

Table 1 Hematological examinations of rats treated orally with C.I. fluorescent brightner 271 in the combined repeated dose and reproductive/developmental toxicity screening test

Dose level	End of administration period				End of recovery period	
	0 mg/kg	20 mg/kg	60 mg/kg	200 mg/kg	0 mg/kg	200 mg/kg
<b>Male</b>						
No. of animals examined	5	5	5	5	5	5
HCT (%)	44.7 ± 1.0	45.2 ± 1.4	44.7 ± 1.3	41.9 ± 4.9	45.5 ± 1.4	36.6 ± 4.5**
HGB (g/dL)	15.7 ± 0.4	15.9 ± 0.3	15.7 ± 0.3	14.9 ± 1.8	15.8 ± 0.5	12.7 ± 1.7**
RBC (×10 <sup>6</sup> /mm <sup>3</sup> )	8.91 ± 0.30	9.08 ± 0.14	8.78 ± 0.20	8.65 ± 0.98	8.98 ± 0.38	7.40 ± 0.88**
MCV (μm <sup>3</sup> )	50.1 ± 1.6	49.8 ± 1.4	50.9 ± 2.5	48.4 ± 1.4	50.8 ± 2.1	49.4 ± 1.9
MCH (pg)	17.7 ± 0.2	17.6 ± 0.4	17.9 ± 0.6	17.2 ± 0.8	17.6 ± 0.6	17.2 ± 0.6
MCHC (%)	35.2 ± 0.8	35.3 ± 0.5	35.2 ± 0.7	35.6 ± 1.5	34.6 ± 0.3	34.8 ± 0.4
WBC (×10 <sup>3</sup> /mm <sup>3</sup> )	9.27 ± 2.71	12.90 ± 3.95	9.80 ± 1.13	14.91 ± 11.42	8.97 ± 1.71	15.29 ± 3.24**
<b>Differential leukocyte</b>						
NEUT (×10 <sup>3</sup> /mm <sup>3</sup> )	1.45 ± 0.50	1.35 ± 0.58	1.65 ± 0.11	2.00 ± 1.76	1.54 ± 0.57	2.32 ± 0.74
(%)	15.6 ± 2.0	11.0 ± 5.2	17.1 ± 3.1	13.1 ± 5.3	16.9 ± 4.0	15.2 ± 4.5
LYMPH (×10 <sup>3</sup> /mm <sup>3</sup> )	7.25 ± 2.01	10.85 ± 3.69	7.61 ± 1.14	12.09 ± 9.07	6.96 ± 1.20	12.18 ± 2.89**
(%)	78.6 ± 1.9	83.7 ± 6.2	77.4 ± 3.3	81.6 ± 6.5	77.9 ± 3.6	79.5 ± 5.0
MONO (×10 <sup>3</sup> /mm <sup>3</sup> )	0.28 ± 0.15	0.35 ± 0.16	0.24 ± 0.07	0.49 ± 0.48	0.25 ± 0.03	0.47 ± 0.13**
(%)	2.9 ± 0.9	2.7 ± 0.5	2.5 ± 0.5	3.0 ± 1.0	2.8 ± 0.6	3.1 ± 0.9
EOSN (×10 <sup>3</sup> /mm <sup>3</sup> )	0.21 ± 0.10	0.23 ± 0.13	0.19 ± 0.03	0.15 ± 0.12	0.11 ± 0.04	0.16 ± 0.04
(%)	2.1 ± 0.7	1.9 ± 1.2	1.9 ± 0.3	1.1 ± 0.6	1.2 ± 0.2	1.1 ± 0.4
BASO (×10 <sup>3</sup> /mm <sup>3</sup> )	0.01 ± 0.00	0.02 ± 0.01	0.01 ± 0.00	0.04 ± 0.04	0.01 ± 0.01	0.02 ± 0.01
(%)	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.2 ± 0.0*	0.1 ± 0.1	0.2 ± 0.1
LUC (×10 <sup>3</sup> /mm <sup>3</sup> )	0.06 ± 0.02	0.09 ± 0.05	0.09 ± 0.05	0.14 ± 0.10	0.09 ± 0.02	0.14 ± 0.06
(%)	0.7 ± 0.1	0.7 ± 0.2	0.9 ± 0.6	1.0 ± 0.2	1.1 ± 0.3	0.9 ± 0.3
PLT (×10 <sup>3</sup> /mm <sup>3</sup> )	1149 ± 82	1061 ± 108	1059 ± 100	1069 ± 130	1048 ± 32	1431 ± 150**
Reticulocyte (%)	2.4 ± 0.5	1.8 ± 0.3*	2.2 ± 0.2	0.8 ± 0.1**	2.0 ± 0.2	3.1 ± 0.8*
<b>Coagulation</b>						
PT (sec.)	18.4 ± 1.6	19.4 ± 2.5	19.7 ± 2.6	22.9 ± 6.0	18.9 ± 2.4	15.9 ± 1.3*
