

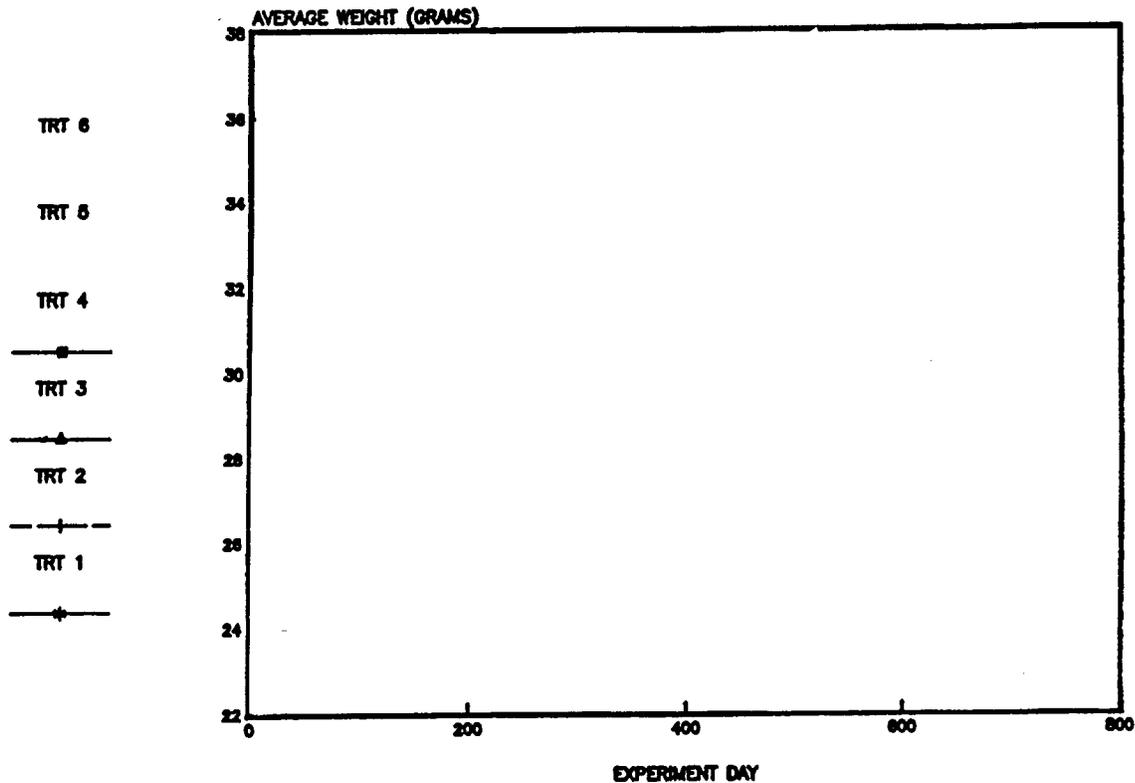
CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 019651/S005

PHARMACOLOGY REVIEW(S)

Pages: 26 through 50

GROWTH CURVES FOR SWISS FEMALE MICE
EXPERIMENT: 090791 PROJECT: 862.09.00-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	9	7	8	7
Histiocytic sarcoma	1	4	0	2	6	6	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.

**APPEARS THIS WAY
ON ORIGINAL**

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	88%	0.27	92%
3	1000	3 Month	5.34	24%	7.29	153%	6.43	124%
4	2000	3 Month	11.36	105%	27.76	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	60%	7.17	101%	8.19	75%
4	2000	6 Month	15.14	39%	17.16	76%	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	78%	1.12	127%
4	2000	9 Month	2.90	64%	1.45	11%	2.26	68%
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	79%	5.11	67%
4	2000	12 Month	12.28	40%	18.02	55%	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%	4.81	36%	5.02	35%
3	1000	3 Month	20.92	25%	14.74	51%	17.49	40%
4	2000	3 Month	23.18	26%	19.58	45%	21.18	35%
1	0	6 Month	0	0%	0	-	0	-
2	200	6 Month	5.03	40%	3.15	56%	4.09	50%
3	1000	6 Month	14.18	28%	13.26	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%	3.56	205%
3	1000	9 Month	5.04	36%	3.92	35%	4.48	36%
4	2000	9 Month	10.31	31%	7.94	46%	8.25	37%
1	0	12 Month	0	-	0	-	0	-
2	200	12 Month	3.65	33%	2.69	60%	3.17	45%
3	1000	12 Month	6.74	18%	16.00	46%	12.63	52%
4	2000	12 Month	11.90	44%	22.92	52%	17.41	60%

* Animals inadvertently received non-medicated feed.

