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APPLICATION NUMBER: 019651/S005

PHARMACOLOGY REVIEW(S)

Pages: 1 through 25

McNeil

PHARMACOLOGIST'S REVIEW OF NDA 19,651
(Supplement SE1-005 dated June 4, 1996)

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APR 21 '97

Sponsor & Address: Procter & Gamble Pharmaceuticals
Cincinnati, OH

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Amendment - July 15, 1996
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Date of Review: April 11, 1997

DRUG: Asacol (Mesalamine) delayed-release tablets.

CATEGORY: Anti-ulcerative colitis agent.

Submission Contents: **(1)** 13-week oral (dietary) dose ranging study in mice, **(2)** 3-month oral (dietary) dose ranging study in mice, **(3)** 3-month oral (dietary) dose ranging study in rats, **(4)** 6-month oral toxicity study in rats, **(5)** 12-month oral toxicity study in dogs, **(6)** 2-year oral (dietary) carcinogenicity study in mice, **(7)** 2-year oral (dietary) carcinogenicity study in rats and **(8)** mutagenicity studies (Ames test, sister-chromatid exchanges (SCE) test in Chinese hamster ovary (CHO) cells, *in vitro* chromosomal aberration tests in CHO cells and human lymphocytes and *in vivo* mouse bone marrow micronucleus test).

The 13-week and 3-month oral (dietary) dose ranging studies in mice Amendments dated June 17, 1993 and June 27, 1994 and reviewed on July 7, 1993 and October 7, 1994, respectively. The pharmacology reviews of these studies are reproduced below.

13-Week Dietary Dose-Ranging Study in Mice (Report # 862.09.00-AC)

Testing Laboratories: Drug Safety Assessment
Procter & Gamble Pharmaceuticals, Inc.
Norwich, NY

Study Started: August 28, 1992

Study Completed: January 8 1993

GLP Requirements: A statement of compliance with the GLP regulations and quality assurance unit was included.

Animals: Male and female CD-1 (Crl:CD-1 (ICR)BR VAF) mice (6-7 weeks old)

Drug Batch No.: 40646/90

Methods: Groups of 15 males and 15 females were given 5-ASA (via diet) at daily doses of 0, 100, 500/2000 (dose was raised to 2000 on day 21 of the study due to lack of observed toxic effects), 1000/3000 (dose was raised to 3000 on day 56 of the study due to lack of observed toxic effects) and 1500 for 13 weeks. Additional animals (only males) were also included in each group for pharmacokinetic study and 2-4 mice/time point/group were used to determine the plasma levels of the drug and its metabolite (acetyl-5-ASA) on days 1, 27, 28 and 84 of the study. All animals were observed once daily for clinical signs and mortality. Body weights and food consumptions were recorded weekly. On day 29 and just before sacrifice blood samples were collected from the orbital sinus and/or by cardiac puncture from 5 mice/sex/group for hematological and serum chemistry tests. Plasma levels of 5-ASA and its metabolite Acetyl-5-ASA were also monitored just before sacrificed. At the end of the study period all animals were sacrificed, and subjected to complete gross and histopathological examinations (except the animals used for monitoring drug levels on days 1, 27, 28 and 84 of the study).

Results:

1. Achieved Doses: The mean intakes of Asacol were within 10% of the intended doses (100 mg/kg/day: mean = 97 mg/kg/day for males and 108 mg/kg/day for females; 1500 mg/kg/day: mean = 1440 mg/kg/day for males and 1502 mg/kg/day for females; 500/2000 mg/kg/day: mean = 467/1866 mg/kg/day for males and 476/2094 mg/kg/day for females; 1000/3000 mg/kg/day: mean = 995/2701 mg/kg/day for males and 1026/2771 mg/kg/day for females). At the highest tested dose (3000 mg/kg/day) about 1.6% of the feed contained the active ingredient (5-ASA).

1. Observed Effects: No treatment related clinical signs were evident in any treated animals.

2. Mortality: None.

3. Body Weight/Food Consumption/Water Consumption: In treated males the body weight gains were reduced by 4%, 3%, 19% and 28% at 100, 1500, 500/2000 and 1000/3000 mg/kg/day respectively. In treated females body weight gains were reduced by 7%, 45% and 18% at 100, 1500 and 500/2000 respectively while body weight gains were increased by 11% in mice treated with 1000/3000 mg/kg/day. Effect on body weight gains were highly variable in females. Food consumptions were not affected by the treatment.

4. Hematology/Coagulation/Bone Marrow: No treatment related effects were seen.

5. Blood Chemistry/Urinalysis: No treatment related effects were seen. Urinalysis were not performed.

6. Organ Weights: At the end of the study period, testis weights were increased by in all treated males.

7. Gross Pathology: No treatment related effects were seen.

8. Histopathology: Multifocal/diffuse mucosal/submucosal subacute/ chronic inflammation of the urinary bladder treated mice (males: control =0/10, 100 mg/kg/day = 1/10, 1500 mg/kg/day = 0/10, 500/2000 mg/kg/day = 1/10 and 1000/3000 mg/kg/day = 2/10; females: control =0/10, 100 mg/kg/day = 0/10, 1500 mg/kg/day = 1/10, 500/2000 mg/kg/day = 2/10 and 1000/3000 mg/kg/day = 2/10). Severity of inflammation was marked at high dose.

9. Plasma Level of the Drug: Levels of drug and it metabolites in plasma samples were highly variable. Nevertheless dose dependent increases in mean plasma levels of 5-ASA were seen in treated mice but increase in mean plasma levels of N-AC-5-ASA was not dose proportional. Thus indicating metabolism saturation at higher dose levels i.e. greater than 1500 mg/kg/day. On day 84 the mean plasma levels (AUC 0-24 hr) of 5-ASA were 1.08 (artificially low value due to the assay sensitivity), 351.57, 496.26 and 892.5 mcg/mlxhr and the mean plasma levels (AUC 0-24 hr) of AC-5-ASA were 56.82, 431.52, 660.42 and 475.71 at 100, 1500, 2000 and 3000 mg/kg/day respectively. The exposure of 5-ASA in mice at 2000 and 3000 mg/kg/day will be about 50 and 89 times higher than that seen in human (according to sponsor, 800 mg Asacol b.i.d.= 32 mg/kg/day assuming 50 kg body weight; the AUC 0-24 hr values: 10 mcg/mlxhr for 5-ASA and 22 mcg/mlxhr for AC-5-ASA) respectively.

