

Final Report

Long term 90 days toxicity study of *Melaleuca Alternifolia* Concentrate (MAC) in male Wistar Rats

General Study Design

The study employed a simple randomized design, using non-pathogenic male Wistar rats, weighing about 100-125 g body weight. Total animal number of 75 rats were divided randomly into 3 treatment groups.

Group 1 (n=25) received a single oral dose of vehicle, 2 mL/ rat/day
Group 2 (n=25) received a single oral dose of MAC 10 mg/kg of rat/day
Group 3 (n=25) received a single oral dose of MAC 30 mg/kg of rat/day

10 mg/kg rat is equivalent to 100 mg/day of 70 kg human.
30 mg/kg rat is equivalent to 300 mg/day of 70 kg human.

Treatment with MAC or vehicle lasted for 90 consecutive days. After treatment, the animals of each dosing group were divided randomly into 2 groups :

- a. The first group were sacrificed for histopathological studies.
- b. The second group underwent 30 days recovery study (without administration of MAC nor vehicle, except water ad libitum and standard food).

(WHO, 1993, Research guidelines for evaluating the safety and efficacy of herbal medicines, Manila).

I. Body weight, food and water intake of male rats after daily administration of MAC orally for 90 days of toxicity test

Table 1. Body weight (g) of male rats after daily administration of MAC orally for 90 days of toxicity test and after cessation of MAC for 30 days (recovery study)

Days with MAC	(Mean \pm SEM; n = 15)		
	Vehicle	10 mg/kg bw	30 mg/kg bw
0	117.8 \pm 3.9	107.3 \pm 3.9	113.9 \pm 5.5
3	128.5 \pm 5.5	118.1 \pm 4.0	132.9 \pm 5.6
6	144.4 \pm 6.8	133.8 \pm 4.3	148.5 \pm 5.6
9	153.9 \pm 6.3	143.5 \pm 4.2	155.2 \pm 5.6
12	173.0 \pm 6.6	154.4 \pm 4.8	168.5 \pm 6.3
15	185.3 \pm 7.1	168.8 \pm 5.2	179.2 \pm 7.1
18	196.3 \pm 8.7	183.2 \pm 5.1	196.5 \pm 6.3
21	210.5 \pm 7.5	188.7 \pm 4.3	201.3 \pm 7.1
24	220.2 \pm 6.9	194.2 \pm 3.7	221.4 \pm 8.0
27	229.7 \pm 6.5	210.7 \pm 6.5	215.8 \pm 7.2
30	233.6 \pm 5.9	205.7 \pm 3.8 ^a	222.1 \pm 7.1
33	245.0 \pm 6.4	212.8 \pm 4.6 ^a	213.4 \pm 15.6
36	253.3 \pm 7.0	218.8 \pm 4.4 ^a	228.5 \pm 8.1
39	262.0 \pm 7.4	229.5 \pm 4.5 ^a	243.5 \pm 7.6
42	268.9 \pm 7.6	233.5 \pm 5.2 ^a	240.5 \pm 7.9
45	261.2 \pm 8.2	226.2 \pm 4.9 ^a	224.8 \pm 17.5
48	260.5 \pm 6.57	224.85 \pm 7.29 ^a	247.73 \pm 7.93
51	272.5 \pm 6.66	241.86 \pm 5.74 ^a	245.50 \pm 6.96
54	280.33 \pm 10.21	244.57 \pm 5.24 ^a	248.23 \pm 7.17
57	282.61 \pm 10.32	244.13 \pm 5.64 ^a	266.67 \pm 6.77
60	295.65 \pm 10.53	253.06 \pm 6.32 ^a	266.08 \pm 10.57
63	295.81 \pm 10.62	256.67 \pm 6.47 ^a	253.65 \pm 12.21
66	304.70 \pm 6.40	262.57 \pm 5.92 ^a	286.64 \pm 8.43
69	308.15 \pm 2.23	265.78 \pm 8.16 ^a	270.47 \pm 12.23 ^a
72	316.44 \pm 14.70*	267.97 \pm 7.70 ^a	273.70 \pm 12.14
75	296.01 \pm 12.96	277.52 \pm 9.84	255.10 \pm 9.09
78	323.10 \pm 12.01	278.58 \pm 9.20	296.25 \pm 12.11
81	324.28 \pm 13.16	295.15 \pm 8.75	298.61 \pm 13.09
84	319.68 \pm 3.52	292.09 \pm 8.54	293.60 \pm 12.09
87	333.22 \pm 14.51	296.78 \pm 9.84	284.86 \pm 9.00 ^a
90	316.88 \pm 13.78	271.76 \pm 9.84	280.64 \pm 10.80
Days without MAC	Recovery study Mean \pm SEM (n = 5)		
3	304.08 \pm 38.11	250.22 \pm 15.63	197.92 \pm 49.03

6	289.60 ± 41.19	253.98 ± 18.50	210.50 ± 51.38
9	309.80 ± 43.07	276.13 ± 8.98**	219.20 ± 52.94
12	343.60 ± 33.99	295.75 ± 5.27	251.00 ± 61.32
15	342.30 ± 31.61	292.75 ± 8.87	243.00 ± 59.34
18	293.58 ± 41.30	291.98 ± 8.72	237.54 ± 55.98
21	346.50 ± 27.21	295.58 ± 8.12	241.18 ± 56.69
24	366.60 ± 28.52	304.25 ± 9.45	248.00 ± 57.46
27	370.00 ± 29.72	309.50 ± 4.57	254.40 ± 58.79
30	350.76 ± 28.57	288.65 ± 8.51	241.94 ± 54.71

* Starting from day-72 with MAC, one animal in the control group died.

** Starting from day-9 without MAC, one animal died.

a : significantly lower than control value (p<0.05).

Table 2. Organs weight (g) of male rats after daily administration of MAC orally for 90 days of toxicity test.

Organs	(Mean ± SEM; n = 15)		
	Vehicle	10 mg/kg bw	30 mg/kg
Heart	1.25 ± 0.11	0.99 ± 0.04	1.01 ± 0.04
Lungs	2.17 ± 0.36	1.45 ± 0.08	1.65 ± 0.12
Liver	10.51 ± 0.58	9.14 ± 0.50	6.38 ± 1.02*
Spleen	1.31 ± 0.64	0.86 ± 0.24	0.58 ± 0.03
Stomach	1.49 ± 0.04	1.39 ± 0.04	1.37 ± 0.04
Duodenum	0.41 ± 0.03	0.41 ± 0.03	0.42 ± 0.03
Ileum	0.49 ± 0.03	0.49 ± 0.03	0.51 ± 0.05
Kidney	1.02 ± 0.04	0.95 ± 0.04	0.98 ± 0.05

* significantly lower than control value (p<0.05).