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.

**APPEARS THIS WAY
ON ORIGINAL**

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: February 22, 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.

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

RAT STUDY DURATION (weeks): 104
STUDY STARTING DATE: October 27, 1992
STUDY ENDING DATE: February 22, 1996
RAT STRAIN: VAF Sprague-Dawley Crl:CD(SD)BR rats
ROUTE: Diet
DOSING COMMENTS:

No. RAT in control (C1): 60	Control2 (C2):
Low Dose (LD): 60	Middle Dose (MD): 60
High Dose (HD): 60	High Dose2 (HD2): 60

RAT Dose Levels (mg/kg/day)

RAT Low Dose: 60	RAT Middle Dose: 120
RAT High Dose: 360	RAT High Dose2: 480

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

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

RAT TUMOR FINDINGS: No

RAT STUDY COMMENTS: 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 based on findings from the 3 month dietary dose ranging study in rats (Report 862.09.00-AG). The high dose (480 mg/kg/day) is considered as MTD based on the renal toxicity. Thus, the dose selection is adequate. The major treatment related non-neoplastic changes were renal toxicity including increased incidences of urothelial hyperplasia, papillary inflammation, edema and necrosis mainly in the 360 and 480 mg/kg/day groups. The incidence of ulcerative and inflammatory lesion of the gastrointestinal tract was also increased dose-dependently. 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 high dose (480 mg/kg/day) is ~2.4 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/m²/day) based on body surface area. In conclusion, asacol was not carcinogenic in this 2-year carcinogenicity study in rats.

**APPEARS THIS WAY
ON ORIGINAL**

COVERSHEET FOR CARCINOGENICITY STUDY IN RATS

1. No. Of Studies: One
2. Name of Laboratory: Procter & Gamble Pharmaceuticals
Norwich, New York 13815
3. Strain: VAF Sprague-Dawley Crl:CD(SD)BR rats
4. No/sex/group: 60
5. Doses (0, L, M, H, H2): 0, 60, 120, 360 and 480 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 42/carcinoma, liver	week 35/mammary fibroadenoma, left axillary
L	week 20/carcinoma, skin, right hip	week 37/adenoma, pituitary
M	week 21/papilloma, skin, lip	week 36/fibrosarcoma, right inguinal
H	week 46/mammary adenocarcinoma, right axillary	week 41/mammary fibroadenoma, right pectoral
H2	week 24/fibrosarcoma, right axillary	week 15/adenoma, pituitary

10. No. Alive at Termination:

mg/kg/day	Male					Female				
	0	60	120	360	480	0	60	120	360	480
No. alive	12	15	15	16	13	19	16	19	16	21
% survival	20	25	25	27	22	32	27	32	27	35

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 II.

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 Crl:CD(SD)BR rats

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	477.5

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:

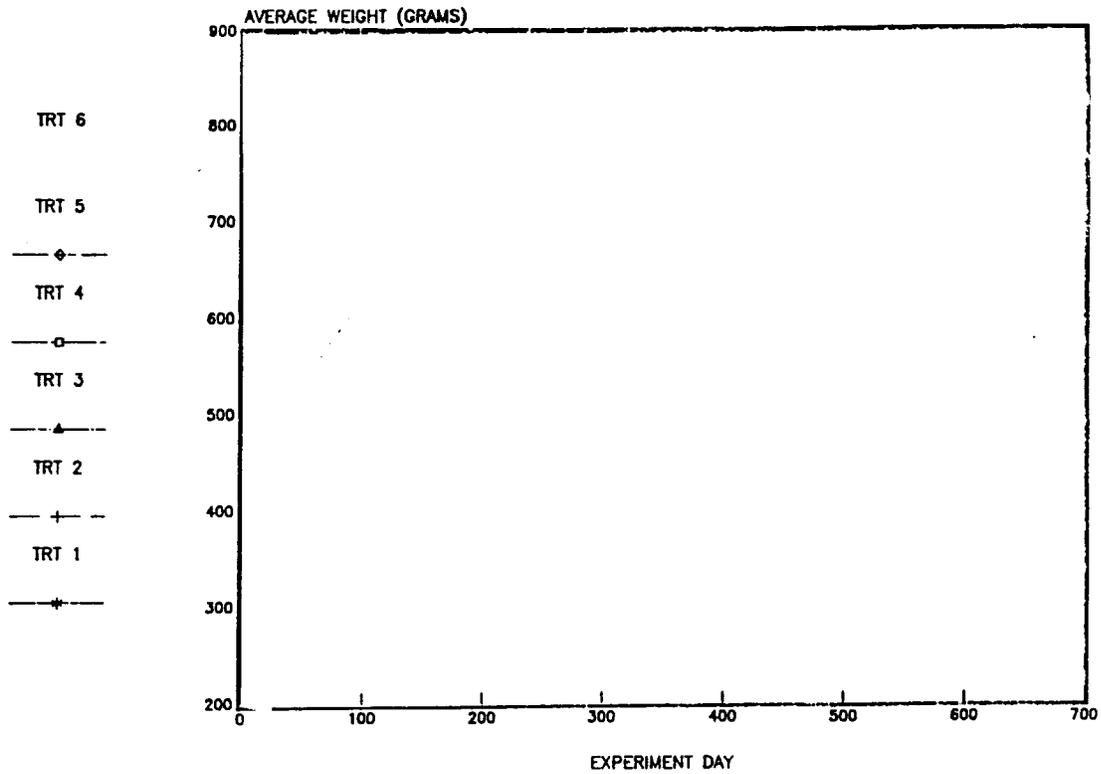
- Clinical Signs: There were no treatment related changes.
- Mortality: Deaths were not dose related and they were 48, 45, 45, 44 and 47 (males) or 41, 44, 41, 44 and 39 (females) in the control, 60, 120, 360 and 480 mg/kg/day groups, respectively. The intercurrent mortality (unscheduled deaths) was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