The data indicates that urinary bladder is the target organ of toxicity and the no effect dose was not established in this study. At 500/2000 mg/kg/day, the body weight gain were reduced by 18-19% in mice of both sexes when compared to the control values. Minimal inflammatory changes in the urine bladder were seen at 500/2000 mg/kg/day in mice of both sexes. Additionally, saturation of conversion of 5-ASA to Ac-5-ASA is indicated at 2000 mg/kg/day dose level and the exposure of 5-ASA in mice at 2000 mg/kg/day will be about 50 higher than that seen in human. Therefore 2000 mg/kg/day can be considered as MTD (animals received 500 mg/kg/day for the first 3 weeks then 2000 mg/kg/day for the remaining 10 weeks).

3-Month Dietary Dose-Ranging Study in Mice (Report # 862.09.00-CC)

Earlier sponsor submitted the result of 13-week dietary dose range study in CD-1 mice in which doses of 0, 100, 500/2000 (dose was raised to 2000 on day 21 of the study due to lack of observed toxic effects), 1000/3000 (dose was raised to 3000 on day 56 of the study due to lack of observed toxic effects) and 1500 were used. The data indicated that 2000 mg/kg/day is the MTD (for detail see review of Amendment dated 6/17/93; date of review: 7/7/93). To confirm the above findings and to better define the MTD, sponsor conducted another 3-month dietary dose range study in CD-1 mice, results of which is being submitted in the present submission.

Testing Laboratories: Drug Safety Assessment
Procter & Gamble Pharmaceuticals, Inc.
Norwich, NY

Study Started: March 18, 1993

Study Completed: February 2, 1994

GLP Requirements: A statement of compliance with the GLP regulations and quality assurance unit was included.

Animals: Male and female CD-1 (Crl:CD-1 (ICR)BR VAF) Swiss mice (35 days old)

Drug Batch No.: 40646/90

Methods: Groups of 10 males and 10 females were given 5-ASA (via diet) at daily doses of 0, ,2000, 4000, 6000, 8000 and 10000 mg/kg/day for 3 months. Additional animals (only males: 2 in the control group and 16/treatment group) were also included in each group for pharmacokinetic study. At the end of treatment period, 4 mice/time point/treatment group were used to determine the plasma levels of the drug and its metabolite (acetyl-5-ASA). The time points were 0, 6,12 and 24 hr after the last dose. On the day of bleeding 2 control animals were bled at time 0. All animals were observed once daily for clinical signs and mortality. Body weights and food consumptions were recorded weekly. At the end of treatment period, just before sacrifice of animals from main groups, blood samples were collected from the orbital sinus and/or by cardiac puncture for hematological and serum chemistry tests. At the end of the study period all animals from main groups were sacrificed, and subjected to complete gross pathological examinations. Only liver, gallbladder, stomach, kidney and urinary bladder from 0, 2000 and 4000 mg/kg/day treated mice were examined microscopically.

Results:

1. **Achieved Doses**: The mean intakes of Asacol were within 10% of the intended doses in the bottom three dose levels (2000 mg/kg/day: mean = 2131 mg/kg/day for males and 2233 mg/kg/day for females; 4000 mg/kg/day: mean = 4123 mg/kg/day for males and 4075 mg/kg/day for females; 6000 mg/kg/day: mean = 5754 mg/kg/day for males and 6201 mg/kg/day for females; 8000 mg/kg/day: mean = 5385 mg/kg/day for males and 7600 mg/kg/day for females; 10000 mg/kg/day: mean = 4585 mg/kg/day for males and 11012 mg/kg/day for females). In the top two dose levels the mean intakes of asacol were significantly reduced in males due to severe decreased food consumptions (see below).

2. **Observed Effects**: No treatment related clinical signs were evident in any mice treated with 2000 mg/kg/day. At 4000 mg/kg/day and higher dose levels, decreased activity, dehydration, ataxia were seen in treated males. Some of the female mice treated with 8000 and 10000 mg/kg/day were moribund.

3. **Mortality**: In the main study, a total of 50 animals (males: 4 in 4000 mg/kg/day group, 7 in 6000 mg/kg/day group, 10 in 8000 mg/kg/day group and 10 in 10000 mg/kg/day group; females: 3 in 6000 mg/kg/day group, 7 in 8000 mg/kg/day group and 9 in 10000 mg/kg/day group) died or killed during the study period. Due to high mortality rates at 6000 mg/kg/day and higher dose levels, remaining mice in these top three dose levels were terminated on day 13 of the study and none of the toxicology parameters were evaluated in these mice. Death occurred after "day 20" of the study in 4000 mg/kg/day group.

3. **Body Weight/Food Consumption/Water Consumption**: In treated males the body weight gains were reduced by 14% and 13% at 2000 and 4000 mg/kg/day respectively, while in treated females body weight gains were increased by 19% and 17% at and 18% at 2000 and 4000 mg/kg/day respectively. Food consumptions were not affected by the treatment in mice treated with up to 4000 mg/kg/day dose levels (during the first 7 days of treatment, food consumptions were decreased by 17%, 45% and 64% in male mice treated with 6000, 8000 and 10000 mg/kg/day respectively).

4. **Hematology/Coagulation/Bone Marrow**: Up to 4000 mg/kg/day, no treatment related effects were seen.

5. **Blood Chemistry/Urinalysis**: At the end of the treatment period, serum aspartate aminotransferase (SGOT) and alanine aminotransferase (SGPT) levels were increased by 45% and 49% respectively in male mice treated with 4000 mg/kg/day. In females, serum glucose levels were increased by 19% and 37% in 2000 and 4000 mg/kg/day treated mice, when compared with the control values.

6. **Organ Weights**: At the end of the study period, liver and testis relative weights were increased by 13% and 18% respectively in male mice treated 4000 mg/kg/day.

7. **Gross Pathology**: Two out of 10 male mice treated with 4000 mg/kg/day had unilateral reduction in kidney size.

8. **Histopathology**: In kidney, at 4000 mg/kg/day, diffuse tubular nephrosis (4/10), multifocal/diffuse tubulo-interstitial inflammation (3/10) and multifocal/diffuse papillary necrosis (5/10) were seen in males. Additionally, increased incidences of chronic inflammation of the urinary bladder were seen in treated female mice (control =2/10, 2000 mg/kg/day = 3/10 and 4000 mg/kg/day = 6/10).

