1580 and 5000 micrograms per plate in triplicate samples. DMSO was used as a negative control, and the positive controls were: methyl methanesulfonate, 9-aminoacridine, 2-nitrofluorene and N-ethyl-N-Nitro-N-Nitrofluorene.

No toxic effect of the test material was observed at any dose. There were no increases in the number of revertant colonies at any dose for any of the bacterial strains. Thus, 5-ASA was not mutagenic in the Ames test either in the presence or absence of metabolic activation.

Mouse Micronucleus Assay with 5-ASA (Project # 014657; [

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The mutagenic potential of 5-ASA was examined in the *in vivo* mouse bone marrow micronucleus assay following oral administration of the drug. Three groups of mice were administered the vehicle (1% Tween 80), 5-ASA (600 mg/kg) or the positive control (cyclophosphamide). Twenty four (24), 48 and 72 hours after treatment, the animals were sacrificed and the bone marrow samples were examined for the presence micronuclei. The ratio of polychromatic to normochromatic erythrocyte was also

assessed.

Following oral administration of a single dose of 600 mg/kg 5-ASA to mice, no increase in the number of micronucleated polychromatic erythrocytes was observed at any time of treatment, when compared with the corresponding negative control group. Thus, 5-ASA was not mutagenic in the mouse bone marrow micronucleus test under the conditions of the experiment.

***In Vivo* Sister Chromatid Exchange Assay in Chinese Hamster Bone Marrow Cells with 5-ASA (Project # 014668; [**

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In this *in vivo* assay, the potential mutagenic activity of 5-ASA was determined in Chinese hamsters after i.p. administration of 24, 122 and 610 mg/kg doses. The negative control group received the vehicle (2% carboxymethylcellulose) and the positive control group received Cyclophosphamide (20 mg/kg, i.p). Twenty two hours after treatment, the animals received a 10 mg/kg i.p. dose of colcemid. Twenty four hours after treatment, all animals were sacrificed, and the femur bone marrow samples were examined. Thirty second-division metaphases per animal were analyzed to determine the sister chromatid exchange rate.

Following administration single i.p. doses of 24, 122 and 610 mg/kg of 5-ASA to Chinese hamsters, no significant increases in the frequency of sister chromatid exchange were observed at any dose, when compared with the negative control group. Thus, under the experimental conditions, 5-ASA was not mutagenic in the sister chromatid exchange assay in the Chinese hamster bone marrow cells.

2.6.6.5 Carcinogenicity

Carcinogenicity:

The sponsor submitted copies of the pharmacology reviews of the 2-year mouse and the 2-year rat carcinogenicity studies with 5-ASA which were reviewed under NDA 19-651. The reviews are incorporated below.

Two Year Carcinogenicity Study of 5-ASA in Diet in Mice
(862.09.00-CD)

Testing Laboratories: Sponsor's Lab
Norwich, New York 13815

Study Start and Completion Dates: June 3, 1993 and March 8, 1996

GLP and OAU Compliance Statement: Sponsor included a statement of compliance with GLP regulation and a quality assurance statement.

Animals: Males (g, -7 weeks)
Females (g, -7 weeks)
Crl:CD-1(ICR) BR VAF Swiss mice

Methods: To determine the carcinogenic potential of 5-ASA, mice (50/sex/group) were treated with 5-ASA in diet at 0, 200, 1000 and 2000 mg/kg/day for 2 years. The study design was summarized in a table on page 217 of volume 18 and this table is attached below.

Treatment Group	Expected mg/kg/day of 5-ASA	Number of Mice Main Study		Proof of Absorption Number of Mice	
		Male	Female	Male	Female
T1	0	50	50	25	25
T2	200	50	50	25	25
T3	1000	50	50	25	25
T4	2000	50	50	25	25

The actual drug consumption was summarized in a table on page 222 of volume 18 and this table is attached below.

Treatment Group	5-ASA Expected mg/kg/day		5-ASA Actual mg/kg/day	
	Male	Female	Male	Female
T1	0	0	0	0
T2	200	200	207.48	203.41
T3	1000	1000	1035.43	1024.77
T4	2000	2000	2048.67	2109.47

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The dose selection was based on findings from the 13 week and 3-month dietary dose ranging studies in mice (Reports 862.09.00-AC and 862.09.00-CC). The high dose of 2000 mg/kg/day was considered as MTD in these studies. In the 2-year carcinogenicity study, clinical signs of toxicity and mortality were observed daily. Body weights were determined weekly. All animals were necropsied at termination. Gross and histopathological examination were performed. Plasma levels of the test drug and its metabolite were determined on day 1 and months 3, 6, 9 and 12 in the satellite animals (25/sex/group). The tumor data were analyzed using the prevalence method of Peto (Peto, R. et.al., Guidelines for simple, sensitive significance tests for carcinogenic effects in long-term animal experiment in Long-term and short term screening assays for carcinogens: a critical appraisal. Geneva: WHO, pp 311-426, 1980) and Life table (death rate) method of Haseman.

Results.

1. Clinical Signs: The major treatment related change was increase in the incidence of ano-genital areas discolored in the treatment groups. The incidence of ano-genital areas discolored was 6, 4, 28 and 27 (males) or 2, 4, 3 and 8 (females) in the control, low, mid and high dose, respectively.

2. Mortality: The intercurrent mortality (unscheduled deaths) was summarized in the following table.

Mortality (unscheduled deaths)								
Days	Males				Females			
	Con	Low	Mid	High	Con	Low	Mid	High
0-365	1	7	5	4	3	3	0	6
366-545	5	8	7	8	10	7	10	8
546-635	6	8	3	7	7	10	6	11
636-737	7	11	11	10	12	14	11	11
Total	19	34	28	29	32	34	27	36

Con, low, mid and high = 0, 200, 1000 and 2000 mg/kg/day

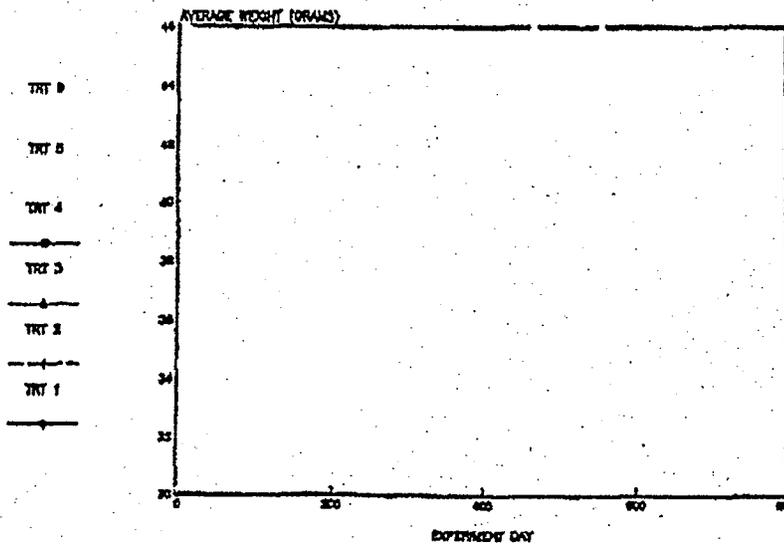
The mortality rate in males was increased in the treatment groups but this increase was not clearly dose-dependent. The possible causes of death include obstructive urologic disease (males), neoplastic and degenerative diseases such as lymphoma, histiocytic sarcoma, renal amyloidosis and chronic progressive glomerulonephropathy (CPG). This information was summarized in a table on page 221 of volume 18. This table is attached below.