Table 3. Food consumption (g) of male rats after daily administration of MAC orally for 90 days of toxicity test and after cessation of MAC for 30 days (recovery study)

Days with MAC	(Mean \pm SEM; n = 15)		
	Vehicle	10 mg/kg bw	30 mg/kg
1	13.22 \pm 0.76	13.00 \pm 0.62	14.86 \pm 1.26
3	14.85 \pm 1.75	13.86 \pm 0.91	14.50 \pm 0.78
6	14.62 \pm 0.83	13.03 \pm 1.30	14.83 \pm 0.67
9	11.90 \pm 0.42	13.05 \pm 1.03	12.79 \pm 0.95
12	14.48 \pm 0.66	12.30 \pm 0.95	12.77 \pm 1.15
15	14.76 \pm 0.83	17.42 \pm 1.77	14.87 \pm 0.63
18	14.77 \pm 0.85	15.88 \pm 1.01	13.15 \pm 0.79
21	15.73 \pm 0.65	15.49 \pm 0.97	15.09 \pm 0.66
24	14.69 \pm 0.61	16.28 \pm 1.42	13.99 \pm 0.71
27	15.21 \pm 0.69	15.62 \pm 1.34	13.32 \pm 0.61
30	13.41 \pm 1.03	15.62 \pm 1.27	12.67 \pm 0.90
33	16.15 \pm 0.68	17.61 \pm 1.71	14.20 \pm 0.56
36	16.87 \pm 0.56	15.54 \pm 1.27	13.61 \pm 0.86
39	17.19 \pm 0.80	17.18 \pm 1.00	14.09 \pm 0.77
42	16.30 \pm 0.85	15.52 \pm 1.12	14.71 \pm 0.66
45	15.73 \pm 1.04	15.27 \pm 1.29	15.21 \pm 1.13
48	16.93 \pm 0.86	15.71 \pm 0.72	15.40 \pm 0.88*
51	15.95 \pm 1.52	14.11 \pm 1.15	14.36 \pm 1.23
54	15.51 \pm 0.77	13.17 \pm 0.91	14.18 \pm 1.07
57	15.84 \pm 0.65	13.98 \pm 0.88	14.41 \pm 1.02
60	18.41 \pm 1.26	14.42 \pm 1.46	15.41 \pm 1.45
63	16.43 \pm 0.77	15.11 \pm 0.76	13.36 \pm 1.77
66	16.30 \pm 0.67	15.30 \pm 0.97	12.06 \pm 1.46
69	17.96 \pm 0.53	16.15 \pm 0.93	16.09 \pm 0.57
72	14.88 \pm 0.96	15.63 \pm 0.82	15.27 \pm 0.72
75	15.79 \pm 0.98	15.06 \pm 0.96	14.96 \pm 1.35
78	15.49 \pm 0.84	14.57 \pm 1.01	18.16 \pm 1.46
81	16.09 \pm 0.69	14.75 \pm 1.08	16.94 \pm 0.62
84	14.99 \pm 0.93	16.71 \pm 1.45	15.41 \pm 0.81
87	16.61 \pm 1.90	15.73 \pm 0.83	14.13 \pm 0.79
90	17.01 \pm 1.19	15.90 \pm 1.20	16.36 \pm 0.62
Days without MAC	Recovery study (Mean \pm SEM; n = 5)		
3	12.8 \pm 4.47	17.34 \pm 1.46	20.38 \pm 3.21
6	13.66 \pm 4.16	16.46 \pm 1.43	16.43 \pm 2.36
9	15.44 \pm 3.69	19.05 \pm 2.42**	15.45 \pm 1.18
12	19.36 \pm 1.33	14.80 \pm 0.54	16.15 \pm 1.27
15	21.02 \pm 2.05	15.75 \pm 1.97	17.45 \pm 2.44
18	19.74 \pm 2.77	12.40 \pm 0.91	17.53 \pm 3.03
21	20.96 \pm 1.41	17.10 \pm 1.24	17.55 \pm 2.72

24	21.76 ± 2.54	16.15 ± 1.17	16.73 ± 1.11
27	25.00 ± 2.17	18.80 ± 1.48	20.25 ± 2.45
30	23.22 ± 2.74	19.00 ± 1.35	20.45 ± 2.86

* Starting from day-46 with MAC, one animal died at the dose 30 mg/kg bw.

** Starting from day-9 without MAC, one animal died.

Table 4. Water consumption (ml) of male rats after daily administration of MAC orally for 90 days of toxicity test and after cessation of MAC for 30 days (recovery study)

Days with MAC	(Mean ± SEM; n = 15)		
	Vehicle	10 mg/kg bw	30 mg/kg
1	19.5 ± 0.74	18.9 ± 1.5	22.7 ± 1.9
3	28.5 ± 7.6	15.7 ± 1.0	21.1 ± 1.6
6	20.2 ± 1.6	17.6 ± 1.4	21.1 ± 1.9
9	17.1 ± 1.1	16.9 ± 0.5	20.3 ± 1.0
12	19.3 ± 1.4	17.5 ± 0.7	21.9 ± 1.6
15	18.2 ± 1.3	15.3 ± 0.6	20.1 ± 1.0
18	21.1 ± 2.2	18.1 ± 0.7	23.7 ± 2.2
21	23.8 ± 1.6	19.9 ± 0.9	27.9 ± 2.7
24	24.9 ± 2.1	19.7 ± 0.9	21.0 ± 2.2
27	25.2 ± 1.9	22.1 ± 1.2	25.4 ± 2.3
30	20.8 ± 1.1	20.5 ± 1.5	25.9 ± 2.0
33	24.1 ± 1.3	22.8 ± 1.5	25.1 ± 2.2
36	21.8 ± 0.8	22.8 ± 1.4	20.7 ± 1.7
39	23.3 ± 1.3	22.2 ± 1.6	24.5 ± 2.4
42	26.0 ± 1.7	24.3 ± 2.3	29.2 ± 2.5
45	29.2 ± 2.4	22.4 ± 1.5	28.0 ± 2.6
48	26.4 ± 2.1	25.2 ± 2.0	29.3 ± 2.4
51	28.2 ± 2.1	24.7 ± 3.0	31.7 ± 3.3
54	28.2 ± 2.2	26.2 ± 3.5	33.7 ± 3.2
57	24.9 ± 1.1	27.3 ± 3.4	30.1 ± 3.2
60	29.6 ± 3.1	27.7 ± 4.0	33.0 ± 3.6
63	26.3 ± 1.3	24.4 ± 2.6	28.7 ± 3.3
66	25.9 ± 1.8	29.5 ± 2.8	32.0 ± 4.1
69	27.4 ± 1.8	31.2 ± 2.6	34.9 ± 3.5
72	28.5 ± 2.3*	29.3 ± 2.1	32.4 ± 3.2
75	29.5 ± 2.0	33.1 ± 3.0	40.4 ± 4.5
78	29.4 ± 2.7	34.8 ± 5.4	39.6 ± 4.2
81	29.5 ± 2.2	30.7 ± 3.0	37.0 ± 5.1
84	29.4 ± 3.6	31.5 ± 3.3	38.1 ± 4.5
87	27.7 ± 3.0	29.6 ± 4.1	40.4 ± 4.5
90	31.6 ± 3.2	31.5 ± 3.1	34.0 ± 4.3