9. **Plasma Level of the Drug**: Data will be reported later.

The data indicate that kidney and urinary bladder are the target organs of toxicity and the no effect dose was not established in this study. Lethality was seen at 4000 mg/kg/day and higher dose levels. The lowest tested dose (2000 mg/kg/day) produced decreased body weight gain in males (14%) and increased body weight gains in females (19%). Additionally, report # 862.09.00-AC indicated that saturation of conversion of 5-ASA to Ac-5-ASA is seen at 2000 mg/kg/day dose level and the exposure of 5-ASA in mice at 2000 mg/kg/day will be about 50 higher than that seen in human (for detail see review of Amendment dated 6/17/93; date of review: 7/7/93). Therefore 2000 mg/kg/day can be considered as MTD. Sponsor has select 2000 mg/kg/day as the highest dose for the planned 24-month carcinogenicity study in mice.

Three Month Oral (dietary) Dose Ranging Study of 5-ASA in Rats (862.09.00-AG)

Testing Laboratories: Sponsor's laboratory

Study Start and Completion Dates: June 29, 1994 and June 6, 1995

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

Animals: Males (206-211 g, 49 days)
Females (165-169 g, 49 days)
Sprague Dawley Crl:CD BR VAF rats

Methods: To determine the dose levels for the two year carcinogenicity study in rats, sponsor conducted a 3-month oral dose (dietary) ranging study in rats (5/sex/group). The intended doses in feed of asacol (5-ASA) were 0, 480, 600, 720, 840, 960 and 1080 mg/kg/day for 3 months. On day 35, the doses of 720 and 960 mg/kg/day were increased to 1500 and 2000 mg/kg/day, respectively due to the absence of significant toxicity. The actual achieved doses were summarized in a table on page 9 in volume 6. This table is attached below.

Treatment Group	Male Day 0-35	Female Day 0-35	Male Day 35-90	Female Day 35-90	Male Day 0-90	Female Day 0-90
T1	0.0	0.0	0.0	0.0	0.0	0.0
T2	472.7	502.4	383.6	421.2	418.1	452.8
T3	614.3	624.6	472.9	535.9	527.8	570.5
T4	737.6	806.9	1205.7	1330.0	1023.4	1127.1
T5	904.1	893.3	680.6	740.7	767.8	800.4
T6	988.3	1055.3	1621.7	1767.2	1375.6	1491.1
T7	1100.2	1229.3	848.7	953.3	946.7	1061.5

Clinical signs of toxicity and mortality were observed daily. Body weights and food consumption were determined weekly. Plasma level of the test drug was determined on days 28, 63 and 84 at ~9:00 am each day. Hematology, clinical chemistry and urinalysis were not determined. All animals were necropsied at termination and the organs were weighed. Histopathological examination was also conducted.

Results:

1. Clinical Signs: There were no treatment related clinical signs of toxicity.

2. Mortality: There were no deaths.

3. Body Weight: There were no treatment related changes. The initial and final body weights in the control group were 209.2 ± 6.3 g and 501 ± 17.1 g for males or 166.6 ± 3.8 g and 285.6 ± 3.9 g for females.

4. Food Consumption: The food consumptions were slightly but significantly increased in the high dose group throughout the study as compared to the control. The average food consumption in the control group was 23-26.8 g/animal/day (males) or 18.6-20.4 g/animal/day (females).

5. Organ Weights: There were no treatment related changes observed during the study.

6. Gross Pathology: There were no treatment related changes observed during the study.

7. Histopathology: The treatment related inflammations were observed in the stomach and kidney. In stomach, there were submucosal and/or mucosal infiltrates of inflammatory cells (focal/multifocal/diffuse), which occasionally extended into the muscular coat of the stomach. The cortical inflammation (focal/multifocal) consisted of aggregates of mononuclear inflammatory cells in the interstitium of the cortex. The peripelvic inflammation was the mixed inflammatory infiltrates subjacent to the partial urothelium of the renal pelvis and the inflammatory cells frequently migrated through the urothelium toward the pelvis. The incidence of these changes were summarized in tables on page 10 in volume 6. These tables are attached below.

Incidence of Gastric Inflammation

	Males							Females						
Dose Group	T1	T2	T3	T4	T5	T6	T7	T1	T2	T3	T4	T5	T6	T7
Dose (mg/kg/day)	0	480	600	1500	840	2000	1080	0	480	600	1500	840	2000	1080
Stomach (n)	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Submucosal inflammation	0	0	0	2	2	1	2	0	0	0	0	0	1	0

Doses shown are the targeted doses at study termination.

Incidence of Selected Renal Lesions

	Males							Females						
Dose Group	T1	T2	T3	T4	T5	T6	T7	T1	T2	T3	T4	T5	T6	T7
Dose (mg/kg/day)	0	480	600	1500	840	2000	1080	0	480	600	1500	840	2000	1080
Kidneys (n)	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Cortical inflammation	1	0	1	0	1	2	4	0	1	0	0	0	0	1
Peripelvic inflammation	0	0	1	0	1	1	3	0	1	1	1	0	2	1

Doses shown are the targeted doses at study termination.

In groups T4 and T6, the initial doses of 720 and 960 mg/kg/day were increased to 1500 and 2000 mg/kg/day on day 35, respectively.

8. 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 females. The plasma concentrations measured on days 62 and 84 were comparable to those on day 28.

These results are summarized in the following table.

Plasma concentration of 5-ASA
(Single sample collected at 9:00 am)

Day 28		Day 62		Day 84	
Dose, mg/kg	Plasma level µg/ml	Dose, mg/kg	Plasma level µg/ml	Dose, mg/kg	Plasma level µg/ml
461	3.63	376	3.27	415	6.45
578	1.63	463	3.31	517	4.81
714	8.54	708	7.95	664	8.66
854	7.61	878	9.62	851	12.44
952	11.59	1187	18.98	1208	24.03
1094	9.83	1600	28.01	1647	37.71
Plasma concentration of AC-5-ASA					
461	9.18	376	7.45	415	11.97
578	4.17	463	8.13	517	10.92
714	12.34	708	12.80	664	13.77
854	13.81	878	11.40	851	14.75
952	14.25	1187	14.73	1208	16.80
1094	14.17	1600	16.03	1647	20.03

In summary, in the 3-month dietary dose ranging study in rats, the major treatment related changes were the gastric and renal inflammation at doses of 840 mg/kg or higher. The target organs of toxicity were the stomach and kidney. Sponsor stated that considering the expected exacerbation of renal effects over the 2-year duration of a carcinogenicity study, the dose of 480 mg/kg/day would be an appropriate choice for the high-dose in the carcinogenicity study.