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Dose (mg/kg/day)	Mortality							
	Males				Females			
	0	200	1000	2000	0	200	1000	2000
Total Early Deaths	19	34	28	29	32	34	28	36
Obstructive urologic disease	0	8	8	8	0	0	0	1
Lymphoma	3	1	1	4	3	5	3	4
Histiocytic sarcoma	0	4	0	2	5	5	6	2
Renal amyloidosis	8	11	3	8	9	8	5	5
GPG	2	0	2	2	5	5	3	4
Other	4	7	8	8	7	5	10	12
Undetermined	4	5	4	1	3	6	2	8

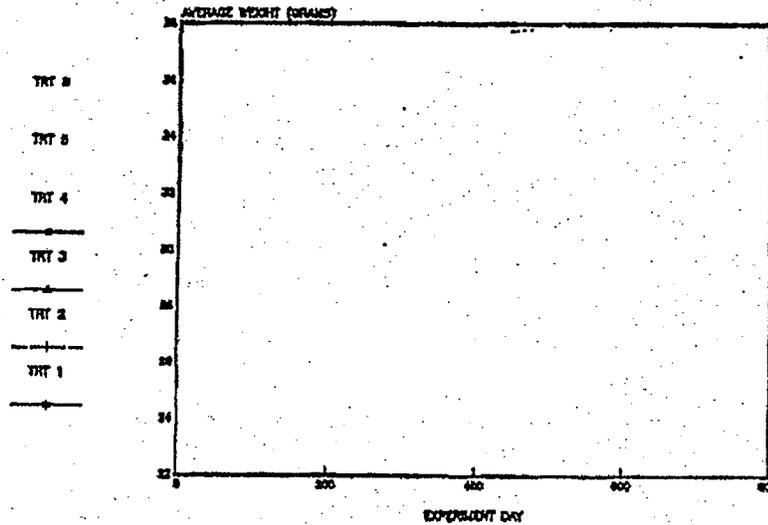
3. Body Weight: The initial and final body weights for the controlled animals were 31.7 and 40.03 g for males or 24.04 and 34.24 g for females. The growth curves depicted in figures 1 and 2 on pages 331 and 333 in volume 19 are attached below.

GROWTH CURVES FOR SWISS MALE MICE
 EXPERIMENT: 090791 PROJECT: 862.08.00-C0



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GROWTH CURVES FOR SWISS FEMALE MICE
 EXPERIMENT: 090791 PROJECT: 892.02.00-CD



There were no significant treatment related changes.

4. Food Consumption: There were no treatment related changes. Average food consumption in the control group was 4.92 (males) or 5.11 (females) g/mouse/day.

5. Gross Pathology: The major treatment related changes were increase in the incidence of urinary bladder distension and dilation in treated males (6, 9, 17 and 13 in control, low, mid and high dose males). The renal pelvic dilation was also noted in the treated groups but not in control (1, 2 and 2 in low, mid and high dose males and 2 high dose females).

6. Histopathology:

Non-neoplastic changes: The incidence of renal pelvic dilation was increased in the treatment groups (0, 0, 3 and 4 in control, low, mid and high dose males and 1, 4, 7 and 6 in control, low, mid and high dose females). There was a total of 50 mice in each group.

Neoplastic changes: The most common tumors were lymphoma and histiocytic sarcoma and the incidence of these tumors were summarized in a table on page 223 of volume 18. This table is attached below.

Incidence of Lymphoma / Histiocytic sarcoma (all organs combined)								
Dose (mg/kg/day)	Males				Females			
	0	200	1000	2000	0	200	1000	2000
Lymphoma	4	2	3	4	0	7	8	7
Histiocytic sarcoma	1	4	0	2	6	8	5	6

The overall incidence of these tumors (all organs combined) was not statistically different between the control and treatment groups. However, the histiocytic sarcoma in the spleen of female mice yielded a p value between 0.025 and 0.05 (0, 0, 1 and 2 for control, low, mid and high dose groups) according to the sponsor's statistical analysis. This is consistent with FDA statistical analysis and not considered significant.

The incidence of neoplastic and non-neoplastic histopathological findings were summarized in sponsor's table 7 on pages 202-300 of volume 19 and this table is attached in Appendix I.

7. Toxicokinetics: AUC values were not provided. The plasma concentrations of 5-ASA and Ac-5-ASA were proportional to the dose administered. The plasma concentrations were not markedly different between males and female. These results were summarized in tables 1 and 2 on pages 293 and 294 in volume 24. These tables are attached below.

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Table 1
Summary of 5-ASA Concentrations in Mice

Treatment Group	Dose (mg/kg)	Sample	5-ASA Concentrations (mcg/ml)					
			Male (mean %CV)		Female (mean %CV)		Overall (mean %CV)	
1	0	Day 1*	0	-	0	-	0	-
2	200	Day 1*	0	-	0	-	0	-
3	1000	Day 1*	0	-	0	-	0	-
4	2000	Day 1*	0	-	0	-	0	-
1	0	3 Month	0	-	0	-	0	-
2	200	3 Month	0.24	-	0.29	69%	0.27	92%
3	1000	3 Month	5.34	24%	7.29	153%	6.43	124%
4	2000	3 Month	11.36	105%	27.78	64%	20.47	83%
1	0	6 Month	0	-	0	-	0	-
2	200	6 Month	0.68	83%	0.27	185%	0.45	119%
3	1000	6 Month	9.21	80%	7.17	101%	8.19	76%
4	2000	6 Month	15.14	39%	17.18	78%	16.15	59%
1	0	9 Month	0	-	0	-	0	-
2	200	9 Month	5.52	200%	0	-	2.76	283%
3	1000	9 Month	1.94	116%	0.63	76%	1.12	127%
4	2000	9 Month	2.80	64%	1.45	11%	2.26	69%
1	0	12 Month	0	-	0	-	0	-
2	200	12 Month	0.41	77%	0.26	157%	0.33	105%
3	1000	12 Month	4.87	59%	5.35	78%	5.11	67%
4	2000	12 Month	12.26	40%	19.02	58%	15.65	54%

* Animals inadvertently received non-medicated feed.

Table 2
Summary of Ac-5-ASA Concentrations in Mice

Treatment Group	Dose (mg/kg)	Sample	Ac-5-ASA Concentrations (mcg/ml)					
			Male (mean %CV)		Female (mean %CV)		Overall (mean %CV)	
1	0	Day 1*	0	-	0	-	0	-
2	200	Day 1*	0	-	0	-	0	-
3	1000	Day 1*	0	-	0	-	0	-
4	2000	Day 1*	0	-	0	-	0	-
1	0	3 Month	0	-	0	-	0	-
2	200	3 Month	5.17	40%	4.91	36%	5.02	35%
3	1000	3 Month	20.82	28%	14.74	51%	17.49	40%
4	2000	3 Month	23.18	26%	19.58	45%	21.16	35%
1	0	6 Month	0	0%	0	-	0	-
2	200	6 Month	5.03	40%	3.15	58%	4.09	50%
3	1000	6 Month	14.18	28%	13.28	46%	13.72	36%
4	2000	6 Month	14.80	45%	21.62	32%	18.21	40%
1	0	9 Month	0	-	0	-	0	-
2	200	9 Month	6.25	161%	0.66	63%	3.59	205%
3	1000	9 Month	5.04	36%	3.92	35%	4.48	36%
4	2000	9 Month	10.31	31%	7.94	46%	9.25	37%
1	0	12 Month	0	-	0	-	0	-
2	200	12 Month	3.65	33%	2.89	80%	3.17	45%
3	1000	12 Month	8.74	18%	16.00	46%	12.63	62%
4	2000	12 Month	11.90	44%	22.92	52%	17.41	60%

* Animals inadvertently received non-medicated feed.

