

Table 1. Food consumption and intake of madder color in rats given madder color for 53 weeks

Group	Food consumption (g/rat/day)		Daily intake (g/rat)		Total intake (g/rat)	
	Male	Female	Male	Female	Male	Female
Control	14.2	9.5	-	-	-	-
0.2%	15.2	9.7	0.03	0.02	11.2	7.2
1.0%	14.9	8.8	0.15	0.09	55.3	32.7
5.0%	13.3	8.1	0.66	0.40	246.5	149.7

Table 2. Hematological and serum biochemical data of F344 male rats given madder color for 53 weeks

Items	Groups			
	Control	0.2%	1%	5%
RBC (x10 ⁴ /μ L)	887.1 ± 40.9 ^{a)}	898.6 ± 38.5	887.4 ± 18.9	832.6 ± 43.0**
Hb (g/dL)	14.7 ± 0.6	15.0 ± 0.8	14.7 ± 0.5	13.9 ± 0.9*
Ht (%)	46.7 ± 1.8	47.0 ± 2.2	46.4 ± 1.1	43.8 ± 2.4**
MCV (fL)	52.6 ± 0.6	52.3 ± 0.4	52.3 ± 0.5	52.6 ± 0.6
MCH (pg)	16.6 ± 0.5	16.7 ± 0.4	16.5 ± 0.4	16.7 ± 0.6
MCHC (g/dL)	31.6 ± 0.8	31.9 ± 0.5	31.6 ± 0.6	31.8 ± 0.8
PLT (x10 ⁴ /μ L)	69.8 ± 4.0	70.0 ± 4.6	68.0 ± 3.7	81.8 ± 6.7**
WBC (x10 ² /μ L)	37.8 ± 3.9	37.9 ± 5.0	38.2 ± 7.6	43.2 ± 10.5
Differential cell counts (%)				
Neut-B	0.8 ± 0.7	1.0 ± 1.0	1.4 ± 0.5	0.8 ± 0.7
Neut-S	30.3 ± 4.2	26.0 ± 4.0	26.8 ± 3.5	37.5 ± 10.1
Eosino	1.5 ± 1.1	0.7 ± 0.6	0.8 ± 0.8	1.2 ± 0.9
Baso	0.1 ± 0.2	0.1 ± 0.2	0.2 ± 0.3	0.3 ± 0.4
Lymph	65.4 ± 4.4	70.2 ± 4.6	68.8 ± 3.3	59.2 ± 11.1
Mono	2.0 ± 1.0	2.0 ± 1.2	2.0 ± 0.7	1.1 ± 0.9
Elb	3.0 ± 2.3	3.3 ± 2.0	3.4 ± 2.6	4.9 ± 5.4
TP (g/dL)	6.8 ± 0.2	6.8 ± 0.2	6.8 ± 0.2	6.8 ± 0.2
A/G	1.9 ± 0.1	2.0 ± 0.2	2.0 ± 0.2	1.7 ± 0.1**
Alb (g/dL)	4.4 ± 0.1	4.5 ± 0.2	4.5 ± 0.1	4.3 ± 0.1**
TG (mg/dL)	108.9 ± 33.4	119.3 ± 31.2	103.3 ± 33.9	69.4 ± 29.5*
TC (mg/dL)	105.9 ± 10.3	104.8 ± 8.9	91.8 ± 9.2*	82.5 ± 14.6**
BUN (mg/dL)	16.7 ± 1.5	16.2 ± 0.6	16.2 ± 0.7	15.7 ± 1.1
CRE (mg/dL)	0.32 ± 0.04	0.32 ± 0.02	0.30 ± 0.02	0.39 ± 0.04**
Na (mEq/dL)	143.9 ± 2.1	144.1 ± 1.4	144.6 ± 0.8	145.2 ± 1.3
Cl (mEq/dL)	102.2 ± 1.5	102.2 ± 1.5	102.6 ± 0.8	102.3 ± 1.9
K (mEq/dL)	4.4 ± 0.3	4.3 ± 0.2	4.5 ± 0.2	4.5 ± 0.3
Ca (mg/dL)	10.5 ± 0.3	10.5 ± 0.2	10.6 ± 0.3	10.4 ± 0.3
IP (mg/dL)	5.3 ± 0.4	5.5 ± 0.3	5.5 ± 0.3	5.9 ± 0.4**
AST (IU/L)	77.9 ± 9.5	75.7 ± 10.7	64.3 ± 7.4**	65.3 ± 7.9**
ALT (IU/L)	52.8 ± 7.9	50.6 ± 9.8	37.6 ± 5.3**	31.1 ± 7.9**
ALP (IU/L)	275.1 ± 45.9	281.9 ± 27.0	239.2 ± 39.9	177.5 ± 34.4**