Six Month Oral Toxicity Study of 5-ASA in Rats
(862.09.00-CE)

Study Start and Completion Dates: June 24, 1993 and February 2, 1996.

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

Animals: Males (213 g, 42 days)
Females (168 g, 42 days)
Sprague Dawley Crl:CD BR rats

Methods: To determine the toxicity of 5-ASA in rats, rats (20/sex/group) were treated with 5-ASA by oral gavage at 0, 80, 170 and 360 mg/kg/day for 6 months. Clinical signs of toxicity and mortality were observed daily. Body weights were determined weekly. Food consumption was determined weekly for the first 13 weeks and monthly thereafter. Plasma levels of the test drug and its metabolites were determined on days 1 and 30 and at termination. Hematology, clinical chemistry and urinalysis were determined at month 3 and termination. All animals were necropsied at termination and the organs were weighed. Histopathological examination was also conducted.

Results:

1. Clinical Signs: The clinical signs of toxicity were mainly in the sacrificed or dead animals and these included ano-genital staining, lethargy, paleness, emaciation and labored breathing.

2. Mortality: In the main study, nine rats died and one was sacrificed in a moribund condition. Oral gavage error was considered as the cause of death for 8 of the nine dead rats. The acute renal failure was responsible for the other death in high dose group (female). Hemorrhagic inflammation of the brain was the cause of the sacrificed female rat (low dose) in a moribund condition. Since this was a single incidence in the low dose group, it may not be directly treatment related. Among the three deaths in the satellite animals, the cause of deaths was not clear for two rats in the high dose group and oral gavage error was responsible for the third death.

3. Body Weight: There were no treatment related changes. Mean body weights were summarized in Fig. 1 on pages 51 and 52 of volume 7. These figures are attached below.

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Figure 1
A Subchronic (6-Month) Oral Toxicity Study
of 5-ASA in the Rat via Oral Gavage Administration