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In summary, in the 2-year dietary carcinogenicity study in mice, mice were treated with asacol in diet at 0, 200, 1000 and 2000 mg/kg/day for 2 years. The dose selection was adequate based on the findings in the 13 week and 3-month dietary dose ranging studies in mice (high dose of 2000 mg/kg/day = MTD). In the current study, the major treatment related non-neoplastic change was renal toxicity including increased incidence of renal pelvic dilation in the treatment groups (0, 0, 3 and 4 in control, low, mid and high dose males and 1, 4, 7 and 6 in control, low, mid and high dose females). This was associated with the increase in the rate of mortality. These results suggest that dose of 1000 mg/kg/day (males) or 200 mg/kg/day (females) produced some toxicities in this study. The treatment with the test drug at doses up to 2000 mg/kg/day for 2 years did not increase the tumor incidence in mice. This study is acceptable.

Two Year Carcinogenicity Study of 5-ASA in Diet in Rats
(862.09.00-CA)

Testing Laboratories: Sponsor's lab (Norwich, New York 13815)

Study Start and Completion Dates: October 27, 1992 and February 22, 1996.

GLP and OAU Compliance Statement: Sponsor included a statement of compliance with GLP regulation and a quality assurance statement.

Animals: Males (-7 weeks)
Females (-7 weeks)
VAF Sprague-Dawley —: CD(SD)BR rats

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Methods: To determine the carcinogenic potential of 5-ASA, rats (60/sex/group) were treated with 5-ASA in diet at 0, 60, 120, 360 and 480 mg/kg/day for 2 years. The actual dose consumed were summarized in a table on page 15 of volume 29 and this table is attached below.

Treatment Group	5-ASA Expected mg/kg/day		5-ASA Actual mg/kg/day	
	Male	Female	Male	Female
T1	0	0	0	0
T2	60	60	65.2	60.3
T3	120	120	125.6	125.9
T4	360	360	372.9	366.0
T5	480	480	514.9	477.6

The dose selection was based on findings from the 3 month dietary dose ranging study in rats (Report 862.09.00-AG). Considering the expected exacerbation of renal effects over the 2-year duration of a carcinogenicity study, sponsor adequately selected 480 mg/kg/day as high dose in the carcinogenicity study. Clinical signs of toxicity and mortality were observed daily. Body weights were determined weekly. Ophthalmology examination was conducted before and at -3, 6, 12, 18 and 24 months after the study started. All rats were topically dilated with tropicamide prior to the ocular evaluation to facilitate the observations with an indirect ophthalmoscope and lenses of 20 and 30 diopters (sponsor stated that methods and materials used were documented in the study notebook). All animals were necropsied at termination and gross and histopathological examinations were conducted. Plasma levels of the test drug and its metabolite were determined on day 1 and

months 1, 3, 6, 9 and 12 in the satellite animals (5/sex/group). The tumor data were analyzed using the prevalence method of Peto (Peto, R. et.al., Guidelines for simple, sensitive significance tests for carcinogenic effects in long-term animal experiment in Long-term and short term screening assays for carcinogens: a critical appraisal. Geneva: WHO, pp 311-426, 1980) and life table (death rate) method of Haseman. The study design was summarized on a table on page 9 in volume 29. This table is attached below.

Treatment Group	Expected mg/kg/day of 5-ASA	Main Study No. of Rats		Proof of Absorption No. of Rats	
		Male	Female	Male	Female
T1	0	60	60	5	5
T2	60	60	60	5	5
T3	120	60	60	5	5
T4	360	60	60	5	5
T5	480	60	60	5	5

Results:

- Clinical Signs:** There were no treatment related changes.
- Mortality:** Deaths were not dose related and they were 48, 45, 45, 44 and 47 (males) or 41, 44, 41, 44 and 39 (females) in the control, 60, 120, 360 and 480 mg/kg/day groups, respectively. The intercurrent mortality (unscheduled deaths) was summarized in the following table.

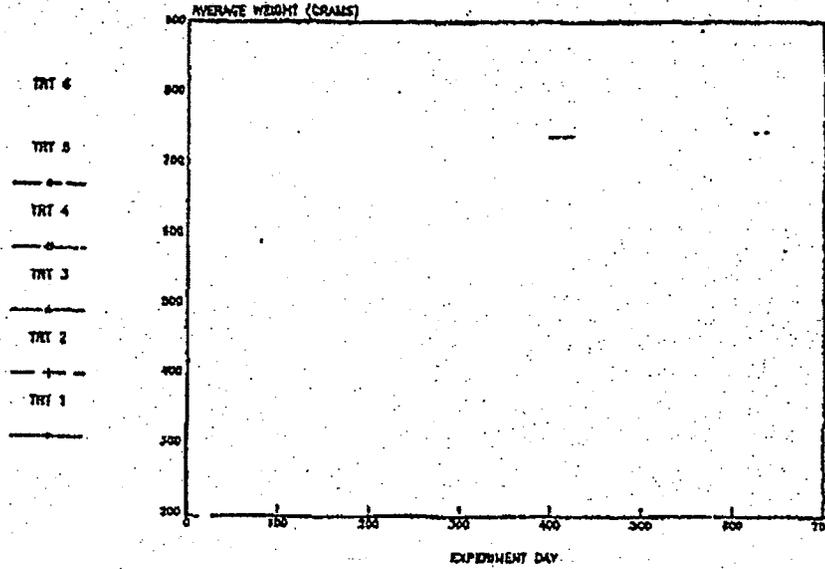
Days	Males					Females				
	con	T1	T2	T3	T4	con	T1	T2	T3	T4
0-365	3	5	2	3	2	5	3	2	3	0
366-545	7	17	10	15	14	10	14	8	13	14
546-635	24	12	18	16	16	15	10	18	13	11
636-737	14	11	15	19	15	11	17	13	14	14
Total	48	45	45	54	47	41	44	41	43	39

con, T1, T2, T3 and T4 = 0, 60, 120, 360 and 480 mg/kg/day

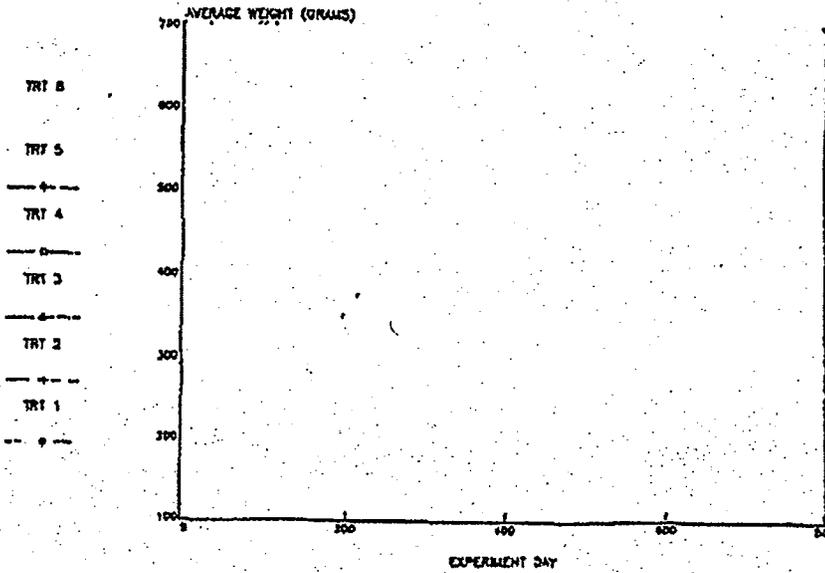
- Body Weight:** There were no treatment related changes. The initial and final body weights in the control groups were 248.7 and 692.8 for males or 178.2 and 571.8 for females. The growth curves depicted in figures 1 and 2 on pages 2 and 4 in volume 30. These figures are attached below.