^{a)} Mean ± SD

*, **: Significantly different from control at p<0.05 and p<0.01, respectively (Dunnet's test)

Table 3. Hematological and serum biochemical data of F344 female rats given madder color for 53 weeks

Items	Groups			
	Control	0.2%	1%	5%
RBC (x10 ⁴ /μL)	781.1 ± 49.5 ^{a)}	787.5 ± 40.1	738.4 ± 57.6	689.8 ± 63.2**
Hb (g/dL)	13.5 ± 1.5	13.6 ± 1.4	13.0 ± 1.0	12.3 ± 1.2
Ht (%)	44.4 ± 2.8	44.9 ± 2.3	42.0 ± 3.2	39.3 ± 3.6**
MCV (fL)	56.8 ± 0.3	57.0 ± 0.3	56.9 ± 0.6	57.0 ± 0.7
MCH (pg)	17.3 ± 1.1	17.3 ± 1.8	17.7 ± 0.2	17.8 ± 0.5
MCHC (g/dL)	30.3 ± 1.9	30.3 ± 3.1	31.0 ± 0.5	31.1 ± 0.9
PLT (x10 ⁴ /μL)	59.2 ± 6.3	61.1 ± 3.4	64.8 ± 5.3	82.6 ± 13.3**
WBC (x10 ² /μL)	21.8 ± 3.5	24.1 ± 2.1	24.3 ± 4.9	32.9 ± 9.4**
Differential cell counts (%)				
Neut-B	1.1 ± 0.7	0.3 ± 0.7**	0.4 ± 0.4**	0.4 ± 0.4*
Neut-S	19.1 ± 6.1	20.0 ± 6.1	20.2 ± 7.8	31.9 ± 7.0**
Eosino	1.0 ± 0.4	0.6 ± 0.6	1.3 ± 0.6	0.7 ± 0.6
Baso	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.3
Lymph	77.7 ± 6.9	76.7 ± 6.9	76.7 ± 7.5	65.4 ± 7.4**
Mono	1.0 ± 0.8	2.4 ± 0.9**	1.3 ± 0.6	1.6 ± 0.6
Elb	6.8 ± 4.2	7.0 ± 4.9	6.8 ± 3.3	8.1 ± 4.0
TP (g/dL)	7.1 ± 0.4	7.0 ± 0.5	6.9 ± 0.3	6.8 ± 0.4
A/G	2.2 ± 0.2	2.3 ± 0.2	2.1 ± 0.2	1.9 ± 0.2**
Alb (g/dL)	4.9 ± 0.4	4.8 ± 0.4	4.7 ± 0.2	4.4 ± 0.2**
TG (mg/dL)	65.9 ± 15.3	47.9 ± 11.7	58.3 ± 18.0	56.1 ± 14.5
TC (mg/dL)	124.9 ± 13.7	111.8 ± 11.7	116.8 ± 16.1	106.0 ± 8.3**
BUN (mg/dL)	17.2 ± 1.8	17.2 ± 2.3	17.1 ± 1.5	19.0 ± 1.3
CRE (mg/dL)	0.35 ± 0.04	0.33 ± 0.02	0.32 ± 0.03	0.33 ± 0.03
Na (mEq/dL)	143.8 ± 1.2	143.9 ± 1.1	144.0 ± 1.2	143.6 ± 1.5
Cl (mEq/dL)	102.5 ± 1.9	103.5 ± 1.8	102.7 ± 2.5	100.5 ± 1.4
K (mEq/dL)	3.9 ± 0.3	4.0 ± 0.2	4.2 ± 0.3**	4.4 ± 0.2**
Ca (mg/dL)	10.5 ± 0.5	10.5 ± 0.5	10.4 ± 0.4	10.2 ± 0.3
IP (mg/dL)	5.0 ± 0.5	5.1 ± 0.6	5.1 ± 0.5	5.9 ± 0.6**
AST (IU/L)	61.7 ± 4.6	66.9 ± 8.8	62.2 ± 4.1	66.0 ± 7.2
ALT (IU/L)	30.7 ± 2.7	30.6 ± 2.4	27.6 ± 5.2	20.2 ± 3.1**
ALP (IU/L)	121.0 ± 19.9	123.6 ± 23.4	120.3 ± 23.1	115.6 ± 16.4