Group Mean Body Weights - Males

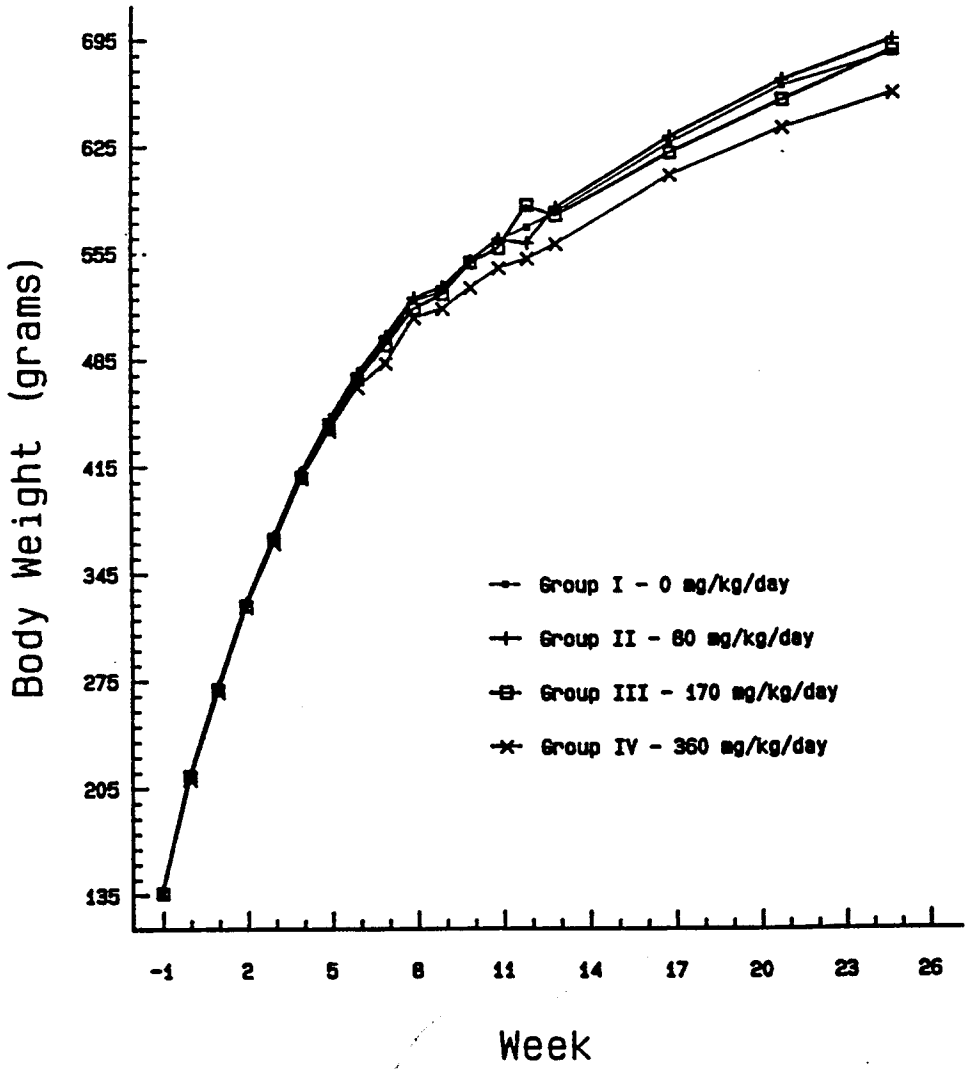

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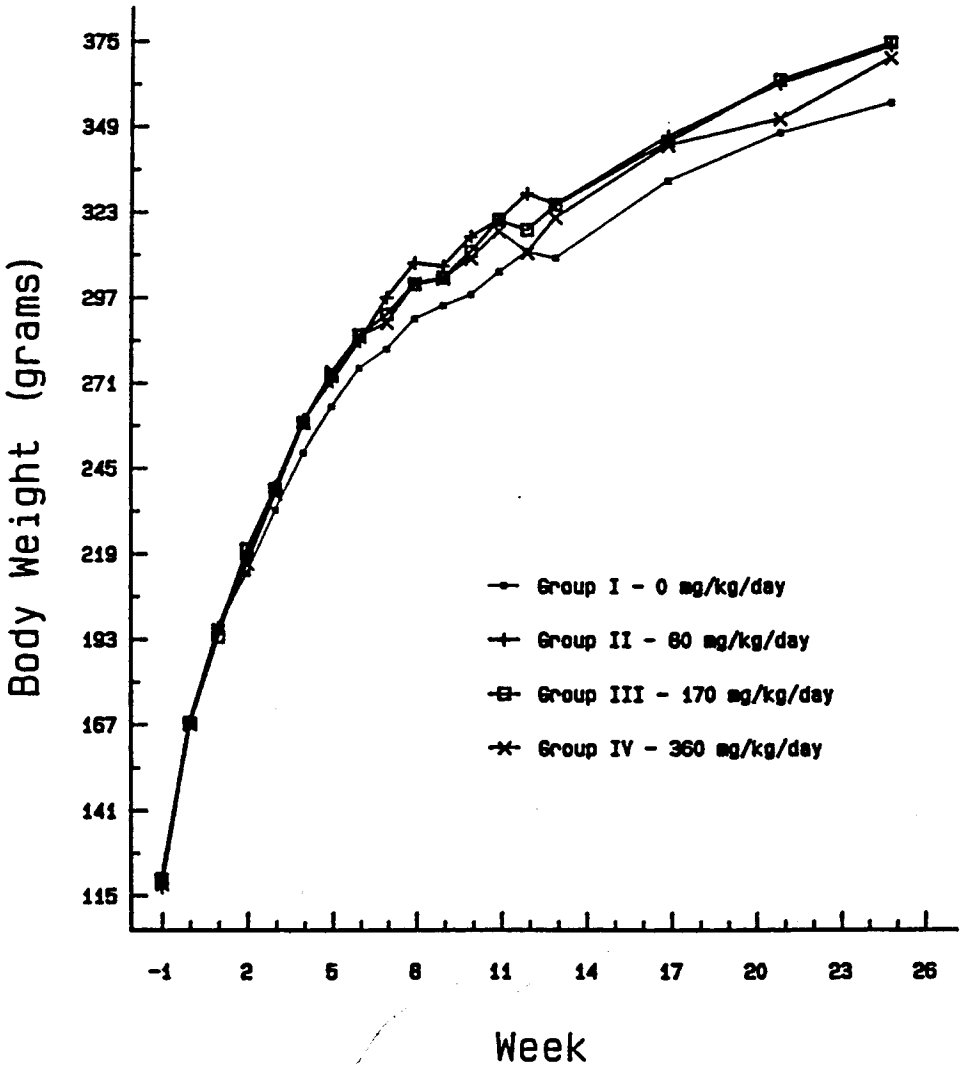
Figure 1 (cont.)
A Subchronic (6-Month) Oral Toxicity Study
of 5-ASA in the Rat via Oral Gavage Administration

Group Mean Body Weights - Females



The initial and final body weights in the control group were 213.6 ± 15 g and 685.5 ± 66.4 g for males or 167.3 ± 10.4 g and 356.6 ± 29.3 g for females.

4. Food Consumption: The food consumptions were slightly but significantly increased mainly in the mid and high dose groups (~5-19% compared to control). The average food consumption in the control group was 35 g/animal/day for males or 21 g/animal/day for females.

5. Ophthalmoscopic examination: There were no treatment related changes.

6. Hematology: There were no treatment related changes at three months after dosing. At termination, slight but significant decrease in hemoglobin (6.5%) and mean corpuscular hemoglobin concentration (2%) was observed in the high dose group (males). A slight but significant increase (18.6%) in the platelet count was also seen in high dose males.

7. Clinical chemistry: At three months after dosing, a significant increase in the blood urea nitrogen (63%), creatinine (40%) and globulin (17%) was seen in the high dose females. These changes were not seen at termination.

8. Urinalysis: There were decreases in urine concentrations of sodium potassium , chloride and calcium) seen mainly in the high dose males and females at three months after dosing and at termination. It appeared that these changes were more severe in females than in males.

9. Organ Weights: The absolute kidney weight , kidney to body weight and kidney to brain weight were significantly increased in the high dose males and females. The change was more severe in the females than in the males. The absolute spleen weight (29%), spleen to body weight (26%) and spleen to brain weight (30%) were also increased in the high dose females (not males).

10. Gross Pathology: Fluid in the lung and/or trachea was found in six animals found dead. Epicardial nodules, thickened pericardium and adhesions of the lung to the thoracic wall were observed in a female that was found dead. There were no other treatment related changes observed during the study.

11. Histopathology: The treatment related renal lesion was observed in the mid and high dose males and females. The major changes seen in the high dose group were necrosis of the renal papilla (10/20 males, 8/20 female) and tubular degeneration of kidney (17/20 males, 10/20 females). Necrosis of the renal papilla consisted of coagulation necrosis or loss of the tip of the papilla sometime associated with foci of mineralization and edema in the interstitium of the remaining papilla. Tubular degeneration consisted of dilated tubules with attenuated epithelium in the

cortex and medulla. Papillary edema without necrosis was seen in 4/20 males and 11/20 females in the high dose group and 4/20 females in the mid dose group. In addition, tubular mineralization (8/20 males and 10/20 females) and urothelial hyperplasia (3/20 males and 4/20 females) were seen in the high dose group. Mucosal/submucosal fibrosis of the stomach and inflammation of the urinary bladder were also observed in the high dose group. The incidence of these findings was summarized in a table on page 87 in volume 9. This table is attached below.

Incidence of Drug-Related Histomorphologic Findings

	Males				Females			
Dose (mg/kg/d)	0	80	170	360	0	80	170	360
Kidney (n)	20	20	20	20	20	20	20	20
Papillary necrosis	-	-	-	10	-	-	-	8
Papillary edema	-	-	4	4	-	-	4	11
Tubular degeneration	-	-	2	17	-	-	1	10
Tubular mineralization	-	-	1	8	3	3	5	10
Urothelial hyperplasia	-	-	2	3	-	1	-	4
Urinary bladder (n)	20	20	20	18	20	18	20	20
Mucosal inflammation	1	1	1	3	-	1	2	2
Stomach (n)	20	20	20	20	20	20	20	20
Mucosal/submucosal fibrosis	-	-	-	1	-	-	-	2

12. 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 two tables on page 46 in volume 7. These table are attached below.