Days without MAC	Recovery study (Mean \pm SEM; n = 5)		
	3	16.8 \pm 5.0	18.2 \pm 3.8
6	20.6 \pm 5.3	20.6 \pm 4.1	27.8 \pm 5.8
9	20.4 \pm 5.4	22.5 \pm 0.9**	27.5 \pm 3.5
12	40.6 \pm 7.3	28.0 \pm 2.9	29.8 \pm 6.1
15	56.2 \pm 13.5	37.8 \pm 12.1	31.5 \pm 4.5
18	37.8 \pm 6.6	28.0 \pm 4.3	31.8 \pm 4.9
21	28.0 \pm 2.4	28.3 \pm 1.5	33.8 \pm 4.4
24	44.6 \pm 6.8	30.8 \pm 1.4	32.5 \pm 4.0
27	43.4 \pm 6.7	28.3 \pm 0.6	39.0 \pm 4.5
30	34.0 \pm 9.0	21.8 \pm 5.2	32.8 \pm 4.3

* Starting from day-72, one animal in the vehicle group died.

** Starting from day-9 without MAC, one animal died.

II. Blood chemistry of male rats after daily administration of MAC orally for 90 days of toxicity test

Table 5. Serum glucose levels (mg/dL) of male rats after daily administration of MAC orally for 90 days of toxicity test and after cessation of MAC (recovery study)

MAC dose (mg/kg)	(Mean \pm SEM; n = 15)			
	Day 0	Day 44	Day 90	Recovery (n=5)
Vehicle	45.12 \pm 4.92	64.31 \pm 5.16	63.99 \pm 4.35	61.26 \pm 5.66
10	53.31 \pm 4.75	51.18 \pm 6.04	63.08 \pm 5.34	61.95 \pm 4.53
30	62.17 \pm 6.22	46.04 \pm 4.78	48.76 \pm 5.12	47.78 \pm 5.33

Table 6. SGPT levels (U/L) of male rats after daily administration of MAC orally for 90 days of toxicity test and after cessation of MAC (recovery study)

MAC dose (mg/kg)	(Mean \pm SEM; n = 15)			
	Day 0	Day 44	Day 90	Recovery (n=5)
Vehicle	34.47 \pm 2.24	37.60 \pm 1.63	44.14 \pm 1.45	33.70 \pm 4.56
10	36.14 \pm 2.32	38.84 \pm 2.27	48.03 \pm 2.02	36.35 \pm 5.56
30	37.09 \pm 2.06	37.25 \pm 2.47	41.43 \pm 2.34	31.85 \pm 5.26

Table 7. SGOT levels (U/L) of male rats after daily administration of MAC orally 90 days of toxicity test and after cessation of MAC (recovery study)

MAC dose (mg/kg)	(Mean ± SEM; n = 15)			
	Day 0	Day 44	Day 90	Recovery (n=5)
Vehicle	179.15 ± 12.27	137.05 ± 8.77	129.08 ± 6.45	160.54 ± 12.14
10	162.23 ± 12.34	150.78 ± 9.38	158.79 ± 7.31	150.70 ± 13.52
30	153.53 ± 12.61	158.28 ± 7.54	178.14 ± 5.67	152.93 ± 11.05

Table 8. Serum triglyceride levels (mg/dL) of male rats after daily administration of MAC orally for 90 days of toxicity test and after cessation of MAC (recovery study)

MAC dose (mg/kg)	(Mean ± SEM; n = 15)			
	Day 0	Day 44	Day 90	Recovery (n=5)
Vehicle	59.81 ± 4.78	91.75 ± 9.54	120.06 ± 10.34	67.48 ± 7.38
10	46.05 ± 8.57	82.66 ± 11.35	115.23 ± 9.56	51.90 ± 6.97
30	39.71 ± 3.84	62.35 ± 4.98	128.77 ± 11.55	67.50 ± 6.73

Table 9. Serum LDL levels (mg/dL) of male rats after daily administration of MAC orally for 90 days of toxicity test and after cessation of MAC (recovery study)

MAC dose (mg/kg)	(Mean ± SEM; n = 15)			
	Day 0	Day 44	Day 90	Recovery (n=5)
Vehicle	26.69 ± 4.18	33.70 ± 2.76	25.42 ± 3.45	21.10 ± 2.79
10	26.41 ± 3.10	40.77 ± 5.98	20.01 ± 2.77	14.30 ± 2.11
30	22.79 ± 3.47	34.07 ± 3.25	16.75 ± 2.86	15.13 ± 2.55

Table 10. Serum HDL levels (mg/dL) of male rats after daily administration of MAC orally for 90 days of toxicity test and after cessation of MAC (recovery study)

MAC dose (mg/kg)	(Mean ± SEM; n = 15)			
	Day 0	Day 44	Day 90	Recovery (n=5)
Vehicle	39.21 ± 5.82	34.47 ± 2.00	34.00 ± 1.98	32.32 ± 2.77
10	37.27 ± 2.64	37.32 ± 2.37	31.67 ± 2.17	37.7 ± 2.35
30	31.86 ± 1.95	33.59 ± 2.24	29.86 ± 2.10	27.33 ± 1.51

Table 11. Serum cholesterol levels (mg/dL) of male rats after daily administration of MAC orally for 90 days of toxicity test and after cessation of MAC (recovery study)

MAC dose (mg/kg)	(Mean \pm SEM; n = 15)			
	Day 0	Day 44	Day 90	Recovery (n=5)
Vehicle	68.00 \pm 4.82	89.71 \pm 5.10	70.47 \pm 4.89	78.08 \pm 8.53
10	70.25 \pm 4.66	101.5 \pm 9.19	69.40 \pm 8.31	73.38 \pm 4.32
30	60.38 \pm 4.64	85.73 \pm 6.30	62.21 \pm 7.68	67.55 \pm 3.55

Table 12. Serum total bilirubin levels (mg/dL) of male rats after daily administration of MAC orally for 90 days of toxicity test and after cessation of MAC (recovery study)

MAC dose (mg/kg)	(Mean \pm SEM; n = 15)			
	Day 0	Day 44	Day 90	Recovery (n=5)
Vehicle	0.56 \pm 0.06	0.61 \pm 0.06	0.42 \pm 0.07	0.38 \pm 0.03
10	0.53 \pm 0.06	0.59 \pm 0.03	0.51 \pm 0.06	0.42 \pm 0.04
30	0.49 \pm 0.04	0.67 \pm 0.07	0.50 \pm 0.04	0.44 \pm 0.06

Table 13. Serum creatinine levels (mg/dL) of male rats after daily administration of MAC orally for 90 days of toxicity test and after cessation of MAC (recovery study)