Mortality (unscheduled deaths)										
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	16	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

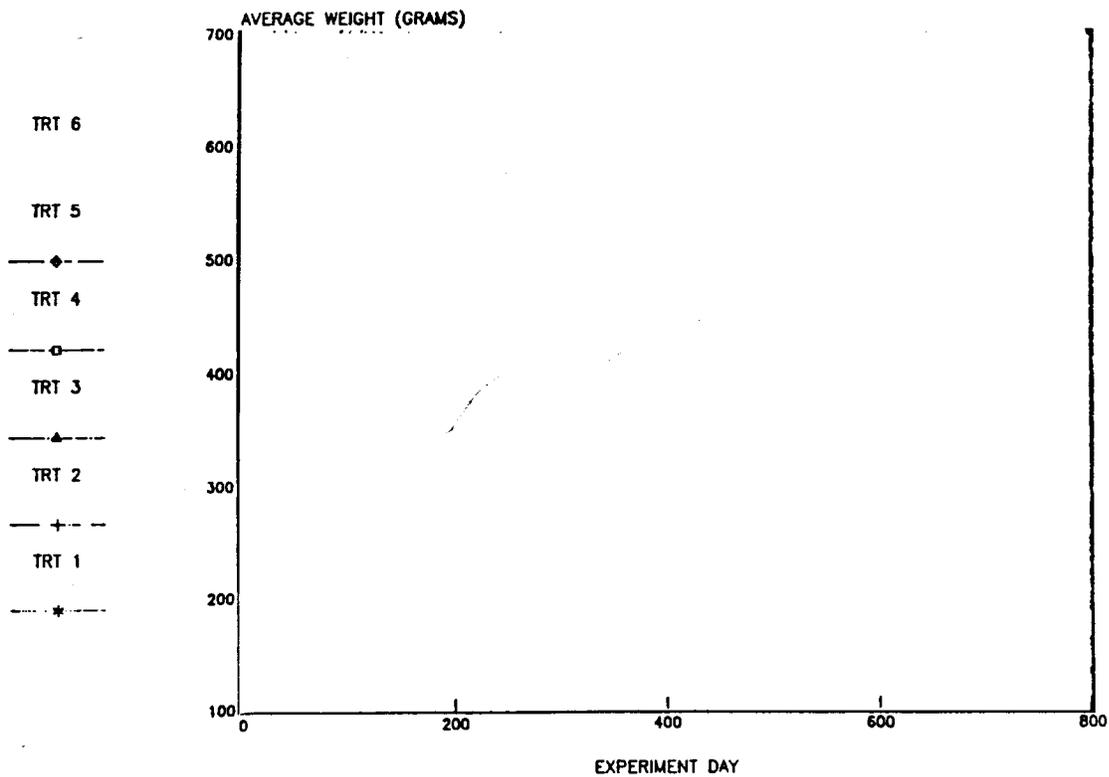
con, T1, T2, T3 and T4 = 0, 60, 120, 360 and 480 mg/kg/day

- 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.