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GROWTH CURVES FOR SPRAGUE-DAWLEY MALE RATS
EXPERIMENT: 090781 PROJECT: 862.09.00-CA



GROWTH CURVES FOR SPRAGUE-DAWLEY FEMALE RATS
EXPERIMENT: 090782 PROJECT: 862.09.00-CA



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This information was also summarized in Table 5 on page 34 of volume 29. This table is attached below.

Mean Body Weight, Food Consumption and Grog Consumption for 724 Days
 Two Year Tumor Study of S-ASA in Sprague-Dawley Rats
 Project No. 882.DG.DD-CA

Treatment Group/Sex	Number Of Rats	S-ASA Average mg/kg/day	Mean Body Weight					Grog	% Control Sub	Food Consumption		
			Day 0	9 Mo. Day 182	12 Mo. Day 354	18 Mo. Day 546	24 Mo. Day 724			g/100g	g/day	
1M	*85	14	0.5	242.7	345.5	391.1	315.1	392.8	456.7	-	31.56	44.42
2M	*85	18	65.2	247.8	347.7	381.4	318.1	348.2	502.2	110.0	31.06	45.89
3M	*85	17	125.9	249.0	324.5	381.3	311.0	341.2	481.7	107.7	31.07	44.81
4M	*85	18	272.8	244.4	371.0	381.1	327.2	318.2	476.7	104.4	30.79	46.14
5M	*85	19	514.9	248.4	305.8	304.0	383.7	362.8	506.3	111.3	31.59	45.08
1F	*85	20	0.0	178.2	304.4	403.2	578.1	371.5	335.5	-	21.70	50.38
2F	*85	18	90.2	180.1	410.0	515.1	614.8	332.0	438.1	115.3	23.04	48.23
3F	*85	19	128.8	179.0	385.5	480.8	565.2	548.7	385.8	92.5	22.18	50.07
4F	*85	17	388.0	179.7	375.8	482.3	554.2	638.4	418.2	105.7	22.48	50.64
5F	*85	21	477.5	182.1	378.1	472.8	548.5	633.4	417.1	105.8	22.12	50.50

* There were 8 rats/sex for the Pharmacokinetics group that were sacrificed on day 371
 ** Males were terminated on day 700

4. Food Consumption: There were no treatment related changes. Average food consumption in the control group was 30.9 (males) or 22.7 (females) g/rat/day.

5. Ophthalmology Examination: There were no treatment related changes.

6. Gross Pathology: There were no treatment related changes

7. Histopathology:

Non-neoplastic changes: The results indicated that the incidences of urothelial hyperplasia, papillary inflammation, edema and necrosis were increased in the treatment groups. The urothelial hyperplasia consisted of areas of proliferating urothelial cells in the renal pelvis on the renal papilla. The incidence of ulcerative and inflammatory lesion of the gastrointestinal tract was also increased in a dose dependent manner. This information was summarized in Table 4 on page 166 of volume 29. This table is included in Appendix II.

Neoplastic changes: No treatment related effects were found. The histiocytic sarcoma and fibrous histiocytoma in the liver of male rats yielded a p value of 0.0331 according to FDA statistical analysis and not considered significant. In this submission, the historical control data of tumor incidence, complete morphological

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description of the lymphoma and histiocytic sarcoma and the tabulated incidence of the tumors of the hematopoietic system by tumor type (whole body counts) were not submitted. These information were requested from sponsor via a letter dated June 26, 1996. In response to our request, sponsor submitted the following information in a table on page 2 in the-amendment dated July 16, 1996. This table is attached below.

Hematopoietic Neoplasms Rat carcinogenicity study (study 862.09.00-CA)										
Dose (mg/kg) n = 60	Males					Females				
	0	60	120	360	480	0	60	120	360	480
Granulocytic leukemia	0	0	0	1	0	0	0	0	0	0
Large granular cell leukemia	2	1	0	1	1	0	0	0	0	0
Lymphoma	2	2	0	1	0	1	1	0	0	0
Histiocytic sarcoma	0	0	2	1	2	0	0	1	0	0
Fibrous histiocytoma	0	0	1	1	1	0	1	2	1	0

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The incidence of neoplastic histopathological findings extracted from sponsor's table 9 on pages 273-287 Of volume 29 is attached in Appendix II.

7. Plasma Levels: The AUC values of 5-ASA and AC-5-ASA from blood samples collected at 12.3 months were summarized in Table 7 on page 75 of volume 34. This tale is attached below.

Table 7
Summary of 5-ASA AUC in Rats at Final Sample (12.3 Months)

Treatment Group	Dose (mg/kg)	5-ASA Mean AUC (ng/ml/h)								
		Male			Female			All		
		Mean	%CV	AUC/dose	Mean	%CV	AUC/dose	Mean	%CV	AUC/dose
2	60	8.04	18%	0.10	8.44	36%	0.11	8.50	25%	0.11
3	120	28.04	44%	0.23	32.87	68%	0.27	30.72	49%	0.25
4	360	112.77	82%	0.91	148.09	37%	0.41	134.05	61%	0.37
5	480	130.69	30%	0.27	208.57	17%	0.45	188.55	32%	0.35

The plasma concentrations of 5-ASA and AC-5-ASA were proportional to the dose administered. The plasma concentrations in females appeared to be slightly higher than those in males.

In summary, in the 2-year dietary carcinogenicity study in rats, 5-ASA was given to rats at 0, 60, 120, 360 and 480 mg/kg/day for 2 years. The dose selection was adequate based on finding in the 3 month dietary dose ranging study in rats (high dose of 480 mg/kg/day = MTD). In the current study, it also produced renal toxicity including increased incidences of urothelial hyperplasia, papillary inflammation, edema and necrosis at doses of 360 and 480 mg/kg/day. The incidence of ulcerative and inflammatory lesion of the gastrointestinal tract was also increased dose-dependently. The treatment with the test drug at doses up to 480 mg/kg/day for 2 years did not increase the tumor incidence in rats. The study is acceptable.

Key study findings: Two 2-year dietary carcinogenicity studies with mesalamine were conducted in mice and rats. In the 2-year carcinogenicity study in mice, mesalamine doses of 0, 200, 1000 and 2000 mg/kg/day were used. Treatment of the animals at doses up to 2000 mg/kg for 2 years was not associated with an increase in any tumor incidences. In a 2-year dietary carcinogenicity study rats, mesalamine doses of 0, 60, 120, 360 and 480 mg/kg/day were used. Treatment of the rats with mesalamine for 2 years did not cause an increase in any tumor incidences.