MAC dose (mg/kg)	(Mean \pm SEM; n = 15)			
	Day 0	Day 44	Day 90	Recovery (n=5)
Vehicle	0.27 \pm 0.04	0.32 \pm 0.04	0.35 \pm 0.03	0.37 \pm 0.05
10	0.21 \pm 0.04	0.34 \pm 0.04	0.29 \pm 0.03	0.35 \pm 0.06
30	0.28 \pm 0.05	0.31 \pm 0.04	0.37 \pm 0.03	0.36 \pm 0.05

Table 14. Serum urea FS levels (mg/dL) of male rats after daily administration of MAC orally for 90 days of toxicity test and after cessation of MAC (recovery study)

MAC dose (mg/kg)	(Mean \pm SEM; n = 15)			
	Day 0	Day 44	Day 90	Recovery (n=5)
Vehicle	36.75 \pm 5.07	29.92 \pm 1.51	34.73 \pm 2.34	43.17 \pm 4.35
10	47.94 \pm 9.26	33.29 \pm 1.30	38.35 \pm 3.55	42.12 \pm 4.67
30	50.95 \pm 9.58	34.05 \pm 1.39	37.24 \pm 3.21	36.55 \pm 5.48

Table 15. Serum uric acid levels (mg/dL) of male rats after daily administration of MAC orally for 90 days of toxicity test and after cessation of MAC (recovery study)

MAC dose (mg/kg)	(Mean \pm SEM; n = 15)			
	Day 0	Day 44	Day 90	Recovery (n=5)
Vehicle	1.45 \pm 0.10	1.12 \pm 0.09	0.91 \pm 0.10	0.70 \pm 0.06
10	1.22 \pm 0.08	1.15 \pm 0.11	0.72 \pm 0.07	0.63 \pm 0.05
30	1.44 \pm 0.07	1.21 \pm 0.08	0.91 \pm 0.08	1.08 \pm 0.11

III. Hematological profiles of male rats after daily administration of MAC orally for 90 days of toxicity test

Table 16. White blood cells of male rats after daily administration of MAC orally for 90 days of toxicity test and after cessation of MAC (recovery study)

MAC dose (mg/kg)	WBC count ($\times 10^3$ /ul) (Mean \pm SEM; n = 15)			
	Day 0	Day 44	Day 90	Recovery (n=5)
Vehicle	7.09 \pm 0.44	11.09 \pm 0.94	7.81 \pm 0.91	6.86 \pm 0.87
10	6.31 \pm 0.47	10.48 \pm 0.89	8.88 \pm 0.64	5.93 \pm 0.78
30	8.30 \pm 0.75	13.10 \pm 0.89	10.35 \pm 0.67	7.53 \pm 0.89

Table 17. Red blood cells of male rats after daily administration of MAC orally for 90 days of toxicity test and after cessation of MAC (recovery study)

MAC dose (mg/kg)	RBC count ($\times 10^6$ /ul) (Mean \pm SEM; n = 15)			
	Day 0	Day 44	Day 90	Recovery (n=5)
Vehicle	7.91 \pm 0.13	8.41 \pm 0.15	8.63 \pm 0.22	5.87 \pm 0.84
10	7.76 \pm 0.18	8.16 \pm 0.25	8.49 \pm 0.19	7.88 \pm 0.66
30	8.53 \pm 0.21	8.39 \pm 0.17	8.30 \pm 0.17	7.54 \pm 0.75

Table 18. Platelet counts of male rats after daily administration of MAC orally for 90 days of toxicity test and after cessation of MAC (recovery study)

MAC dose (mg/kg)	Platelet count ($\times 10^3$ /ul) (Mean \pm SEM; n = 15)			
	Day 0	Day 44	Day 90	Recovery (n=5)
Vehicle	1161.87 \pm 46.37	1041.93 \pm 47.35	702.43 \pm 50.45	607.80 \pm 45.62
10	1165.27 \pm 58.71	993.73 \pm 78.78	796.60 \pm 34.87	942.75 \pm 46.89
30	1135.07 \pm 37.19	1058.47 \pm 39.58	846.57 \pm 46.01	717.65 \pm 38.79

Table 19. Hemoglobin levels (g/dL) of male rats after daily administration of MAC orally for 90 days of toxicity test and after cessation of MAC (recovery study)

MAC dose (mg/kg)	(Mean \pm SEM; n = 15)			
	Day 0	Day 44	Day 90	Recovery (n=5)
Vehicle	16.07 \pm 0.21	16.66 \pm 0.27	16.14 \pm 0.45	13.56 \pm 0.52
10	15.10 \pm 0.69	16.28 \pm 0.42	15.62 \pm 0.39	14.44 \pm 0.33
30	16.23 \pm 0.40	16.57 \pm 0.26	15.54 \pm 0.32	14.25 \pm 0.48

Table 20. Hematocrite levels (%) of male rats after daily administration of MAC orally for 90 days of toxicity test and after cessation of MAC (recovery study)

MAC dose (mg/kg)	(Mean \pm SEM; n = 15)			
	Day 0	Day 44	Day 90	Recovery (n=5)
Vehicle	44.58 \pm 0.90	45.88 \pm 0.79	48.26 \pm 1.02	40.32 \pm 1.23
10	43.97 \pm 0.86	44.73 \pm 1.28	46.71 \pm 1.33	43.20 \pm 1.65
30	47.22 \pm 1.15	45.82 \pm 0.82	45.73 \pm 0.98	42.05 \pm 1.87

Table 21. Lymphocyte levels (%) of male rats after daily administration of MAC orally for 90 days of toxicity test and after cessation of MAC (recovery study)

MAC dose (mg/kg)	(Mean \pm SEM; n = 15)			
	Day 0	Day 44	Day 90	Recovery (n=5)
Vehicle	83.87 \pm 1.54	80.26 \pm 1.69	70.41 \pm 1.76	70.86 \pm 5.67
10	85.15 \pm 1.13	78.81 \pm 1.36	70.84 \pm 2.60	74.90 \pm 4.69
30	76.79 \pm 3.84	74.77 \pm 2.38	69.40 \pm 2.31	68.95 \pm 5.52

Table 22. Neutrophil levels (%) of male rats after daily administration of MAC orally for 90 days of toxicity test and after cessation of MAC (recovery study)

MAC dose (mg/kg)	(Mean \pm SEM; n = 15)			
	Day 0	Day 44	Day 90	Recovery (n=5)
Vehicle	16.07 \pm 1.54	19.73 \pm 1.69	29.95 \pm 1.43	39.19 \pm 5.67
10	14.85 \pm 1.14	20.69 \pm 1.33	29.16 \pm 2.60	35.10 \pm 3.87
30	22.73 \pm 3.59	25.23 \pm 2.38	38.84 \pm 2.02	31.05 \pm 4.62

Table 23. Mean Corpuscular Volume (fL) of male rats after daily administration of MAC orally for 90 days of toxicity test and after cessation of MAC (recovery study)