APTT (sec.)	28.6 ± 2.1	26.5 ± 2.1	29.1 ± 1.7	28.4 ± 3.5	28.2 ± 2.0	23.3 ± 4.4
<b>Female</b>						
No. of animals examined	6	5	5	5	5	5
HCT (%)	38.5 ± 1.2 (5)	40.1 ± 2.4	40.0 ± 1.2	32.5 ± 1.3**	43.1 ± 1.6	37.1 ± 2.4**
HGB (g/dL)	13.2 ± 0.6 (5)	13.7 ± 0.8	13.5 ± 0.5	11.4 ± 0.4**	14.7 ± 0.5	13.1 ± 0.8**
RBC (×10 <sup>6</sup> /mm <sup>3</sup> )	6.81 ± 0.30 (5)	6.86 ± 0.39	7.22 ± 0.29	6.16 ± 0.27*	8.24 ± 0.24	7.22 ± 0.44**
MCV (μm <sup>3</sup> )	56.6 ± 1.3 (5)	58.4 ± 1.5	55.5 ± 1.5	52.8 ± 1.3**	52.3 ± 1.6	51.3 ± 0.5
MCH (pg)	19.4 ± 0.3 (5)	20.0 ± 0.5	18.7 ± 0.6*	18.6 ± 0.4*	17.9 ± 0.6	18.1 ± 0.2
MCHC (%)	34.4 ± 0.6 (5)	34.2 ± 0.5	33.6 ± 0.5	35.1 ± 0.5	34.1 ± 0.3	35.2 ± 0.4**
WBC (×10 <sup>3</sup> /mm <sup>3</sup> )	10.95 ± 2.38 (5)	10.99 ± 4.79	11.27 ± 3.56	9.72 ± 1.84	4.76 ± 1.84	5.58 ± 1.33
<b>Differential leukocyte</b>						
NEUT (×10 <sup>3</sup> /mm <sup>3</sup> )	4.23 ± 1.57 (5)	3.92 ± 2.36	3.85 ± 0.66	2.75 ± 1.25	0.86 ± 0.49	1.06 ± 0.22
(%)	38.0 ± 8.8 (5)	34.6 ± 6.9	35.6 ± 7.4	27.5 ± 8.2	17.2 ± 4.9	19.2 ± 2.6
LYMPH (×10 <sup>3</sup> /mm <sup>3</sup> )	6.29 ± 1.18 (5)	6.62 ± 2.42	6.86 ± 3.00	6.55 ± 0.91	3.63 ± 1.30	4.28 ± 1.09
(%)	58.0 ± 8.2 (5)	61.4 ± 6.2	59.6 ± 8.2	68.2 ± 6.8	77.2 ± 4.9	76.4 ± 2.7
MONO (×10 <sup>3</sup> /mm <sup>3</sup> )	0.25 ± 0.12 (5)	0.28 ± 0.15	0.35 ± 0.21	0.25 ± 0.14	0.13 ± 0.06	0.13 ± 0.05
(%)	2.3 ± 0.8 (5)	2.5 ± 0.7	3.0 ± 1.3	2.7 ± 1.5	2.6 ± 0.4	2.4 ± 0.5
EOSN (×10 <sup>3</sup> /mm <sup>3</sup> )	0.08 ± 0.01 (5)	0.09 ± 0.03	0.10 ± 0.04	0.08 ± 0.11	0.09 ± 0.03	0.06 ± 0.02
(%)	0.7 ± 0.1 (5)	0.9 ± 0.4	0.9 ± 0.4	0.8 ± 1.0	1.9 ± 0.3	1.0 ± 0.3**
BASO (×10 <sup>3</sup> /mm <sup>3</sup> )	0.01 ± 0.00 (5)	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.01	0.03 ± 0.01
(%)	0.1 ± 0.0 (5)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0
LUC (×10 <sup>3</sup> /mm <sup>3</sup> )	0.09 ± 0.03 (5)	0.06 ± 0.04	0.10 ± 0.06	0.07 ± 0.02	0.05 ± 0.04	0.05 ± 0.02
(%)	0.9 ± 0.3 (5)	0.5 ± 0.2	0.9 ± 0.4	0.7 ± 0.3	1.0 ± 0.5	0.9 ± 0.2
PLT (×10 <sup>3</sup> /mm <sup>3</sup> )	1131 ± 80 (5)	1108 ± 105	1175 ± 263	1397 ± 109*	1017 ± 108	1295 ± 76**
Reticulocyte (%)	7.8 ± 0.7 (5)	7.8 ± 1.6	6.5 ± 1.4	3.8 ± 1.7**	1.8 ± 0.6	3.0 ± 0.6*
<b>Coagulation</b>						
PT (sec.)	17.4 ± 0.4 (5)	17.9 ± 0.5	17.4 ± 0.8	17.5 ± 0.4	16.1 ± 0.9	15.4 ± 0.8
APTT (sec.)	19.6 ± 1.2 (5)	18.8 ± 1.5	17.6 ± 1.6	15.7 ± 0.7**	19.4 ± 1.7	18.6 ± 1.9