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



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 Drug Consumption for 724 Days
Two Year Tumor Study of 5-ASA in Sprague-Dawley Rats
Project No. 862.09.00-CA

Treatment Group/Sex	Number Of Rats		5-ASA Average mg/kg/day	Mean Body Weight						% Control Gain	Food Consumption Average	
	Day 0	**Day 724		Day 0	8 Mo. Day 182	12 Mo. Day 364	18 Mo. Day 548	24 Mo. **Day 724	Gain		g/rat/day	g/kg/day
1M	* 85	14	0.0	248.7	885.5	791.1	815.1	892.8	458.7	—	30.88	44.43
2M	* 85	18	85.2	247.0	887.9	781.4	768.1	748.2	502.2	110.0	31.08	45.58
3M	* 85	17	125.8	248.0	884.8	781.3	811.0	741.2	491.7	107.7	31.07	44.81
4M	* 85	18	372.9	244.4	878.0	781.1	787.3	718.2	478.7	104.4	30.78	45.14
5M	* 85	13	514.8	248.4	895.8	804.0	783.7	752.5	508.3	111.3	31.50	45.08
1F	* 85	20	0.0	178.2	384.4	493.2	578.1	571.8	385.5	—	22.70	50.38
2F	* 85	18	80.3	180.1	410.0	515.1	614.9	632.0	458.1	115.3	23.04	48.83
3F	* 85	19	125.9	179.0	385.5	480.9	555.2	548.7	385.9	82.5	22.18	50.07
4F	* 85	17	388.0	179.7	375.8	488.3	554.2	599.4	418.2	105.7	22.40	50.64
5F	* 85	21	477.5	180.1	378.1	472.8	549.8	593.4	417.1	105.5	22.12	50.60

* There were 5 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

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

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 (mco/ml*hr)								
		Male	%CV	AUC/dose	Female	%CV	AUC/dose	All	%CV	AUC/dose
2	60	6.05	15%	0.10	6.84	36%	0.11	6.50	28%	0.11
3	120	28.04	44%	0.23	32.41	58%	0.27	30.22	49%	0.25
4	360	112.77	52%	0.31	148.66	37%	0.41	133.95	41%	0.37
5	480	130.49	30%	0.27	208.57	17%	0.43	168.53	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.

MUTAGENICITY STUDIES:

On the Mutagenic Action of Some Enzyme Immunoassay Substrates
(J. Immunological Methods, 36:55-61, 1980)

Methods: This is a published report. In this report, the potential mutagenic effects of 5-ASA was examined in an Ames test using the direct plate incorporation method in three strains salmonella typhimurium (TA98, TA100 and TA1537) in the presence and absence of metabolic activation, S-9 mix from rat liver. The following concentrations of 5-ASA were tested: 0, 0.1, 0.2, 0.5, 1, 2, 5 and 10 g/l with and without S-9 (each plate containing 23 ml medium and mixture). The basis of selection of these concentrations was not provided. However, the highest concentration appeared to be toxic to the bacteria. Positive control (2-amino-anthracene, 10 µg/plate) was tested. The result was considered positive if the test substance induced at least two fold increases in revertant colonies (for TA 98 and TA 1537) compared to the spontaneous rate (1.5 fold for TA 100).

Results: The results indicated that 5-ASA did not significantly increase the colonies. The positive control, however, significantly increased it.

In conclusion, the results suggest that 5-ASA was not mutagenic in this test system.

In a published report (mutation Research, 283:59-64, 1992), the following three tests were reported: sister-chromatid exchanges (SCE) test and chromosomal aberration test using Chinese hamster ovary (CHO) cells in vitro and mouse bone marrow micronucleus test in vivo. These three tests are reviewed below.

Sister-Chromatid Exchange (SCE) Test With 5-ACA

Methods: The test was conducted with and without S9 mix from rat liver. Chinese hamster ovary cells (CHO) were treated with 5-ASA at 28, 93 and 280 $\mu\text{g/ml}$ (duration not specified) and cultured for 26 hours (second metaphase) and then treated with colcemid (0.1 $\mu\text{g/ml}$). Fifty cells per dose group were scored. The sister-chromatid exchanges were observed and compared between the control and test groups. Positive controls (cyclophosphamide, 0.125 and 0.5 $\mu\text{g/ml}$ and mitomycin C, 0.001 and 0.004 $\mu\text{g/ml}$) were also tested.