Group (mg/kg)	Plasma Levels of 5-ASA (μg/mL) ± SD		
	Day 1	Day 30	Day 180
	Males		
I (0)	ND	ND	ND
II (80)	15.4 ± 6.14	22.2 ± 7.51	29.0 ± 10.2
III (170)	32.8 ± 3.82	47.0 ± 14.8	70.8 ± 14.6
IV (360)	74.3 ± 20.8	81.7 ± 17.5	91.2 ± 11.4
	Females		
I (0)	ND	ND	ND
II (80)	12.7 ± 4.11	19.4 ± 2.27	24.4 ± 9.90
III (170)	40.6 ± 13.9	55.3 ± 9.69	82.0 ± 19.1
IV (360)	84.8 ± 10.4	96.9 ± 14.5	119 ± 47.3

Group (mg/kg)	Plasma Levels of Ac-5-ASA (μg/mL) ± SD		
	Day 1	Day 30	Day 180
	Males		
I (0)	ND	ND	ND
II (80)	24.8 ± 4.99	26.2 ± 7.47	21.0 ± 3.14
III (170)	30.6 ± 14.5	34.3 ± 12.6	25.6 ± 6.40
IV (360)	26.6 ± 6.51	28.7 ± 7.39	31.3 ± 3.54
	Females		
I (0)	ND	ND	ND
II (80)	23.4 ± 4.47	22.5 ± 6.24	21.9 ± 3.80
III (170)	23.4 ± 5.45	26.6 ± 6.59	27.4 ± 8.00
IV (360)	28.2 ± 7.01	30.1 ± 12.6	32.0 ± 11.9

ND = none detected

In summary, in the 6-month oral toxicity study (oral gavage) the major treatment related changes were the renal lesion seen at the mid and high doses (170 and 360 mg/kg/day). These were evidenced by increase in the blood urea nitrogen and creatinine and kidney weight (absolute and relative to body and brain weights) and histopathological findings. The histopathological changes were papillary edema, necrosis of the renal papilla and tubular degeneration of kidney. The tubular mineralization and urothelial hyperplasia were seen in the high dose males and females. Mucosal/submucosal fibrosis of the stomach and inflammation of the urinary bladder were also observed in the high dose males and females. The low dose (80 mg/kg/day) was no effect dose. The stomach and kidney were the target organs of toxicity.

One Year Oral Toxicity Study of 5-ASA in Dogs
(862.09.00-AI)

Study Start and Completion Dates: July 15, 1994 and March 8, 1996

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

Animals: Males
Females
Beagle dogs

Methods: To determine the toxicity of 5-ASA in dogs, dogs (4/sex/group) were treated with 5-ASA by oral gavage at 40, 80 and 160 mg/kg/day for 12 months. Clinical signs of toxicity and mortality were observed daily. Body weights were determined weekly. Food consumption was determined daily. Ophthalmologic examination and ECG were performed before, 3, 6 and 9 months after the treatment and at termination. Plasma levels of the test drug were determined on days 0, 6 and 30, after 6 months and at termination. Hematology and clinical chemistry were determined before, 1, 3, 6 and 9 months after the treatment and at termination. Urinalysis was determined before, 3, 6 and 9 months after the treatment and at termination. All animals were necropsied at termination and the organs were weighed. Histopathological examination was also conducted.

Results:

1. Clinical Signs: One female in high dose group had decreased activity, dehydration and hypovolemia.

2. Mortality: There were no deaths.

3. Body Weight: There were no treatment related changes.

4. Food Consumption: There were no treatment related changes.

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

6. ECG: There were no treatment related changes.

7. Hematology: There were no treatment related changes.

8. Clinical Chemistry: The major treatment related changes were increases in the blood urea nitrogen and creatinine observed in the mid and high dose groups throughout the treatment period.

9. Urinalysis: The major treatment related change was decrease in the specific gravity in the mid (1.013) and high (1.011) dose groups as compared to the control at termination.

10. Organ Weights: There were no treatment related changes.

11. Gross Pathology: The major treatment related changes were observed in the kidney in 2/4 males in the mid dose group and all males and females in the high dose group. These changes included one or more of the following: depressed or pitted areas on the surfaces of the kidney, pale streaking in the areas of the cortices and medullae and papillary necrosis.

12. Histopathology: The histopathological examination revealed chronic nephritis (in 1/4 mid dose males, 2/4 high dose males, 2/4 mid dose females and 3/4 high dose females). This was often consistent with the gross pathological changes in the mid and high dose groups. The detail description of this lesion was not provided in this submission.

13. Toxicokinetics: There was very little Ac-5-ASA detected at all dose levels and all sampling times. The plasma concentrations of 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 1-5 on pages 10-14 in volume 16. These tables are attached below.

Table 1. Summary of Day 1 Pharmacokinetic Parameters from Dogs given Once-Daily Oral Doses of 5-ASA of 40, 80, or 160 mg/kg for 53 Weeks.

	Sex - Dose (mg/kg/day)					
PK Parameters	Male - 40	Female - 40	Male - 80	Female - 80	Male - 160	Female - 160
$t_{1/2}$ (hr)	1.15 (25.2)[a]	2.09 (57.1)	2.17 (43.1)	1.93 (30.8)	2.45 (7.6)	1.99 (22.5)
T_{max} (hr)	1.0 (69.2)	0.6 (40.0)	0.9 (26.6)	1.3 (40.0)	1.8 (28.8)	2.4 (46.7)
C_{max} (µg/ml)	52.15 (20.0)	58.15 (7.9)	67.44 (28.3)	96.53 (24.5)	128.5 (12.1)	114.3 (24.8)
C_{max}/Dose	1.29 (21.0)	1.45 (7.9)	0.84 (28.3)	1.25 (24.5)	0.80 (12.1)	0.71 (24.8)
AUC ($\mu g \cdot ml^{-1} \cdot hr$)	137.2 (29.3)	167.7 (6.2)	218.2 (18.0)	429.9 (43.8)	707.9 (20.9)	588.9 (14.7)
AUC/Dose	3.43 (29.3)	4.19 (6.2)	2.73 (18.0)	5.37 (43.8)	4.42 (20.9)	3.68 (14.7)
Cl_T/F ($ml \cdot min^{-1} \cdot kg^{-1}$)	5.15 (25.6)	3.99 (6.0)	6.25 (16.4)	3.64 (45.1)	3.88 (19.2)	4.62 (17.1)
V_β/F(ml)	488 (9.3)	734 (61.3)	1190 (46.3)	543 (14.6)	839 (20.6)	808 (34.6)

[a] Values are mean (%coefficient of variation); n = 4.