2.6.6.6 Reproductive and developmental toxicology

Reproduction and Fertility Study with 5-ASA in Rats (Project # 014938;

b(4)

The study was reviewed in an earlier submission, and is summarized below.

In the oral Segment I fertility and general reproductive performance study in male and female Wistar (KFM-HAN) rats, groups of animals (24/sex/group) received 0, 80, 160 and 320 mg/kg/day doses. Dosing of the male animals started 60 days before pairing (21 days maximum) and continued through the pairing period. Dosing of females started 14 days before pairing, and continued through the pairing and post-partum (21 days) period. Male animals were sacrificed approximately 120 days after initiation of treatment. Half of the mated females were sacrificed on gestation day 21 and the fetuses removed by cesarean section. The dams were subjected to postmortem examinations, including gross macroscopic examinations of all internal organs, with emphasis on the uterus, uterine contents, position of the fetuses and the number of corpora lutea. The fetuses were examined for external malformations and visceral and skeletal abnormalities. The other half of the animals was allowed to bear and rear the F1 pups. F1 animals were examined for developmental and behavioral parameters before being sacrificed on Day 21 post-partum.

There were no treatment-related mortalities or clinical signs. No treatment-related effects on body weight or food consumption were observed. Treatment of the male and female rats with 5-ASA at oral doses up to 320 mg/kg, did not affect the fertility or reproductive performance of the animals. There were no effects on the body weights, sex ratios, and the developmental and behavioral parameters of the F1 pups. 5-ASA had no teratogenic effects in rats.

Embryotoxicity and Teratogenicity Study with 5-ASA in Rats (Project # 014940;

b(4)

The study was reviewed in an earlier submission, and is summarized below.

The embryotoxic and teratogenic potential of Salofalk (5-ASA) was assessed in Wistar rats (25/group) following administration of 80, 160 and 320 mg/kg/day doses. Mated females were administered 5-ASA from gestation day 6 through 25 by oral gavage (10 ml/kg, in 2% CMC). Treated females were mated with sexually mature males (1:1 ratio). The animals were observed for mortality and clinical signs twice daily, and the body weights and food consumption were recorded daily. On Day 21 after mating, the females were sacrificed and fetuses removed by cesarean section. Following examinations of the ovaries (corpora lutea) and uterus (mucosa and contents, placenta, abortions, resorptions), the fetuses were removed, weighed and examined externally. One third of the fetuses were examined for visceral abnormalities and the remaining two thirds were examined for skeletal abnormalities.

There were no mortalities in any group and no treatment-related clinical signs were observed. Treatment with 5-ASA was not associated with any changes in body weight gain or food consumption in any group. No significant changes in the number of corpora lutea or pre- and post- implantation losses were observed in any group. The mean fetal body weights from different groups were similar, and no significant differences in the sex ratio of the fetuses were observed. Treatment of the pregnant female rats with 5-ASA at doses up to 320 mg/kg was not associated with any visceral or skeletal abnormalities in F1 fetuses. Thus, 5-ASA was not teratogenic in rats.

Embryotoxicity and Teratogenicity Study with Salofalk (5-ASA) in Rabbits (Project # 014962;

b(4)

The study was reviewed in an earlier submission, and is summarized below.

In the Segment II teratogenicity study in rabbits, 5-ASA was administered by oral gavage to pregnant rabbits (6 animals/group) at 0, 55, 165 and 495 mg/kg/day doses on Days 6 through 18 of gestation. On Day 28 of gestation, the dams were killed and the fetuses removed by cesarean section. The dams were examined for any abnormalities, including the number of corpora lutea, implantation sites, abortions and resorptions. The fetuses were removed, weighed, examined externally and for visceral and skeletal abnormalities.

Treatment of the pregnant animals with 5-ASA was not associated with any treatment related mortality, clinical signs, body weight or food consumption in any group. There were no effects on the number of corpora lutea or pre- and post- implantation losses were observed in any group. The mean fetal body weights from different groups were similar, and no significant differences in the sex ratio of the fetuses were observed. Treatment of the pregnant female rabbits with 5-ASA was not associated with any visceral or skeletal

abnormalities in F1 fetuses. 5-ASA, at oral doses up to 495 mg/kg/day, was not maternotoxic, embryotoxic or teratogenic in rabbits.

Peri- and Post-natal Study (Segment III) with 5-ASA in the Rat (Project # 014951; []

b(4)

The study was reviewed in an earlier submission and is summarized below.

In the Segment III peri- and post-natal developmental study, 5-ASA was administered orally to female rats (25 animals/group) at 0, 80, 160 and 320 mg/kg/day (10 ml/kg, by oral gavage) doses from Day 16 of gestation through Day 21 of lactation. The animals were observed twice daily for clinical signs or mortality. Following parturition, the pups from each litter were counted, sexed, and examined for anomalies and malformations. The development of the pups was observed daily. Any abnormal signs and symptoms were recorded. The body weights of the pups were recorded on Days 1, 4, 7, 14 and 21 after parturition. At the end of the lactation period (Day 21), the dams and the pups were sacrificed and necropsies done. The uteri of the dams were examined for the number of implantation sites.

Treatment with 5-ASA had no effects on the dams during gestation, parturition and lactation periods. The reproduction and rearing data of all groups were comparable. No treatment-related external malformations and/or anomalies were observed in pups from any group. No treatment-related differences of litter sizes, neonatal mortality, development and growth of pups were observed in any group. On necropsy, no abnormalities in pups were observed in any group.

Reproductive and developmental toxicology summary:

In the oral segment I fertility and reproductive performance study in the male and female rats, 5-ASA was administered at oral doses of 0, 80, 160 and 320 mg/kg/day. 5-ASA, at oral doses up to 320 mg/kg/day did not affect fertility or reproductive performance in the rat. 5-ASA, at oral doses of 0, 80, 160 and 320 mg/kg/day, was not teratogenic in rats, and in rabbits, it was not teratogenic at doses up to 495 mg/kg/day. In the segment III peri- and postnatal development study in rats, 5-ASA, at oral doses up to 320 mg/kg/day, had no influence on any reproductive parameters and was devoid of any maternal or fetal toxicities. No differences in the development and growth of F1 pups were observed in any group.

Reproductive and developmental toxicology conclusions: Reproductive and development toxicology studies with 5-ASA in rats and rabbits do not suggest any reproductive risk for the drug in humans.

2.6.6.7 Local tolerance

No studies were submitted.

2.6.6.8 Special toxicology studies**Primary Eye Irritation after Single Application with 5-ASA in Rabbits**
(Project # 018955; [

b(4)

The eye irritation potential of 5-ASA was examined in rabbits after application of the drug (0.1 g/animal) into the conjunctival sac of the left eye. The right eye was left untreated and served as the control. The eyes were examined at 1, 24, 48 and 72 hours after application, and the ocular lesions were graded using a subjective numerical scoring system.

Moderate redness of the conjunctiva, clearly visible vesicles, slight lacrimation and the presence of the drug substance in the conjunctival sac were observed at 1 hour after application. These changes gradually disappeared with time, and no changes were observed after 72 hours. Thus, 5-ASA was minimal to slight irritant, when applied directly to rabbit's eye.

5-Day Rectal Tolerability with Salofalk (5-ASA) suppositories in the Rabbit (Project # 021014; [

b(4)

Key study findings: Intrarectal administration of 5-ASA (250 mg) suppositories to rabbits did not cause any irritation.