MAC dose (mg/kg)	(Mean \pm SEM; n = 15)			
	Day 0	Day 44	Day 90	Recovery (n=5)
Vehicle	56.35 \pm 0.39	54.58 \pm 0.31	55.93 \pm 0.54	55.48 \pm 0.63
10	56.73 \pm 0.46	54.89 \pm 0.36	54.95 \pm 0.60	54.83 \pm 0.44
30	55.37 \pm 0.41	54.66 \pm 0.37	55.16 \pm 0.45	55.83 \pm 0.61

Table 24. Mean Corpuscular Hemoglobin (pg) of male rats after daily administration of MAC orally for 90 days of toxicity test and after cessation of MAC (recovery study)

MAC dose (mg/kg)	(Mean \pm SEM; n = 15)			
	Day 0	Day 44	Day 90	Recovery (n=5)
Vehicle	20.34 \pm 0.15	19.82 \pm 0.10	18.71 \pm 0.57	18.68 \pm 0.66
10	20.02 \pm 0.39	20.02 \pm 0.24	18.42 \pm 0.27	18.28 \pm 0.43
30	19.84 \pm 0.18	19.78 \pm 0.17	18.74 \pm 0.46	18.95 \pm 0.56

Table 25. Mean Corpuscular Hemoglobin Concentration (g/dl) of male rats after daily administration of MAC orally for 90 days of toxicity test and after cessation of MAC (recovery study)

MAC dose (mg/kg)	(Mean \pm SEM; n = 15)			
	Day 0	Day 44	Day 90	Recovery (n=5)
Vehicle	36.13 \pm 0.38	36.33 \pm 0.13	33.44 \pm 0.41	33.56 \pm 0.35
10	35.63 \pm 0.58	36.46 \pm 0.25	33.49 \pm 0.24	33.35 \pm 0.32
30	35.85 \pm 0.14	36.17 \pm 0.15	33.99 \pm 0.36	33.90 \pm 0.37

IV. Urine chemistry of male rats after daily administration of MAC orally for 90 days of toxicity test

Table 26. Urine status of male rats after daily administration of MAC orally for 90 days of toxicity test and after cessation of MAC (recovery study)

MAC dose (mg/kg)	Urine status (n = 15)			
	Day 0	Day 44	Day 90	Recovery (n=5)
Urine colour				
Vehicle	normal	normal	normal	normal
10	normal	normal	normal	normal
30	normal	normal	normal	normal
Urine pH				
Vehicle	8.5	8.5	8.5	8.5
10	8.5	8.5	8.5	8.5
30	8.5	8.5	8.5	8.5
Urine specific gravity				
Vehicle	1.00	1.00	1.00	1.00
10	1.00	1.00	1.00	1.00
30	1.00	1.00	1.00	1.00
Protein				
Vehicle	normal	normal	normal	normal
10	normal	normal	normal	normal
30	normal	normal	normal	normal
Urobilinogen				
Vehicle	normal	normal	normal	normal
10	normal	normal	normal	normal
30	normal	normal	normal	normal
Sediments (epithels and crystals of triple phosphate or calcium oxalate)				
Vehicle	normal	normal	normal	normal
10	normal	normal	normal	normal
30	normal	normal	normal	normal

V. Histopathology of male rat organs after daily administration of MAC orally for 90 days of toxicity test

Organs	Vehicle (n = 10)	MAC 10 mg/kg (n = 10)	MAC 30 mg/kg (n = 10)
Liver	Normal	Normal	Normal
Kidney	Normal	Normal	Normal
Spleen	Normal	Normal	Normal
Heart	Normal	Normal	Normal
Lungs	Normal	Normal	Normal
Stomach	Normal	Inflammation (n=3)*	Inflammation (n=1)*
Intestine	Normal	Inflammation (n=5)**	Inflammation (n=4)**

* Submucosal gastric inflammation occurred in 3 rats (10 mg/kg), and in one rat (30 mg/kg)

** Mucosal intestinal inflammation in 5 rats (10 mg/kg), and in 4 rat (30 mg/kg)

VI. Histopathology of male rat organs during recovery study for 30 days (after cessation of MAC)

Organs	Vehicle (n = 4)	After MAC 10 mg/kg (n = 6)	After MAC 30 mg/kg (n = 5)
Liver	Normal	Normal	Normal
Kidney	Normal	Normal	Normal
Spleen	Normal	Normal	Normal
Heart	Normal	Normal	Normal
Lungs	Normal	Normal	Normal
Stomach	Normal	Inflammation (n=3)*	Normal
Intestine	Normal	Inflammation (n=5)**	Inflammation (n=1)**

* Submucosal gastric inflammation remained in 3 rats (10 mg/kg).

** Mucosal intestinal inflammation in 5 rats (10 mg/kg), and in 1 rat (30 mg/kg)

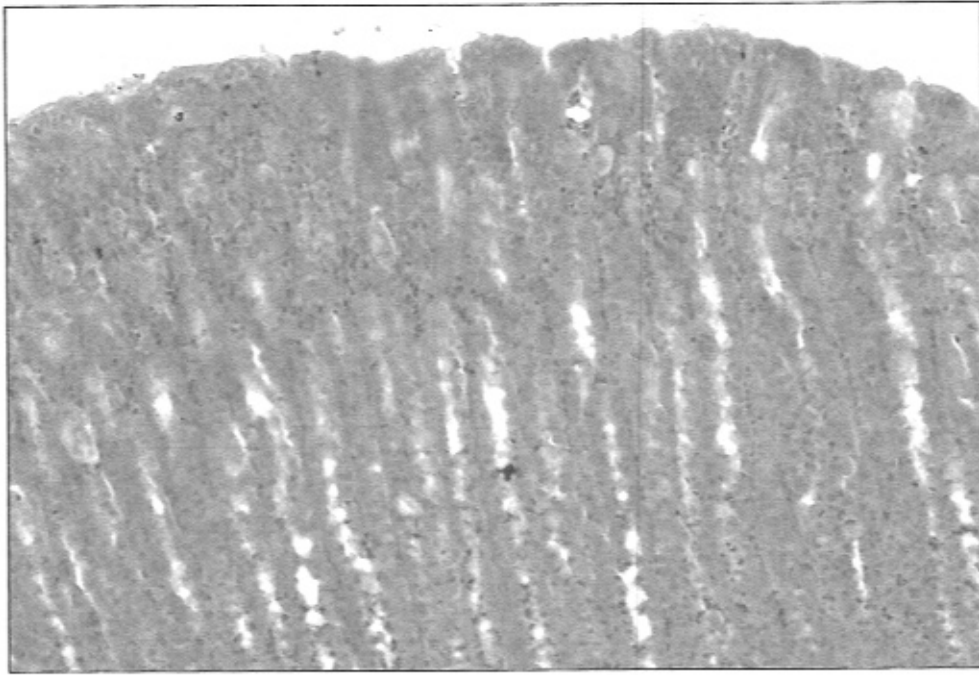
Frequency of inflammation
90 days with MAC and 30 days without MAC

With oral MAC (n=10)