Table 2. Summary of Day 6 Pharmacokinetic Parameters from Dogs given Once-Daily Oral Doses of 5-ASA of 40, 80, or 160 mg/kg for 53 Weeks.

	Sex - Dose (mg/kg/day)					
PK Parameters	Male - 40	Female - 40	Male - 80	Female - 80	Male - 160	Female - 160
$t_{1/2}$ (hr)	1.54 (25.0)[a]	1.21 (35.2)	1.90 (32.2)	2.62 (36.1)	2.11 (42.1)	1.90 (14.9)
T_{max} (hr)	0.6 (40.0)	1.0 (70.7)	1.4 (45.8)	1.0 (40.8)	1.3 (40.0)	1.8 (16.5)
C_{max} (µg/ml)	42.97 (49.0)	56.60 (35.0)	78.91 (19.3)	82.62 (23.8)	116.5 (30.7)	104.1 (18.5)
C_{max}/Dose	1.07 (49.0)	1.41 (35.0)	0.99 (19.3)	1.03 (23.8)	0.73 (30.7)	0.65 (18.5)
AUC ($\mu g \cdot ml^{-1} \cdot hr$)	128.1 (46.3)	164.0 (51.9)	297.3 (26.6)	327.9 (15.2)	536.8 (52.7)	457.6 (23.7)
AUC/Dose	3.20 (46.3)	4.10 (51.9)	3.72 (26.6)	4.10 (15.2)	3.36 (52.7)	2.86 (23.7)
Cl_T/F ($ml \cdot min^{-1} \cdot kg^{-1}$)	5.88 (34.0)	4.76 (39.3)	4.99 (34.5)	4.15 (17.1)	5.78 (35.8)	6.13 (27.8)
V_β/F(ml)	750 (29.5)	535 (59.4)	778 (43.1)	940 (39.5)	1040 (63.0)	981 (14.9)

[a] Values are mean (%coefficient of variation); n = 4.

Table 3. Summary of Day 29 Pharmacokinetic Parameters from Dogs given Once-Daily Oral Doses of 5-ASA of 40, 80, or 160 mg/kg for 53 Weeks.

	Sex - Dose (mg/kg/day)					
PK Parameters	Male - 40	Female - 40	Male - 80	Female - 80	Male - 160	Female - 160
$t_{1/2}$ (hr)	1.41 (16.1)[a]	2.25 (49.1)	1.69 (15.6)	3.31 (70.9)	2.02 (32.5)	2.52 (20.5)
T_{max} (hr)	0.9 (54.7)	1.0 (40.8)	1.0 (40.8)	1.4 (18.2)	1.5 (27.2)	1.6 (29.5)
C_{max} (µg/ml)	47.83 (39.1)	41.73 (19.5)	78.45 (30.1)	69.91 (21.6)	109.6 (26.2)	107.1 (23.1)
C_{max}/Dose	1.20 (39.1)	1.04 (19.5)	0.98 (30.1)	0.87 (21.6)	0.68 (26.2)	0.67 (23.1)
AUC ($\mu g \cdot ml^{-1} \cdot hr$)	132.6 (42.9)	153.3 (19.3)	246.7 (18.8)	272.9 (26.0)	459.4 (24.3)	511.6 (24.6)
AUC/Dose	3.32 (42.9)	3.83 (19.3)	3.08 (18.8)	3.41 (26.0)	2.87 (24.3)	3.20 (24.6)
Cl_T/F ($ml \cdot min^{-1} \cdot kg^{-1}$)	5.62 (33.3)	4.47 (19.2)	5.55 (19.0)	5.14 (25.8)	6.03 (20.2)	5.45 (24.1)
V_β/F(ml)	678 (37.1)	867 (46.8)	800 (15.4)	1320 (51.7)	1010 (23.0)	1150 (8.6)

[a] Values are mean (%coefficient of variation); n = 4.

Table 4. Summary of Day 189 Pharmacokinetic Parameters from Dogs given Once-Daily Oral Doses of 5-ASA of 40, 80, or 160 mg/kg for 53 Weeks.

	Sex - Dose (mg/kg/day)					
PK Parameters	Male - 40	Female - 40	Male - 80	Female - 80	Male - 160	Female - 160
$t_{1/2}$ (hr)	2.95 (48.2)[a]	1.50 (8.0)[b]	3.16 (36.8)	4.60 (64.5)	3.38 (16.3)	2.89 (43.1)
T_{max} (hr)	1.3 (23.1)	1.5 (0)[b]	1.6 (29.5)	1.1 (22.2)	1.4 (34.8)	1.3 (51.6)
C_{max} (µg/ml)	60.90 (16.6)	65.95 (5.9)[b]	99.34 (15.0)	82.15 (18.6)	116.6 (12.6)	100.6 (29.0)
C_{max}/Dose	1.52 (16.6)	1.65 (5.9)[b]	1.24 (15.0)	1.03 (18.6)	0.73 (12.6)	0.63 (29.0)
AUC ($\mu g \cdot ml^{-1} \cdot hr$)	177.3 (16.2)	192.6 (12.6)[b]	353.6 (13.1)	308.2 (24.7)	479.8 (17.3)	521.6 (35.1)
AUC/Dose	4.43 (16.2)	4.82 (12.6)[b]	4.42 (13.1)	3.85 (24.7)	3.00 (17.3)	3.26 (35.1)
Cl_T/F ($ml \cdot min^{-1} \cdot kg^{-1}$)	3.83 (15.5)	3.51 (13.5)[b]	3.82 (12.3)	4.52 (22.9)	5.68 (17.5)	5.56 (33.9)
V_β/F(ml)	944 (43.6)	455 (15.8)[b]	1030 (35.9)	1940 (85.7)	1680 (26.9)	1300 (31.0)

[a] Values are mean (%coefficient of variation); n = 4, except where noted.
[b] n = 3.

Table 5. Summary of Day 370 Pharmacokinetic Parameters from Dogs given Once-Daily Oral Doses of 5-ASA of 40, 80, or 160 mg/kg for 53 Weeks.