Study no: 021014

Volume #, and page #: volume #14, Page # 1

Conducting laboratory and location: [

b(4)

Date of study initiation: May 18, 1983

GLP compliance: Yes

QA reports: yes (X) no ():

Drug, lot #, radiolabel, and % purity: 5-aminosalicylic acid (Salofalk) Suppositories; Batch no. 830106.

Formulation/vehicle: N/A

Methods: Adult New Zealand albino rabbits (approximately 19 weeks old) were used in the study. The treatment group animals were administered intrarectally a 250 mg 5-ASA suppository, once a day for 5 consecutive days. The control animals received the suppository without any active drug. Following 5 days of administration, the animals were sacrificed and detailed macroscopic examinations were conducted. The rectum of each rabbit was examined histologically.

Results: Intrarectal administration of 5-ASA (250 mg) suppositories to rabbits for 5 days showed no signs of irritation. No treatment-related histopathological changes were observed in the rectum of the treated animals.

Summary of individual study findings: Intrarectal administration of 5-ASA (250 mg) suppositories to rabbits did not cause any irritation.

Rectal Mucosal Tissue Irritancy Testing with 5-ASA in Dogs:

Key Study Findings: Intrarectal administration of 5-ASA suspension to dogs for 28 days produced histopathological changes in the rectum/colon, such as, mucoid proctitis and edema of the lamina propria. Proctoscopic examinations did not show any differences between the control and the treatment group.

Study no: S40202-C03-6483

Conducting laboratory and location: []

b(4)

Date of study initiation: October 05, 1983

GLP compliance: Yes

QA reports: yes (Y) no ():

Drug, lot #, radiolabel, and % purity: 5-aminosalicylic acid (5-ASA) Rectal Suspension; Batch no. not known.

Methods: The suspension of 5-ASA was administered intrarectally to adult male and female mongrel dogs (3g/30 mL) for 28 days. Control animals were administered the vehicle. The drug was introduced approximately 3 to 5 cm into the rectum using an applicator. A biopsy of the rectal tissues and a proctoscopic examination were performed 7 days prior to dosing and on Days 15 and 30 of dosing. Histopathological examinations of all biopsy specimens were conducted to determine any treatment-related changes.

Results: The average daily dose of 5-ASA administered to each animal was 21.9 ± 1.6 ml (2.19 ± 0.16 grams). Proctoscopic examinations of the rectum/colon of the control and 5-ASA treated animals did not show any differences in the incidence and severity of the lesions. An increased incidence and degree of severity of hyperplasia of mucosal goblet cells (mucoid proctitis) and increased fluid in the lamina propria of the rectum (edema) were observed in males and females in Weeks 2 and 4. The histopathological findings in the control and treatment group animals are summarized in the Table below.

Sex	Biopsy Time	Mucoïd Proctitis		Edema	
		Incidences	Severity	Incidences	Severity
Male	Predose	1/8	1.0	2/8	1.5
	2 weeks	8/9	1.8	7/9	1.1
	4 weeks	8/9	1.8	7/9	1.3
Female	Predose	4/8	1.3	3/8	1.0
	2 weeks	8/9	1.9	7/9	1.1
	4 weeks	9/9	1.9	6/9	1.5

Severity, 1=minimal; 4=severe.

Summary of individual study findings: In the rectal mucosal irritability study with 5ASA in dogs, a suspension of the drug was intrarectally administered to the animals. Proctoscopic examinations of the rectum/colon did not show any significant differences between the control and the treatment group animals. However, histopathological examinations of the biopsy specimens showed higher incidences and severity of mucoïd proctitis and edema of the lamina propria from the treatment groups.

Test For Delayed Contact Hypersensitivity in the Guinea Pig with 5-ASA (Project # 018336; [redacted])

b(4)

The skin sensitizing potential for 5-ASA was examined in guinea pigs following intracutaneous injections (0.1 ml of 0.1% solution) to the animals. Following sensitization, the animals were challenged twice with the drug.

5-ASA had no skin sensitizing potential in the delayed hypersensitivity test in the guinea pig.

Other Studies:

[redacted] is used as a coating for the mesalamine formulation. The following toxicology studies with [redacted] were submitted in the NDA submission.

b(4)

Study title: [redacted] 4-Week (Preliminary) Oral Toxicity Study in Dogs (Final Report).

b(4)

Key study findings: [redacted], administered at oral doses of 40 and 180 mg/kg/day for 4 weeks, was well-tolerated in dogs. No target organ of toxicity was identified, and the NOAEL was not established. This is not a complete toxicology study, as only 2 animals/sex/group were used, and histopathological examinations were limited only to the GI tract, liver and the kidney.

Study no.: 57750EXT

Volume #, and page #: vol 14, page 1.

Conducting laboratory and location: []

GLP compliance: yes

QA report: yes (X) no ()

Drug, lot #, and % purity: _____ in the form of coated pellets, containing
[] with reference to the dry
substance, about [] (batch # B060232001)

b(4)

b(4)

Methods

Doses: 0, 45 and 180 mg/kg/day (in terms of _____ dry substance); 0, 200 and 800 mg/kg/day (in terms of test item as supplied).

b(4)

Species/strain: beagle dogs

Number/sex/group or time point (main study): 2 animals/sex/group

Route, formulation, volume, and infusion rate: The test substance was administered orally as capsules.

Satellite groups used for toxicokinetics or recovery: None

Age: 24 weeks

Weight: 4.0 to 6.5 kg.

Observation and Times:

Clinical signs: The animals were observed twice a day for clinical signs

Body weights: Body weights were recorded weekly

Food consumption: Food and water consumption for each animal was recorded daily

Hematology: Blood samples for hematology examinations were collected before initiation of treatment and at the end of the 4-week treatment period.

Clinical chemistry: Blood samples for clinical chemistry examinations were collected before initiation of treatment and at the end of the 4-week treatment period.

Urinalysis: Urine samples were collected at the end of the treatment period.

Gross pathology: At the end of the treatment period, all animals were sacrificed and complete necropsies performed.

Organ weights: The weights of the following organs were recorded: Adrenal glands, brain, heart, kidneys, liver, ovaries, pituitary glands, stomach, testes, thymus, thyroid gland.

Histopathology: The following organs from all animals were fixed for histopathological examinations: Abnormalities, adrenal glands, aorta, bone marrow, brain, cecum, colon, duodenum, epididymis, eyes, femur with joint, gall bladder, heart, ileum, jejunum, kidneys, larynx, liver, lungs, lymph nodes, mammary area, esophagus, optic nerves, ovaries, oviducts, pancreas, parathyroid glands, pituitary gland, prostate, rectum, salivary glands, sciatic nerve, skeletal muscle, skin, spinal column, spinal cord, spleen, stomach, testes, thymus, thyroid gland, tongue.

The following tissues from all animals were examined microscopically: abnormalities, cecum, colon, duodenum, ileum, jejunum, kidneys, liver and salivary glands.

Adequate Battery: yes (), no (X)—Only the GI tract, liver and salivary glands were examined.

Peer review: yes (), no (X)

Results:

Mortality: There were no mortalities in any group.

Clinical signs: No treatment-related clinical signs were observed in any group.

Body weights: The mean body weights of the control male and female animals were 6.91 and 6.29 kg before initiation of dosing, and 7.70 and 7.28 kg on Day 29, respectively. No treatment-related changes in body weight were observed in any group.