^{a)} Mean ± SD

*,**: Significantly different from control at p<0.05 and p<0.01, respectively (Dunnet's test)

Table 4. Absolute organ weights of F344 rats given madder color for 53 weeks (g)

		Groups			
		Control	0.2%	1%	5%
Male	Body weight	396.1 ± 31.9 ^{a)}	412.4 ± 22.5	400.8 ± 21.4	326.4 ± 21.3**
	Brain	2.01 ± 0.08	2.05 ± 0.04	2.07 ± 0.07	2.02 ± 0.04
	Lung (R)	0.75 ± 0.07	0.75 ± 0.05	0.76 ± 0.06	0.70 ± 0.05
	Lung (L)	0.39 ± 0.03	0.39 ± 0.03	0.39 ± 0.09	0.37 ± 0.03
	Heart	1.07 ± 0.09	1.09 ± 0.06	1.11 ± 0.09	0.98 ± 0.08*
	Spleen	0.70 ± 0.06	0.74 ± 0.06	0.76 ± 0.07	0.73 ± 0.06
	Liver	8.7 ± 0.7	9.5 ± 0.7	10.0 ± 0.9**	8.6 ± 1.2
	Adrenal (R)	0.017 ± 0.005	0.018 ± 0.004	0.018 ± 0.002	0.017 ± 0.003
	Adrenal (L)	0.017 ± 0.003	0.019 ± 0.002	0.018 ± 0.004	0.017 ± 0.003
	Kidney (R)	1.1 ± 0.08	1.2 ± 0.08	1.3 ± 0.11**	1.2 ± 0.11
	Kidney (L)	1.1 ± 0.08	1.2 ± 0.06	1.4 ± 0.27**	1.2 ± 0.11
	Testis (R)	1.4 ± 0.4	1.8 ± 0.2	1.7 ± 0.1	1.7 ± 0.1
	Testis (L)	1.6 ± 0.3	1.6 ± 0.4	1.7 ± 0.1	1.8 ± 0.7
Females	Body weight	200.4 ± 6.3	192.2 ± 10.4	176.8 ± 8.2**	160.1 ± 5.4**
	Brain	1.85 ± 0.03	1.85 ± 0.04	1.77 ± 0.21	1.82 ± 0.05
	Lung (R)	0.52 ± 0.03	0.51 ± 0.02	0.51 ± 0.03	0.45 ± 0.02**
	Lung (L)	0.29 ± 0.02	0.27 ± 0.01	0.28 ± 0.02	0.25 ± 0.02**
	Heart	0.64 ± 0.03	0.63 ± 0.04	0.66 ± 0.06	0.58 ± 0.04*
	Spleen	0.46 ± 0.03	0.45 ± 0.04	0.45 ± 0.03	0.43 ± 0.03
	Liver	4.4 ± 0.2	4.3 ± 0.3	4.3 ± 0.4	4.2 ± 0.2
	Adrenal (R)	0.018 ± 0.003	0.019 ± 0.004	0.020 ± 0.056	0.017 ± 0.002
	Adrenal (L)	0.018 ± 0.002	0.020 ± 0.004	0.021 ± 0.004	0.016 ± 0.003
	Kidney (R)	0.63 ± 0.02	0.64 ± 0.04	0.73 ± 0.05**	0.69 ± 0.05*
	Kidney (L)	0.63 ± 0.03	0.65 ± 0.04	0.73 ± 0.05**	0.69 ± 0.04**

^{a)} Mean ± SD

*, **: Significantly different from control at p<0.05 and p<0.01, respectively (Dunnet's test)

Table 5. Relative organ weights of F344 rats given madder color for 53 weeks (g)