	Sex - Dose (mg/kg/day)					
PK Parameters	Male - 40	Female - 40	Male - 80	Female - 80	Male - 160	Female - 160
$t_{1/2}$ (hr)	4.28 (83.5)	3.33 (110)	3.07 (46.4)	3.03 (15.3)	2.70 (4.0)	2.48 (22.0)
T_{max} (hr)	0.8 (66.7)	1.1 (22.2)	0.9 (85.7)	1.4 (18.2)	1.4 (34.8)	1.9 (13.3)
C_{max} (µg/ml)	66.30 (36.1)	51.70 (17.2)	103.0 (9.9)	107.7 (38.4)	132.2 (11.0)	104.9 (21.0)
C_{max}/Dose	1.66 (36.1)	1.29 (17.2)	1.29 (9.9)	1.35 (38.4)	0.83 (11.0)	0.66 (21.0)
AUC ($\mu g \cdot ml^{-1} \cdot hr$)	177.5 (31.3)	144.0 (22.8)	360.0 (23.5)	424.2 (43.3)	606.3 (7.1)	461.2 (21.3)
AUC/Dose	4.44 (31.3)	3.60 (22.8)	4.50 (23.5)	5.30 (43.3)	3.79 (7.1)	2.88 (21.3)
Cl_T/F ($ml \cdot min^{-1} \cdot kg^{-1}$)	3.99 (25.0)	4.81 (22.6)	3.84 (21.1)	3.61 (42.8)	4.42 (7.3)	5.97 (19.3)
V_β/F(ml)	827 (113)	535 (28.1)	999 (49.0)	928 (35.3)	1030 (10.6)	1270 (24.1)

[a] Values are mean (%coefficient of variation); n = 4.

In summary, in the 1-year oral toxicity study in dogs, dogs (4/sex/group) were treated with 5-ASA by oral gavage at 0, 40, 80 and 160 mg/kg/day for 12 months. The major treatment related changes were chronic nephritis in the mid and high dose groups. This was evidenced by increases in the blood urea nitrogen and creatinine and gross and histopathological findings. No effect dose was identified at 40 mg/kg/day. The kidney was the target organ of toxicity.

FDA CDER CARCINOGENICITY ASSESSMENT COMMITTEE (CAC/CAC-EC)
RODENT CARCINOGENICITY FACTSHEET

NDA: 19,651 (SE1/005)
CAS #:
DIVISION(s): HFD-180
DRUG NAME(S): Asacol/mesalamine
SPONSOR: Procter & Gamble Pharmaceuticals, Inc.
LABORATORY: Sponsor's lab at Norwich, New York 13815

P/T REVIEWER(s): Ke Zhang
P/T REVIEW DATE: April 11, 1997
CARCINOGENICITY STUDY REPORT DATE: March 8, 1996

THERAPEUTIC CATEGORY: Anti-ulcerative colitis

PHARMACOLOGICAL/CHEMICAL CLASSIFICATION: Antiinflammatory agent

PRIOR FDA DOSE CONCURRENCE (Div./CAC)? (Y/N; Date): No

MUTAGENIC/GENOTOXIC (Y/N/EQUIVOCAL/Na; assay): Negative in Ames test, sister-chromatid exchanges (SCE) test in Chinese hamster ovary (CHO) cells, *in vitro* chromosomal aberration tests in CHO cells and human lymphocytes and *in vivo* mouse bone marrow micronucleus test.

MOUSE CARCINOGENICITY STUDY (multiple studies? Std1, Std2 etc):

MOUSE STUDY DURATION (weeks): 104
STUDY STARTING DATE: June 3, 1993
STUDY ENDING DATE: March 8, 1996
MOUSE STRAIN: Crl:CD-1(ICR) BR VAF Swiss mice
ROUTE: Diet
DOSING COMMENTS:

No. Mice in control (C1): 50
Low Dose (LD): 50
High Dose (HD): 50
Control2 (C2):
Middle Dose (MD): 50
High Dose2 (HD2):

Mouse Dose Levels: (mg/kg/day)

Mouse Low Dose: 200
Mouse High Dose: 2000
Mouse Middle Dose: 1000
Mouse High Dose2:

Basis for doses selected (MTD, AUC ratio, saturation, maximum feasible): MTD

MOUSE CARCINOGENICITY (negative, positive, MF, M, F): Negative (MF)

MOUSE TUMOR FINDINGS: No

MOUSE STUDY COMMENTS: 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 high dose of 2000 mg/kg/day was MTD based on the findings in the 13 week and 3-month dietary dose ranging studies in mice. Therefore, the dose selection was adequate. 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. 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. The high dose (2000 mg/kg/day) is ~5.1 folds higher than the maximum recommended human maintenance dose (1.6 g/day, 32 mg/kg/day if 50 kg body weight assumed or 1184 mg/m^2/day) based on body surface area. In conclusion, asacol was not carcinogenic in this 2-year carcinogenicity study in mice.

COVERSHEET FOR CARCINOGENICITY STUDY IN MICE

1. No. Of Studies: One

2. Name of Laboratory: Procter & Gamble Pharmaceuticals at Norwich, New York 13815

3. Strain: Crl:CD-1(ICR) BR VAF Swiss mice

4. No/sex/group: 50

5. Doses (0, L, M, H): 0, 200, 1000 and 2000 mg/kg/day

6. Basis for Dose Selection Stated: Yes

7. Interim Sacrifice: No

8. Total Duration (weeks): 104

9. Week/Site for First Tumor:

Group	Male	Female
0	week 28/lymphoma, multiple organs	week 22/lymphoma, multiple organs
L	week 56/histiocytic sarcoma, liver	week 50/lymphoma, multiple organs
M	week 74/mesothelioma, lung	week 70/lymphoma, multiple organs
H	week 11/lymphoma, multiple organs	week 69/adenoma, lung

10. No. Alive at Termination:

	Male				Female			
mg/kg/day	0	200	1000	2000	0	200	1000	2000
No. alive	31	16	22	21	18	16	22	14
% survival	62	32	44	42	36	32	44	28

11. Statistical Methods used: 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.

12. Attach Tumor and Non-tumor Data for Each Tissue: Tumor and non-tumor data attached in Appendix I.

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 QAU 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

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)								
	Males				Females			
Days	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	5	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.

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