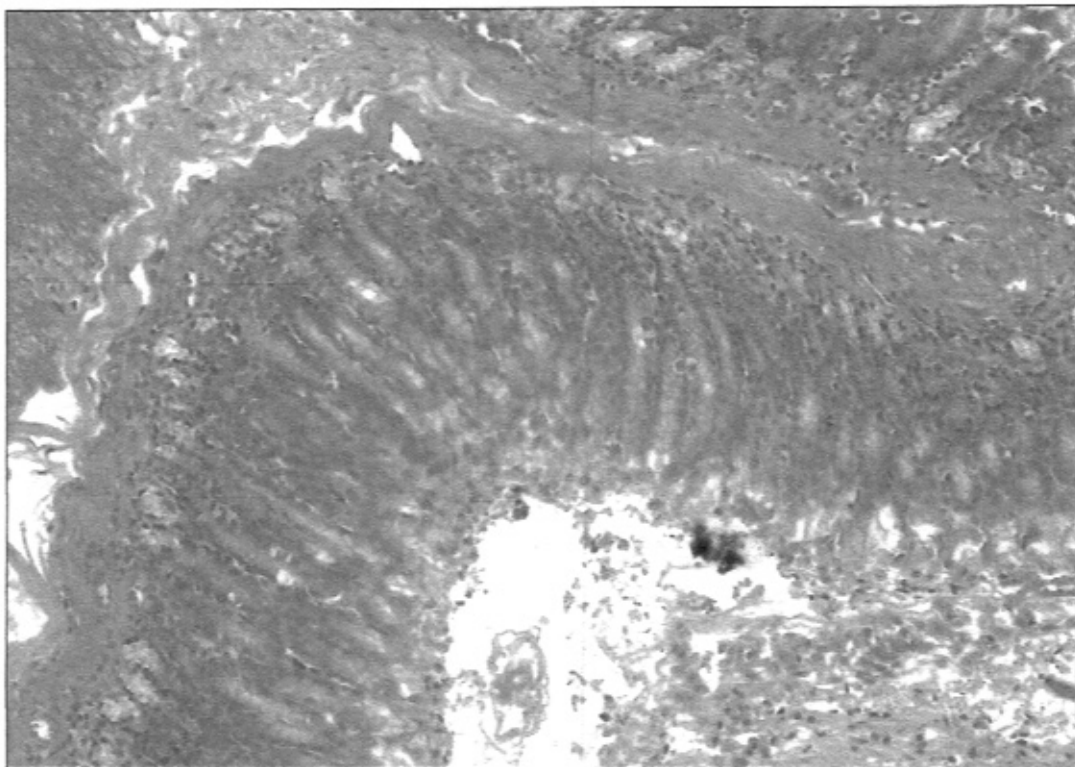
Organs	10 mg/kg	30 mg/kg
Stomach	0	0
Intestine	0	1
Stomach + Intestine	4	2

Recovery Without MAC (n=5)

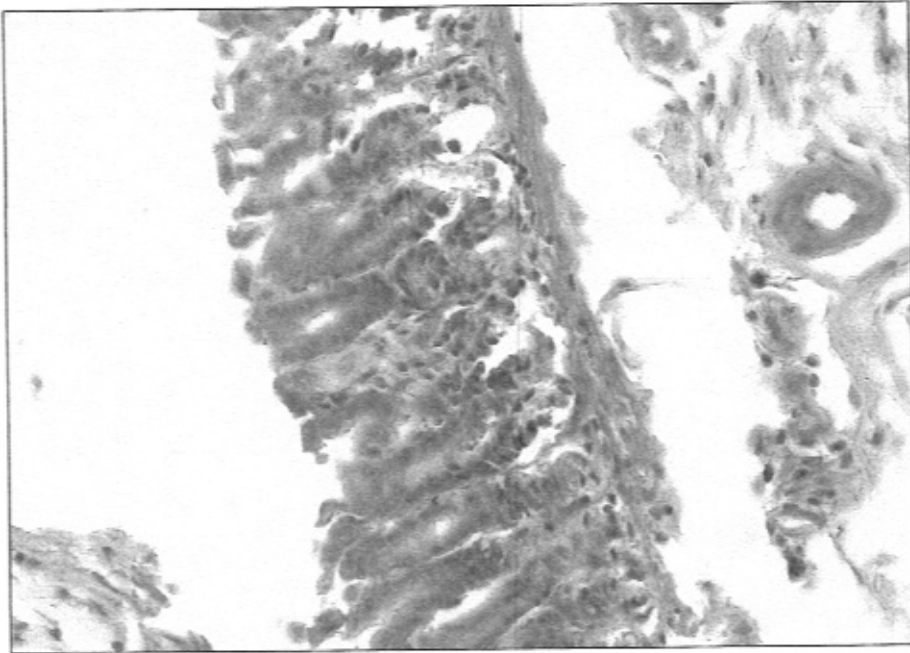
Organs	10 mg/kg	30 mg/kg
Stomach	0	0
Intestine	0	1
Stomach + Intestine	4	1