			Groups			
			Control	0.2%	1%	5%
Male	Brain	(g/100g b.w.)	0.51 ± 0.04 ^{a)}	0.50 ± 0.03	0.52 ± 0.02	0.62 ± 0.03**
	Lung (R)	(g/100g b.w.)	0.19 ± 0.01	0.18 ± 0.01	0.19 ± 0.01	0.22 ± 0.01**
	Lung (L)	(g/100g b.w.)	0.10 ± 0.01	0.10 ± 0.01	0.10 ± 0.02	0.11 ± 0.01**
	Heart	(g/100g b.w.)	0.27 ± 0.02	0.27 ± 0.01	0.28 ± 0.02	0.30 ± 0.02**
	Spleen	(g/100g b.w.)	0.18 ± 0.01	0.18 ± 0.01	0.19 ± 0.011*	0.22 ± 0.01**
	Liver	(g/100g b.w.)	2.20 ± 0.09	2.31 ± 0.07	2.49 ± 0.13**	2.63 ± 0.22**
	Adrenal (R)	(mg/100g b.w.)	0.004 ± 0.001	0.004 ± 0.001	0.004 ± 0.001	0.005 ± 0.001*
	Adrenal (L)	(mg/100g b.w.)	0.004 ± 0.001	0.005 ± 0.001	0.005 ± 0.001	0.005 ± 0.001
	Kidney (R)	(g/100g b.w.)	0.27 ± 0.02	0.28 ± 0.01	0.32 ± 0.02**	0.36 ± 0.02**
	Kidney (L)	(g/100g b.w.)	0.27 ± 0.02	0.28 ± 0.02	0.36 ± 0.07**	0.36 ± 0.02**
	Testis (R)	(g/100g b.w.)	0.36 ± 0.11	0.43 ± 0.03	0.42 ± 0.03	0.51 ± 0.03**
	Testis (L)	(g/100g b.w.)	0.40 ± 0.08	0.38 ± 0.09	0.43 ± 0.55	0.55 ± 0.19**
Females	Brain	(g/100g b.w.)	0.93 ± 0.03	0.97 ± 0.07	1.01 ± 0.14*	1.14 ± 0.05**
	Lung (R)	(g/100g b.w.)	0.26 ± 0.02	0.26 ± 0.02	0.29 ± 0.01**	0.28 ± 0.01*
	Lung (L)	(g/100g b.w.)	0.14 ± 0.01	0.14 ± 0.01	0.16 ± 0.01*	0.16 ± 0.01*
	Heart	(g/100g b.w.)	0.32 ± 0.01	0.33 ± 0.02	0.37 ± 0.03**	0.36 ± 0.02**
	Spleen	(g/100g b.w.)	0.23 ± 0.02	0.23 ± 0.02	0.25 ± 0.01*	0.27 ± 0.02**
	Liver	(g/100g b.w.)	2.21 ± 0.08	2.25 ± 0.11	2.44 ± 0.15**	2.60 ± 0.10**
	Adrenal (R)	(mg/100g b.w.)	0.009 ± 0.002	0.010 ± 0.002	0.011 ± 0.003	0.010 ± 0.001
	Adrenal (L)	(mg/100g b.w.)	0.009 ± 0.001	0.012 ± 0.002	0.012 ± 0.002**	0.010 ± 0.002
	Kidney (R)	(g/100g b.w.)	0.31 ± 0.01	0.33 ± 0.020	0.41 ± 0.02**	0.43 ± 0.03**
	Kidney (L)	(g/100g b.w.)	0.32 ± 0.01	0.34 ± 0.02*	0.41 ± 0.02**	0.43 ± 0.02**

^{a)} Mean ± SD

*, **: Significantly different from control at p<0.05 and p<0.01, respectively (Dunnet's test)

Table 6. Histopathological findings in the kidneys and mesenteric lymph nodes in male and female rats given madder color for 53 weeks