Results: In this test, 5-ASA did not significantly increase the frequency of the sister-chromatid exchanges in CHO cells with or without metabolic activation. However, the positive controls, mitomycin and cyclophosphamide, significantly increased the frequency of the sister-chromatid exchanges in CHO cells.

In conclusion, the results suggest that 5-ASA is not mutagenic in this test system.

In Vitro Chromosomal Aberration Test With 5-ACA in CHO Cells

Methods: The test was conducted with and without S9 mix from rat liver. Chinese hamster ovary (CHO) cells were treated with 5-ASA at 61, 130 and 280 $\mu\text{g/ml}$ (duration no specified) and were cultured for 12 hours (first metaphase) and then treated with colcemid (0.1 $\mu\text{g/ml}$). Two hundred cells per dose group were scored. The chromosomal aberrations were observed and compared between the control and test groups. Positive controls (cyclophosphamide, 20 $\mu\text{g/ml}$ and mitomycin C, 0.4 $\mu\text{g/ml}$) were also tested.

Results: In this test, 5-ASA did not significantly increase the frequency of the chromosomal aberration in CHO cells with or without metabolic activation. However, the positive controls, mitomycin and cyclophosphamide, significantly increased the frequency of the chromosomal aberration in CHO cells.

In conclusion, the results suggest that 5-ASA is not mutagenic in this test system.

In Vivo Mouse Micronucleus Test With 5-ACA

Methods: B6C3F₁ mice were given 5-ASA at 125, 187.5 and 250 mg/kg/day for 3 days by i.p. injection. The authors stated that the selection of doses was based on the lethality seen in an initial toxicity evaluation. Positive control (dimethylbenzanthracene, 12.5 mg/kg) was also tested. Mice were sacrificed 24 hours after the last dose and bone marrow was collected. The frequency of micronucleated polychromatic erythrocytes was then determined.

Results: In this test, 5-ASA did not significantly increase the frequency of micronucleated polychromatic erythrocytes compared to the control. The positive control, however, significantly increased it compared to the control.

In conclusion, the results suggest that 5-ASA is not mutagenic in this test system.

Proposed Labeling:

The labeling is according to 21 CFR, Subpart B. The following revisions in the labeling are recommended:

Sponsor's Version:

CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY:

Dietary mesalamine was not carcinogenic in rats at doses as high as 480 mg/kg/day, or in mice at 2000 mg/kg/day. When compared on a mg/m² basis, these doses are 9.3 and 12.3 times the recommended human maintenance dose of Asacol of 473 mg/m² (0.8 g/day), respectively. Mesalamine was negative in the Ames assay for mutagenesis, negative for induction of sister chromatid exchanges (SCE) and chromosomal aberrations in Chinese hamster ovary cells in vitro, and negative for induction of micronuclei (MN) in mouse bone marrow polychromatic erythrocytes. Mesalamine was also negative for SCE and MN in human lymphocytes. Mesalamine, at oral doses up to 480 mg/kg/day, had no adverse effect on fertility or reproductive performance of male and female rats.

Evaluation: The maximum recommended human maintenance dose (1.6 g/day) should be used in calculation of the relative dose level in animals to that in humans. The sentence "mesalamine was also negative for SCE and MN in human lymphocytes" needs to be removed since it was not conducted in accordance with current guidelines and does not add any new information.

Suggested Version: Dietary mesalamine was not carcinogenic in rats at doses as high as 480 mg/kg/day, or in mice at 2000 mg/kg/day. These doses are 2.4 and 5.1 times the maximum recommended human maintenance dose of Asacol of 1.6 g/day (32 mg/kg/day if 50 kg body weight assumed or 1184 mg/m²), respectively, based on body surface area. Mesalamine was negative in the Ames assay for mutagenesis, negative for induction of sister chromatid exchanges (SCE) and chromosomal aberrations in Chinese hamster ovary cells in vitro, and negative for induction of micronuclei (MN) in mouse bone marrow polychromatic erythrocytes. Mesalamine, at oral doses up to 480 mg/kg/day, had no adverse effect on fertility or reproductive performance of male and female rats.

SUMMARY AND EVALUATION:

Asacol is an approved anti-inflammatory drug for treatment of active ulcerative colitis in the U.S. In the present NDA supplement, sponsor is seeking for approval to market asacol for maintenance of remission of ulcerative colitis. The maximum proposed clinical dose is 1.6 g/day or 32 mg/kg/day (50 kg body weight assumed). In support of this indication, following preclinical studies were submitted: a 6-month oral toxicity study in rats, 1-year oral toxicity study in dogs, 13-week and 3-month dietary dose ranging studies in mice, 13-week dietary dose ranging study in rats, 2-year carcinogenicity studies in mice and rats and published reports of mutagenicity studies including Ames test, sister-chromatid exchanges (SCE) test in Chinese hamster ovary (CHO) cells, *in vitro* chromosomal aberration tests in CHO cells and *in vivo* mouse bone marrow micronucleus test.