Food consumption: No significant changes in the food consumption were observed in the treated animals when compared with the controls.

Hematology: Treatment group males had higher WBC levels (48% and 31% at 45 and 180 mg/kg/day, respectively) at the end of the treatment period. High dose females had higher platelet levels (14%).

Clinical chemistry: No significant clinical chemistry changes were observed in any group at the end of the treatment period.

Urinalysis: No treatment-related changes were observed at the end of the treatment period as compared with controls.

Gross pathology: White granular content in the colon and rectum was observed in one male animal receiving the 45 mg/kg/day dose. Dark granular content was observed in the gall bladder of 2 treated females at 45 and 180 mg/kg/day, respectively. Splenic congestion was observed in one low and one high dose male.

Organ weights: The relative spleen weights of males were higher than that of controls (control 0.299, 61% and 82% increases at 45 and 180 mg/kg/day, respectively). The relative heart, thyroid and kidney weights of high dose males were lower than that of controls (10%, 29% and 13%, respectively).

Histopathology: Histopathological examination was restricted to the gastrointestinal tract, kidneys, liver and abnormalities detected in all animals. No treatment-related histopathological changes were observed in any group.

Thus, _____ administered at oral doses of 40 and 180 mg/kg/day for 4 weeks, was well-tolerated in dogs. No target organ of toxicity was identified, and the NOAEL was not established. This is not a complete toxicology study, as only 2 animals/sex/group were used, and histopathological examinations were limited only to the GI tract, liver and

b(4)

the kidney. Dose selection was not appropriate, and no basis for dose selection was mentioned. The sponsor should have used the maximum feasible dose or a dose producing limiting toxicity as the high dose.

Toxicokinetics: Not conducted.

Study title: _____ : **26-Week Oral Toxicity Study in Dogs Followed by a 3-Week Recovery Period.**

b(4)

Key study findings: _____, administered at oral doses of 50, 125 and 250 mg/kg/day (equivalent to 200, 500 and 1000 mg/kg/day, respectively, in terms of test item as supplied) for 26 weeks, was well-tolerated in dogs. Decreased body weight (about 10%) was observed in animals treated with the 250 mg/kg/day dose. The gall bladder, spleen and the urinary bladder were the target organs of toxicity, and the 250 mg/kg/day dose was a tolerated dose. The sponsor did not examine sufficiently high doses of Eudrgit NE 40D in this study.

b(4)

Study no.: 53990

Volume #, and page #: vol 15, page 1.

Conducting laboratory and location:

GLP compliance: yes

QA report: yes (X) no ()

Drug, lot #, and % purity: _____ in the form of coated pellets, containing _____ with reference to the dry substance, about _____ (batch # B060232001)

b(4)

b(4)

Methods

Doses: 0, 50, 125 and 250 mg/kg/day (in terms of _____ dry substance); 0, 200, 500 and 1000 mg/kg/day (in terms of test item as supplied).

Species/strain: Beagle dogs.

Number/sex/group or time point (main study): 4 animals/sex/group.

Route, formulation, volume, and infusion rate: The test substance was administered daily orally as capsules.

Satellite groups used for toxicokinetics or recovery: Control and high dose groups had 3 animals/sex for a 3-week recovery period.

Age: 24 weeks

Weight: 6.7 to 8.8 kg.

b(4)

Observation and Times:

Clinical signs: The animals were observed twice a day for clinical signs and mortality.

Body weights: Body weights were recorded weekly.

Food consumption: Food and water consumption for each animal was recorded daily.

Ophthalmology: Ophthalmologic examinations of each animal were conducted prior to initiation of dosing, and during Weeks 12 and 25 of dosing period.

Electrocardiography: ECG recordings were conducted on each animal prior to initiation of dosing, and during Weeks 12 and 26 (before dosing and approximately 2 hours after administration).

Hematology: Blood samples for hematology examinations were collected before initiation of treatment and during Weeks 13 and 26 of the dosing period.

Clinical chemistry: Blood samples for clinical chemistry examinations were collected before initiation of treatment and during Weeks 13 and 26 of the dosing period.

Urinalysis: Urine samples were collected prior to initiation of dosing and during Weeks 13 and 26. Fecal samples were also collected at the same time to test for fecal occult blood.

Gross pathology: At the end of the treatment period, all animals were sacrificed and complete necropsies performed.

Organ weights: The weights of the following organs were recorded: Adrenal glands, brain, epididymis, heart, kidneys, liver, lungs, ovaries, pituitary glands, prostate gland, salivary glands, spleen, testes, thymus, thyroid gland and uterus-cervix.

Histopathology: The following organs from all animals were fixed for histopathological examinations: Abnormalities, adrenal glands, aorta, bone marrow, brain, cecum, colon, duodenum, epididymis, eyes, femur with joint, gall bladder, heart, ileum, jejunum, kidneys, larynx, liver, lungs, lymph nodes, mammary area, esophagus, optic nerves, ovaries, oviducts, pancreas, parathyroid glands, pituitary gland, prostate gland, rectum, salivary glands, sciatic nerve, skeletal muscle, skin, spinal column, spinal cord, spleen, stomach, testes, thymus, thyroid gland, tongue, trachea, uterus, urinary bladder, uterus-cervix, vagina.

All tissues (except larynx) from all animals were examined microscopically.

Adequate Battery: yes (X), no ()

Peer review: yes (), no (X)

Results:

Mortality: There were no mortalities in any group.

Clinical signs: No treatment-related clinical signs were observed in any group. One female animal treated with the 250 mg/kg/day dose had one episode of emesis on Day 32 of study.

Body weights: The mean body weights of the control male and female animals were 8.02 ± 0.35 and 10.79 ± 0.52 kg before initiation of dosing, and 8.30 ± 0.60 and 9.91 ± 1.5 kg on Day 182, respectively. Mean body weights of the high dose male (-11%) and female (-9%) animals were lower than that of controls during the course of the study.

Food consumption: The mean food consumptions of the control male and female animals were 285.33 ± 34.23 and 277.48 ± 28.59 g/animal/day on Day 1, and 291.43 ± 22.73 and 300.36 ± 0.57 g/animal/day at Week 27, respectively. Slight reduction in food consumption was observed during the treatment period in males receiving 250 mg/kg/day (11.3%), and all female treatment groups (5.6% to 20.5%, not dose-dependent) when compared to controls.

Ophthalmology: No treatment-related ophthalmologic changes were observed in any group.

Electrocardiography: No treatment-related electrocardiographic changes were observed in any group.

Hematology: No significant changes in hematology parameters were observed in the treatment group animals at the end of the study period compared to control or pretreatment values.

Clinical chemistry: No significant clinical chemistry changes were observed in any group at the end of the treatment period.

Urinalysis: Increased urine volume (18% and 20% at mid and high doses, respectively) and decreased specific gravity were observed in females in week-13; males also had slightly increased urine volume at this time. At Week 26, males had increased urine volume at all doses.

Gross pathology: White granular content in the gastrointestinal tract was observed in one male animal receiving the 50 mg/kg/day dose and one male and one female receiving the 125 mg/kg/day dose.

Organ weights: Treatment group males had decreased adrenal (18% at mid and high doses), testes (17.4% at high dose), thymus (8.4% at high dose) and thyroid (13.5% at high dose) weights. Females had increased thymus weight (45% at high dose). At the end of the recovery period, high dose males had lower epididymis (19.4%), thymus (43%), adrenal (22.7%) and prostate gland (66%) weights, and females had increased uterus (179%) weight.