Sex Group No. of animals examined	Males				Females					
	Control	0.2%	1%	5%	Control	0.2%	1%	5%		
	10	10	10	10	10	10	10	10		
Kidneys										
Tubular regeneration, focal	Total incidence	1	1	4	10**,##	0	1	0	1	
	Grade	±	1	1	4	0	0	0	1	
		+	0	0	0	10	0	1	0	0
		++	0	0	0	0	0	0	0	0
Infiltration of mononuclear cells, inters	Total incidence	2	2	5	10**,##	1	1	0	1	
	Grade	±	2	2	4	6	0	0	0	0
		+	0	0	1	4	1	1	0	1
		++	0	0	0	0	0	0	0	0
Vacuolar degeneration, proximal tubul epithelium	Total incidence	0	0	10**	10**	5	4	10**,##	10**,##	
	Grade	±	0	0	1	0	5	4	1	0
		+	0	0	9	10	0	0	9	10
		++	0	0	0	0	0	0	0	0
Anisokaryosis, proximal tubular epithe outer medulla	Total incidence	1	0	1	10**,##	0	0	1	10**,##	
	Grade	±	1	0	1	4	0	0	1	8
		+	0	0	0	6	0	0	0	2
		++	0	0	0	0	0	0	0	0
Atypical tubules, outer medulla	Total incidence	0	0	2	9**	0	0	0	5*	
Adenoma, renal tubules	Total incidence	0	0	0	1	0	0	0	0	
Mesenteric lymph node										
Dilation, medullary sinus	Total incidence	5	3	6	10**,##	3	2	2	1	
	Grade	±	5	3	6	4	3	2	2	1
		+	0	0	0	3	0	0	0	0
		++	0	0	0	3	0	0	0	0

±:very slight, +:slight, ++:moderate

*,**.:Significantly different from control at p<0.05 and p<0.01, respectively (Fisher's exact probability test)

#,##.:Significantly different from control at p<0.05 and p<0.01, respectively (Mann-Whitney's U-test)

Table 7. Final body, absolute and relative kidney weights of F344 rats given madder color for 104 weeks

Sex	Male			Female		
	Control	2.5%	5%	Control	2.5%	5%
Group						
No. of animals examined ^{a)}	39	42	46	43	44	47
Body weight g	416 ± 47.2 ^{b)}	365.5 ± 28.8**	337 ± 19.9**	259 ± 26.2	206 ± 22.6**	187 ± 11.8**
Kidneys (absolute) g	2.54 ± 0.23	3.01 ± 1.03**	3.36 ± 2.89**	1.64 ± 0.12	2.04 ± 0.66**	1.81 ± 0.14**
Kidneys (relative) g/100g b.w.	0.61 ± 0.07	0.82 ± 0.26**	0.99 ± 0.76**	0.64 ± 0.05	1.00 ± 0.35**	0.97 ± 0.07**

^{a)} Animals those survived until the end of the experiment.

^{b)} Mean ± SD

** : Significantly different from the controls at p<0.01 (Dunnet's test).

Table 8. Incidences of non-neoplastic and neoplastic lesions in the kidney in male and female rats treated with madder color for 104 weeks

Sex Group	Male (%)			Female (%)		
	Control	2.5%	5%	Control	2.5%	5%
No. of animals examined	50	47 ^{b)}	50	50	47 ^{a)b)}	48 ^{b)}
Kidneys						
Anisokaryosis, renal tubules	0	39 (83)**	50 (100)** [†]	0	47 (100)**	48 (100)**
Atypical tubules	0	47 (100)**	50 (100)**	0	47 (100)**	48 (100)**
Adenoma or Carcinoma, renal tubule	0	19 (40)**	43 (86)** [§]	0	8 (16)**	15 (31)**
Adenoma	0	18 (38)**	39 (78)** [§]	0	5 (10)*	13 (27)**
Carcinoma	0	3 (6)	16 (32)** [§]	0	3 (6)	2 (4)
Mesenchymal tumors	0	1 (2)	0	1 (2)	0	0
Transitional cell carcinoma, renal pel0	0	1 (2)	0	0	0	0

^{a)} One animal which died before 52 weeks was not included.

^{b)} Two or three animals were not examined because of post-mortem degeneration.

*, **: Significantly different from control at p<0.05 and p<0.01, respectively (Fisher's exact probability test)

[†]: Significantly different from 2.5% group at p<0.01 (Fisher's exact probability test)

[§]: Significantly different from 2.5% group at p<0.01 (Chi-square test for independence)