In the 6-month oral toxicity study (oral gavage) in rats (862.09.00-CE), 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 and necrosis and tubular degeneration of kidney. The tubular mineralization, urothelial hyperplasia, mucosal/submucosal fibrosis of the stomach and inflammation of the urinary bladder were also seen in the high dose group. The low dose (80 mg/kg/day) was no effect dose. The stomach and kidney were the target organs of toxicity.

In the 1-year oral toxicity study in dogs (862.09.00-AI), dogs (4/sex/group) were treated with 5-ASA by oral gavage at 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 (31-64%) and creatinine (20-25%) and chronic nephritis. No effect dose was identified at 40 mg/kg/day. The kidney was the target organ of toxicity. The renal toxicity (renal papillary necrosis) was noted in rats and dogs previously (see labeling).

In the 13-week dietary dose ranging study in mice (862.09.00-AC), mice were treated with 5-ASA at 0, 100, 500/2000, 1000/3000, and 1500 mg/kg/day for 13 weeks. The animals in the 500/2000 mg/kg/day group were treated with 500 mg/kg/day for the first 21 days and then the dose was increased to 2000 mg/kg/day for the rest of the study. The results suggested that the dose of 2000 mg/kg/day was MTD. This was based on the reduction of body weight gain (18-19%) in this group (both males and females) and the ratio of the exposure level (AUC) of 5-ASA in mice to that in humans (50 folds). To confirm this finding and to better define the MTD, a 3-month dietary dose ranging study was conducted in mice (862.09.00-CC).

In the 3-month dietary dose ranging study in mice (862.09.00-CC), mice were treated with 5-ASA at 0, 2000, 4000, 6000, 8000 and 10,000 mg/kg/day for 3 months. No effect dose was not identified. The test drug was lethal at 4000 mg/kg/day or higher. The lowest dose tested (2000 mg/kg/day) was identified as MTD. This was based on the decreased body weight gain (14%) in males (the body weight gain increased by ~19% in females) and the findings in the other 13-week dietary dose ranging study in mice (862.09.00-AC).

In the 2-year dietary carcinogenicity study in mice (862.09.00-CD), 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. 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) or higher produced some toxicities in the study. Therefore, the dose selection was adequate and the study is acceptable. 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/kg or 32 mg/kg/day if 50 kg body weight assumed or 1184 mg/m²/day) based on body surface area.

In the 3-month dietary dose ranging study in rats (862.09.00-AG), the major treatment related changes were the gastric and renal inflammation at doses of 840 mg/kg or higher. No effect dose was identified at 600 mg/kg/day. The target organs of toxicity were the stomach and kidney. Sponsor selected the dose of 480 mg/kg/day as the high-dose in the carcinogenicity study considering the expected exacerbation of renal effects over the 2-year duration of a carcinogenicity study.

In the 2-year dietary carcinogenicity study in rats (862.09.00-CA), 5-ASA was given to rats at 0, 60, 120, 360 and 480 mg/kg/day for 2 years. The dose selection was based on findings from the 3 month dietary dose ranging study in rats (Report 862.09.00-AG). The high dose (480 mg/kg/day) is considered as MTD based on the renal toxicity. Thus, the dose selection is adequate. 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. The high dose (480 mg/kg/day) is ~2.4 folds higher

than the maximum recommended human maintenance dose (1.6 g/kg or 32 mg/kg/day if 50 kg body weight assumed or 1184 mg/m²/day) based on body surface area.

5-ASA was not mutagenic in all genetic toxicity studies conducted including Ames test, sister-chromatid exchanges (SCE) test in Chinese hamster ovary (CHO) cells, *in vitro* chromosomal aberration tests in CHO cells and *in vivo* mouse bone marrow micronucleus test.

The kidney was the major target organ of toxicity in mice, rats and dogs. This was evidenced by increases incidence of renal pelvic dilation (mice), papillary inflammation, edema and necrosis (rats) and chronic nephritis (dogs). The increased blood urea nitrogen and creatinine were also found in rats and dogs. The renal toxicity was seen in the acute oral toxicity study in dogs (bilateral renal papillary necrosis at doses of 208-750 mg/kg), 14-day oral toxicity studies in rats and rabbits (1080 mg/kg/day), 6-month oral toxicity study in rats (papillary necrosis at 170 mg/kg/day or higher) and 1-year oral toxicity study in dogs (chronic nephritis at 80 mg/kg/day or higher). There were no additional organs of toxicity found in the long term toxicity studies as compared to the short term studies.