Histopathology: High dose females had higher incidences of lymphoid aggregations in the gall bladder (3/4, compared to 1/4 in control and other dose groups). Splenic congestion/hemorrhage was observed in males receiving _____ (1/4, 3/4 and 1/4 at low, mid and high doses, respectively; none in control). Slightly higher incidences

b(4)

of vascular degenerative changes in the urinary bladder was observed in mid and high dose males (1/4, 0/4, 2/4 and 2/4 in control, low, mid and high doses, respectively).

Thus, _____ administered at oral doses of 50, 125 and 250 mg/kg/day (equivalent to 200, 500 and 1000 mg/kg/day, respectively, in terms of test item as supplied) for 26 weeks, was well-tolerated in dogs. Decreased body weights (about 10%) was observed in animals treated with the 250 mg/kg/day dose. The gall bladder, spleen and the urinary bladder were the target organs of toxicity, and the 250 mg/kg/day dose was the tolerated dose. The sponsor did not examine sufficiently high doses of Eudrgit NE 40D in this study.

b(4)

Toxicokinetics: Not conducted.

2.6.6.8 Discussion and Conclusions

In toxicology studies with mesalamine, kidney and the GI tract were the target organs of toxicity. In acute toxicity studies, oral doses of 800 and 1800 mg/kg were lethal in mice and rats, respectively, and the target organs were the kidney and GI tract. Repeat dose toxicology studies in rats and dogs (13-week and 26-week oral toxicity studies in rats and 26-week and 52-week oral toxicity studies in dogs), have shown the kidney to be the major target organ of toxicity. Oral doses of 40 mg/kg/day produced minimal to slight tubular injury and doses of 160 mg/kg/day or higher in rats produced renal lesions including tubular degeneration, tubular mineralization, and papillary necrosis. Oral doses of 60 mg/kg/day or higher in dogs also produced renal lesions including tubular atrophy, interstitial cell infiltration, chronic nephritis, and papillary necrosis. Lesions in the kidneys and the GI tract were also observed in mice and rats in 2-year carcinogenicity studies. Thus, encapsulated mesalamine granules have the potential for causing adverse effects on the kidneys of patients taking the drug.

2.6.6.10 Tables and Figures

2.6.7 TOXICOLOGY TABULATED SUMMARY

N/A

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Oral mesalamine (5-aminosalicylic acid; 5-ASA) formulations have been used as the treatment for the induction and maintenance of remission of ulcerative colitis for many years. The sponsor submitted NDA 22-301 for encapsulated mesalamine granules pursuant to Section 505(b)(2) of the Federal Food, Drug and Cosmetics Act. A reference was made to the toxicology data for mesalamine submitted under NDA 19-651 (Procter and Gamble Pharmaceuticals, Asacol) and NDA 21-252 (Axcan Scandipharm Inc., Canasa). Original study reports of the toxicology studies were also submitted. In

addition, a 4-week oral toxicity study and a 26-week oral toxicity study in dogs with _____ were submitted under the NDA.

b(4)

Acute oral and intravenous toxicity studies with mesalamine were conducted in rats and mice. The clinical signs following oral and intravenous treatment in both rats and mice were similar, and included sedation, dyspnea, weight loss, coma (mice only) and ventral/curved body position. The kidney and the GI tract were the target organs of toxicity.

Repeat dose toxicity studies were conducted in rats (including a 13-week and a 26-week oral gavage and a 52-week dietary admixture) and dogs (including a 26-week and a 52-week oral toxicity study). The kidney was the target organ of toxicity in all studies in both rats and dogs. In the rat, a dose of 640 mg/kg (13 weeks) resulted in a number of deaths attributed to renal failure, urinary changes (polyurea, proteinuria and hematuria) and marked increases in serum LDH levels. Kidney weights were significantly increased and the histopathological changes comprised of renal lesions involving the papillary and cortical regions and characterized by areas of slight to extremely severe necrosis. A moderate papillary necrosis was also observed in male rats from the 13-week study at a lower dose of 160 mg/kg. In the 26-week rat study, papillary necrosis was observed at a dose of 320 mg/kg. Other renal changes observed in the 26-week rat study at a dose of 360 mg/kg included tubular degeneration of the kidney, tubular mineralization and urothelial hyperplasia. Increases in blood urea nitrogen, creatinine and globulin levels were observed at 360 mg/kg females in the same study. Papillary edema was observed in female rats at 170 mg/kg in the 26-week study, and minimal to slight tubular injury was observed in males receiving the drug for 13 weeks. In the 52-week oral toxicity study in rats, hyalinization of the tubular basement membrane and Bowman's capsule was observed at 100 and 320 mg/kg doses. Other treatment-related findings observed in rats included hemorrhagic changes in the gastric mucosa at 640 mg/kg (13-week study), mucosal/submucosal fibrosis of the stomach and inflammation of the urinary bladder at 360 mg/kg (26-week study), and ulceration of the stomach at 320 mg/kg (52-week study). In dogs, polyurea and an increased urinary excretion of gamma-glutamyltransferase and lactate dehydrogenase was observed at 120 mg/kg in the 26-week toxicity study. Papillary necrosis was observed at 60 mg/kg and higher doses in all three chronic toxicity studies in dogs. Chronic nephritis was observed in the 52-week oral toxicity study in dogs. Adverse effects on the kidney and GI tract were also observed in mice and rats in the 2-year dietary carcinogenicity studies.

The genotoxic potential of mesalamine was examined in the Ames test, the *in vivo* mouse micronucleus assay, and the *in vivo* sister chromatid exchange assay in Chinese hamster bone marrow cells. Mesalamine was not genotoxic in any of the genotoxicity assays.

Carcinogenicity studies with mesalamine were conducted in rats and mice following dietary administration of the drug for 2 years. Mesalamine was not carcinogenic in rats at doses up to 480 mg/kg/day, or in mice at doses up to 2000 mg/kg/day.

Mesalamine had no effect on fertility or reproductive performance of male and female rats at oral doses up to 480 mg/kg/day. It was not teratogenic in rats and rabbits at oral doses up to 480 mg/kg/day. In the Segment III pre- and post- natal developmental study in rats, oral mesalamine had no effects on the pre- and post- natal development at doses up to 320 mg/kg/day.

Mesalamine was minimally to slight irritant, when applied directly to the eye of rabbits. In a rectal mucosal irritability study in dogs, intrarectally administered mesalamine caused higher incidences and severity of mucoid proctitis and edema of the lamina propria in treated animals as compared to controls. 5-ASA had no skin sensitizing potential in the delayed hypersensitivity test in the guinea pig following intracutaneous injections.

Conclusions: Oral mesalamine formulations have been used for the treatment and maintenance of remission of ulcerative colitis for many years. The pharmacological and toxicological properties of mesalamine have been studied extensively in nonclinical studies. Toxicology studies in animals have identified the kidney and the GI tract as major target organs of toxicity.

Unresolved toxicology issues (if any): None

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

Suggested labeling: See the 'Recommendations on Labeling' under the Executive Summary section of the review.

Signatures (optional):

Reviewer Signature _____

Supervisor Signature _____ Concurrence Yes ___ No ___

APPENDIX/ATTACHMENTS: N/A

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/s/

Sushanta Chakder
9/25/2008 04:52:00 PM
PHARMACOLOGIST