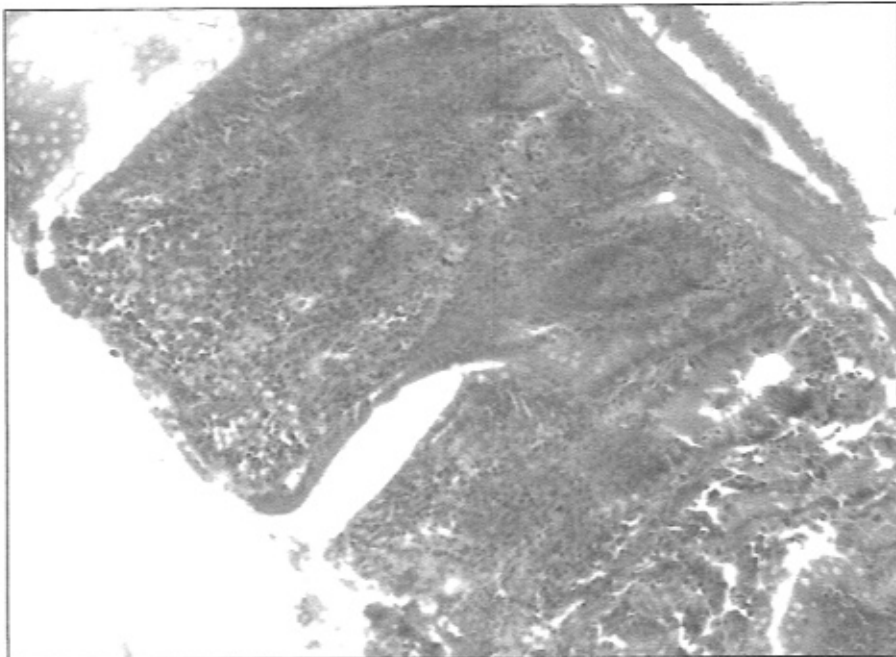
Normal Gastrium



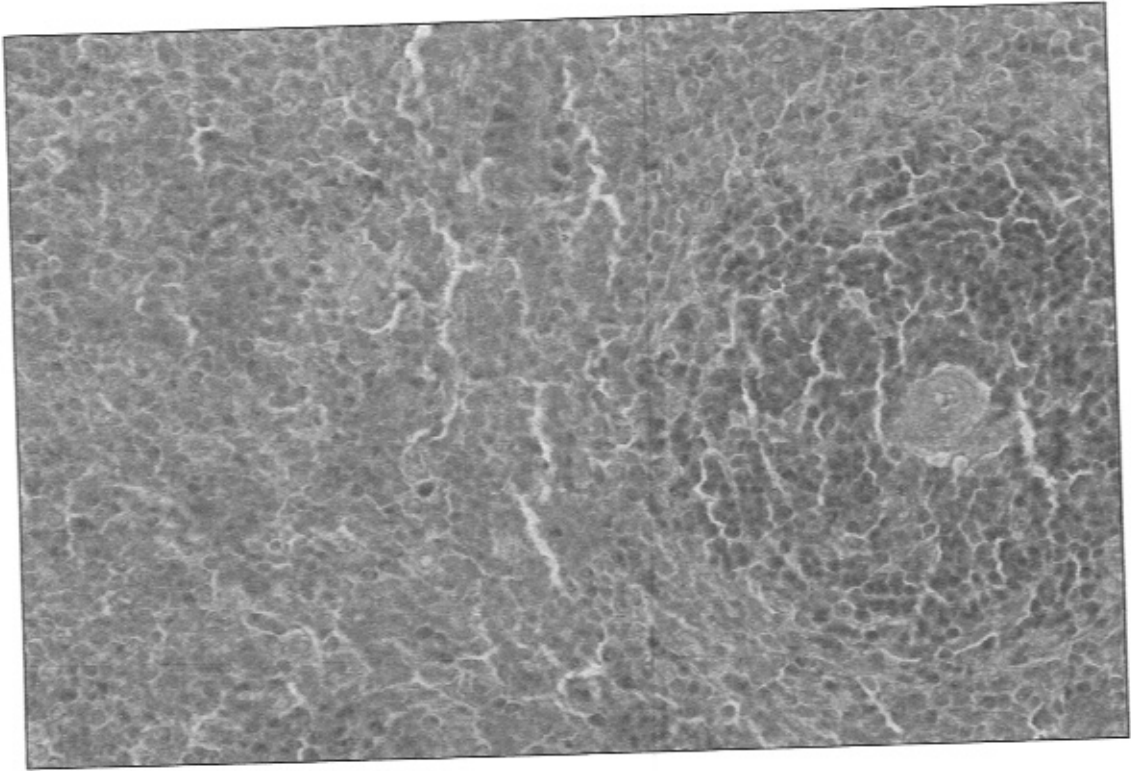
Gastritis



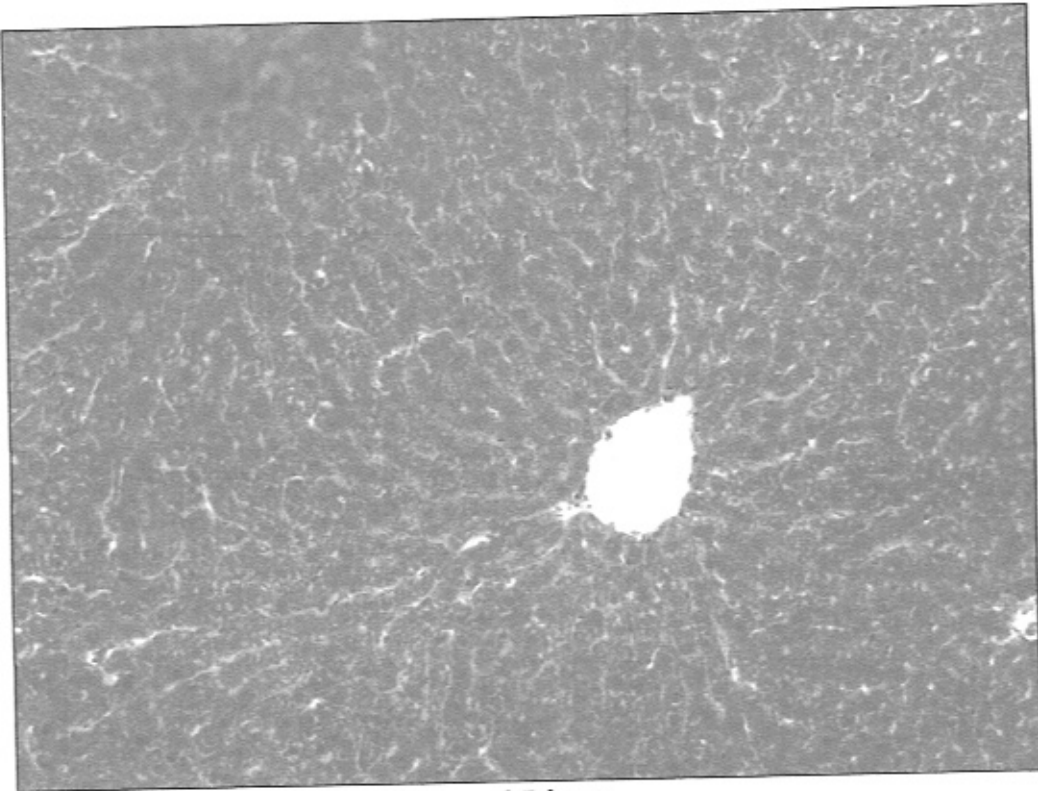
Normal Intestine



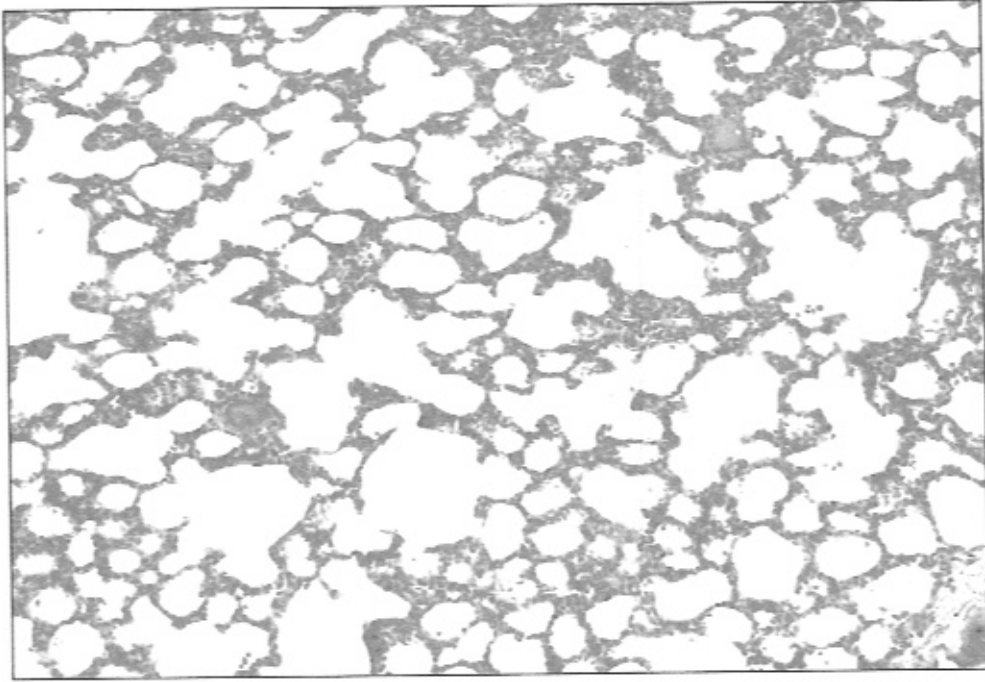
Inflamed intestine



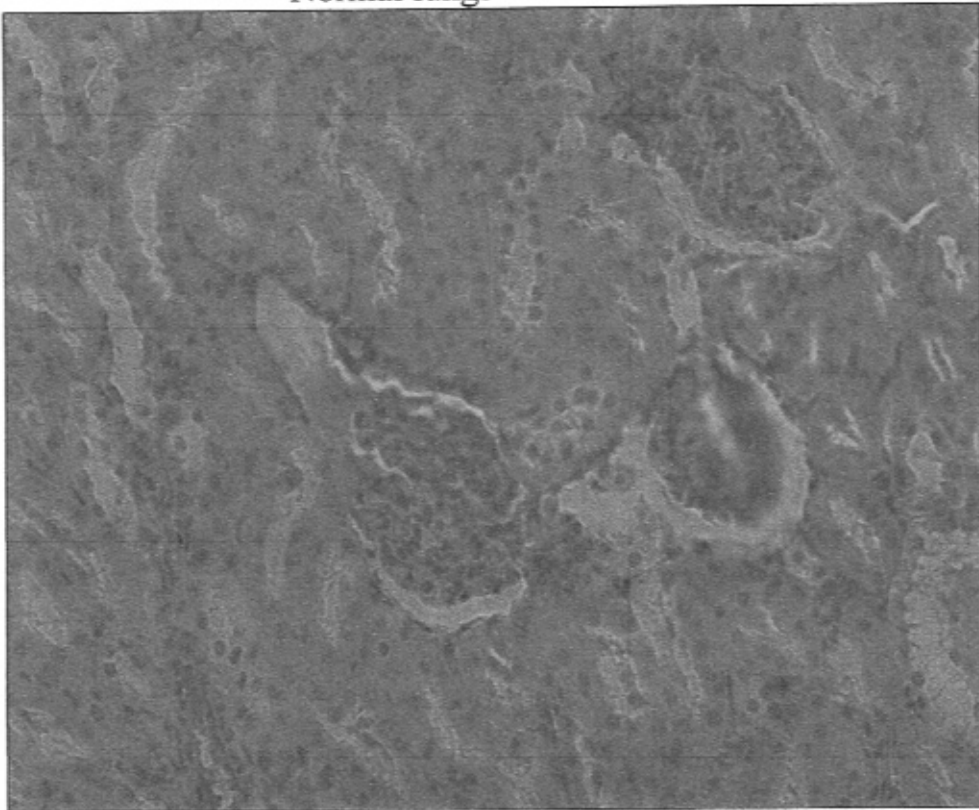
Normal Spleen



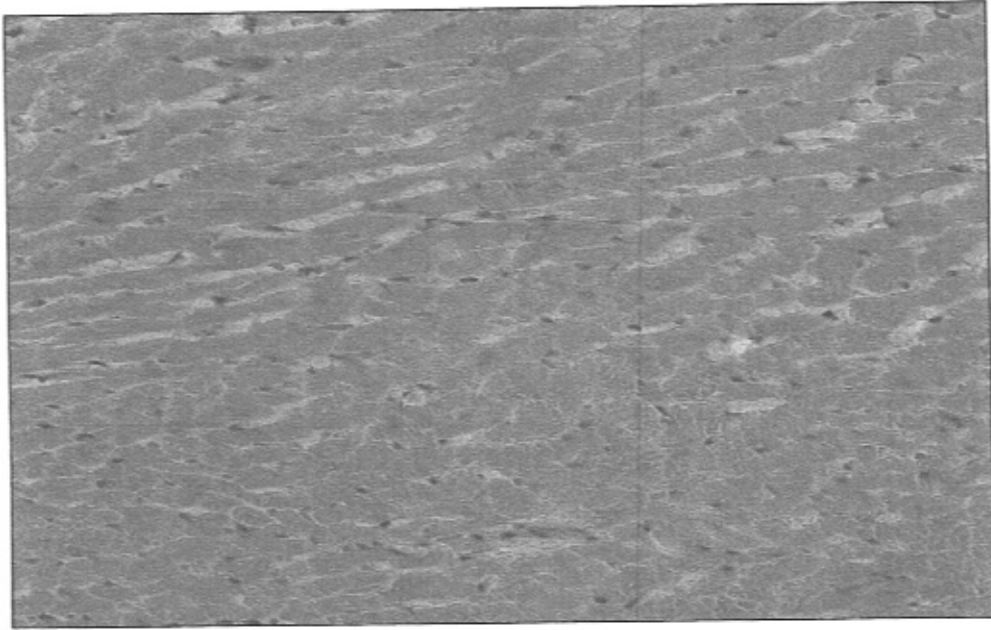
Normal Liver



Normal lungs



Normal kidney



Normal Myocardium

Conclusions

After daily administration of MAC orally in male Wistar rats for 90 days and after cessation of MAC (recovery study), the following results have been obtained :

1. Body weights of the rats tend to decrease with increasing doses.
2. Weights of organs (heart, lungs, spleen, stomach, duodenum, ileum and kidney) including liver at 10 mg/kgbw were similar to the corresponding control values. Liver weight after MAC at 30 mg/kg bw was significantly lower than that of control value ($p < 0.05$).
3. Food and water intakes were normal or very similar to control animals.
4. Blood chemistry :
 - a. Glucose levels were normal or unchanged.
 - b. Liver function (SGOT, SGPT, total bilirubin) was normal.
 - c. Kidney function (urea and creatinine) was normal.
 - d. Uric acid levels were normal (similar to control level).
 - e. Lipid levels (triglyceride and cholesterol) increased in control and MAC groups, but LDL-C and HDL-C level were unchanged.
5. Hematology :
WBC, RBC, hemoglobin, hematocrite, lymphocyte, neutrophile, MCV, MCH, MCHC were unchanged.
6. All parameters of urine chemistry were normal.
7. Histology of liver, kidney, spleen, heart and lungs were normal.
8. Sub-mucosal gastric inflammation occurred in 3 rats (10 mg/kg), and in 1 rat (30 mg/kg) out of 10 rats.
9. Mucosal intestinal inflammation in 5 rats (10 mg/kg), and in 4 rat (30 mg/kg).