The labeling should be revised as suggested in the labeling portion.

RECOMMENDATION:

From a preclinical standpoint, this application is approvable. Sponsor should be asked to revise the labeling as recommended.

**APPEARS THIS WAY
ON ORIGINAL**

/S/

4/11/97

Ke Zhang, Ph.D.

Attachments: Appendix I, Pages 44 - 143
Appendix II, Pages 144 - 163

cc:
IND
HFD-180
HFD-180/Dr. Choudary
HFD-180/Dr. Fredd
HFD-180/Dr. Zhang
HFD-345/Dr. Viswanathan

**APPEARS THIS WAY
ON ORIGINAL**

/S/

4/21/97

R/D Init.: J. Choudary 3/30/97

KZ/hw/4/10/97

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Appendix I.

Incidence of Neoplastic and Non-neoplastic
Histopathological Findings (from sponsor's pathology
table 7 on pages 202-300 of volume 19)

**APPEARS THIS WAY
ON ORIGINAL**

DA 19,651
Page 45

Pathology Table 7:

Summary of all Histomorphologic Findings

Oncogenicity Study (24 Months) of 5-ASA in the Diet of Swiss Mice

Project No. 862.09.00-CD

98 pages

**APPEARS THIS WAY
ON ORIGINAL**

22-FEB-96
PLACES V8.200

Oncogenicity Study (24-Months) of 5-ASA in the Diet of Swiss Mice
Project No. 862.09.00-CD
Pathology Table 7
Summary of all Histomorphologic Findings

OBSERVATIONS	TREATMENT	INCIDENCE OF OBSERVATION (NUMERIC)									
		MALES					FEMALES				
		0 mg/kg	200 mg/kg	1000 mg/kg	2000 mg/kg	#	0 mg/kg	200 mg/kg	1000 mg/kg	2000 mg/kg	#
CARDIOVASCULAR SYSTEM:-											
AORTA:											
No abnormality detected		(47)	(41)	(47)	(48)	#	#	(46)	(45)	(48)	#
Metastatic site: HISTIOCYTIC SARCOMA (M)		44	35	42	42	#	#	39	37	36	#
Focal/multifocal/mural acute/chronic inflammation		1	2	3	2	#	#	1	3	2	#
Multifocal medial mineralization				1		#	#	2	4	5	#
Metastatic site: MESOTHELIOMA (M)				1		#	#				#
LYMPHOMA (M)		2	1	1	1	#	#	4	2	1	#
Diffuse adventitial fibrosis					1	#	#				#
Dilation			1			#	#				#
Multifocal mural degeneration		1				#	#				#

Figures in brackets represent the number of animals from which this tissue was examined microscopically
The absence of a numeral indicates that the lesion specified was not identified

90790 (Study Locked)

NDA 19,651
Page 47

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PLACES VB.200

Oncogenicity Study (24-Months) of S-ASA in the Diet of Swiss Mice
Project No. 862.09.00-CD
Pathology Table 7
Summary of all Histomorphologic Findings

OBSERVATIONS	TREATMENT	INCIDENCE OF OBSERVATION (NUMERIC)											
		MALES						FEMALES					
		0 mg/kg	200 mg/kg	1000 mg/kg	2000 mg/kg	#	0 mg/kg	200 mg/kg	1000 mg/kg	2000 mg/kg	#		
CARDIOVASCULAR SYSTEM:-													
AORTA:													
Metastatic site: broncholar-alveolar ADENOCARCINOMA (M)	(47)	(41)	(47)	(48)	1	(47)	(46)	(45)	(48)				
Metastatic site: ADENOCARCINOMA (M)	(50)	(50)	(50)	(50)	1	(50)	(49)	(50)	(50)				
HEART:													
No abnormality detected	21	14	16	15		18	19	22	22				
Metastatic site: HISTIOCYTIC SARCOMA (M)	2	2				2	1	2	2				
Metastatic site: GRANULOCYTIC LEUKEMIA (M)		1											
Focal/multifocal vasculitis	2	1	2	2		1	2	1	2				
Focal/multifocal/MOS atrial thrombosis	3	4	3	4		2	4	3	4				
Focal myocardial necrosis		1					1						

Figures in brackets represent the number of animals from which this tissue was examined microscopically
The absence of a numeral indicates that the lesion specified was not identified

90790 (Study Locked)

22-FEB-96
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PLACES V8.200

Oncogenicity Study (24-Months) of 5-ASA in the Diet of Swiss Mice
Project No. 862.09.00-CD
Pathology Table 7
Summary of all Histomorphologic Findings

OBSERVATIONS	TREATMENT	INCIDENCE OF OBSERVATION (NUMERIC)																		
		MALES					FEMALES													
		0 mg/kg	200 mg/kg	1000 mg/kg	2000 mg/kg	#	0 mg/kg	200 mg/kg	1000 mg/kg	2000 mg/kg	#									
CARDIOVASCULAR SYSTEM:-																				
HEART:		(50)	(50)	(50)	(50)	(50)	(50)	(49)	(50)	(50)										
Multifocal myocardial mineralization		1			1					3										
focal valvular cartilaginous metaplasia				1																
LYMPHOMA (M)		3		1	1					2										
focal/multifocal valvular/epicardial/perivascular/NOS acute/subacute/chronic inflammation/lymphocytic infiltration		9	11	16	14					10	12	10	11							
focal hemorrhage				2																
Focal/multifocal myocardial/epicardial/NOS fibrosis		8	4	5	7					4	4	2	6							
Right ventricular dilation																				
Focal/multifocal/NOS valvular degeneration		5	5	7	4					1	4	2	1							

Figures in brackets represent the number of animals from which this tissue was examined microscopically . . .
The absence of a numeral indicates that the lesion specified was not identified

90790 (Study Locked)

22-FEB-96
PLACES VB.200

Oncogenicity Study (24-Months) of S-ASA in the Diet of Swiss Mice
Project No. 862.09.00-CD
Pathology Table 7
Summary of all Histomorphologic Findings

OBSERVATIONS	TREATMENT	INCIDENCE OF OBSERVATION (NUMERIC)									
		MALES					FEMALES				
		0 mg/kg	200 mg/kg	1000 mg/kg	2000 mg/kg	#	0 mg/kg	200 mg/kg	1000 mg/kg	2000 mg/kg	#
CARDIOVASCULAR SYSTEM:-											
HEART:											
Focal/multifocal myofiber degeneration		(50)	(50)	(50)	(50)	(50)	(49)	(50)	(50)	(50)	
Autolysis		6	9	2	6	2	1	1	1	1	
Focal/multifocal/diffuse valvular/myocardial amyloidosis		9	15	9	9	15	13	10	9	9	
DIGESTIVE SYSTEM:-											
CECUM:											
No abnormality detected		(50)	(49)	(47)	(50)	(49)	(50)	(49)	(50)	(50)	
Metastatic site: HISTIOCYTIC SARCOMA (M)		37	32	37	35	30	32	36	35	35	
Metastatic site: GRANULOCYTTIC LEUKEMIA (M)			1			1	3	1			
Focal/multifocal/diffuse submucosal/NOS acute/chronic inflammation			3	1	1			1			

Figures in brackets represent the number of animals from which this tissue was examined microscopically. The absence of a numeral indicates that the lesion specified was not identified.

90790 (Study Locked)

22-FEB-96
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PLACES VOL.200

Oncogenicity Study (24-Months) of 5-ASA in the Diet of Swiss Mice
Project No. 662.09.00-CD
Pathology Table 7
Summary of all Histomorphologic Findings

OBSERVATIONS	TREATMENT	INCIDENCE OF OBSERVATION (NUMERIC)																
		MALES				FEMALES												
		0 mg/kg	200 mg/kg	1000 mg/kg	2000 mg/kg	0 mg/kg	200 mg/kg	1000 mg/kg	2000 mg/kg									
DIGESTIVE SYSTEM:-																		
CECUM:																		
Multifocal ulceration(s)		(50)	(49)	(47)	(50)					(49)	(50)	(49)	(50)					
Multifocal/diffuse submucosal edema		2	3	2	1					5	3	4	1					
LYMPHOMA (N)		2	1							2	1		2					
Focal/MOS lymphoid hyperplasia		2		2														
Dilatation		1								1								
Multifocal hemosiderin deposition											1							
Autolysis		5	11	6	12					10	10	7	12					
Multifocal amyloidosis		1								3	2	1	1					

Figures in brackets represent the number of animals from which this tissue was examined microscopically. The absence of a numeral indicates that the lesion specified was not identified.

90790 (Study Locked)