NEUT: Neutrophil LYMPH: Lymphocyte MONO: Monocyte EOSN: Eosinophil BASO: Basophil LUC: Large unstained cells

Values are expressed as Mean ± S.D.

Values in parentheses are expressed no. of animals examined

Significant difference from the control group; \*p ≤ 0.05, \*\*p ≤ 0.01.

Table 2 Blood chemical examinations of rats treated orally with C.I. fluorescent brightner 271 in the combined repeated dose and reproductive/developmental toxicity screening test

Dose level	End of administration period				End of recovery period	
	0 mg/kg	20 mg/kg	60 mg/kg	200 mg/kg	0 mg/kg	200 mg/kg
<b>Male</b>						
No. of animals examined	5	5	5	5	5	5
T.protein (g/dL)	6.15 ± 0.19	5.99 ± 0.20	6.17 ± 0.15	6.50 ± 0.47	6.04 ± 0.16	6.12 ± 0.71
Albumin (g/dL)	3.23 ± 0.05	3.11 ± 0.07*	3.22 ± 0.09	3.35 ± 0.33	3.11 ± 0.08	2.63 ± 0.89
A/G	1.11 ± 0.09	1.08 ± 0.05	1.09 ± 0.05	1.06 ± 0.08	1.06 ± 0.04	0.77 ± 0.30
Glucose (mg/dL)	160 ± 12	149 ± 13	154 ± 19	145 ± 18	161 ± 15	153 ± 22
Triglyceride (mg/dL)	44.1 ± 15.1	43.3 ± 11.6	55.1 ± 16.8	45.6 ± 34.4	47.3 ± 8.4	177.9 ± 188.1
T.cholesterol (mg/dL)	66 ± 7	58 ± 10	56 ± 9	81 ± 26	71 ± 18	252 ± 207**
BUN (mg/dL)	13.2 ± 1.7	12.3 ± 0.6	13.6 ± 1.0	22.7 ± 17.7	13.1 ± 1.1	22.5 ± 14.8*
Creatinine (mg/dL)	0.30 ± 0.02	0.27 ± 0.02	0.31 ± 0.03	0.72 ± 0.60*	0.26 ± 0.03	0.89 ± 0.52**
T.bilirubin (mg/dL)	0.06 ± 0.01	0.05 ± 0.01	0.05 ± 0.02	0.04 ± 0.01	0.04 ± 0.01	0.02 ± 0.02*
Total bile acid (μmol/L)	13.3 ± 5.6	12.4 ± 5.2	12.2 ± 6.8	22.3 ± 16.2	13.4 ± 6.1	19.7 ± 11.6
AST (U/L)	87 ± 13	84 ± 6	83 ± 7	110 ± 38	104 ± 29	109 ± 70
ALT (U/L)	30 ± 3	30 ± 3	32 ± 3	45 ± 21	31 ± 2	60 ± 30**
ALP (U/L)	445 ± 43	411 ± 134	445 ± 61	645 ± 308	332 ± 14	379 ± 176
Gamma-GTP (U/L)	0.6 ± 0.2	0.6 ± 0.1	0.6 ± 0.1	1.1 ± 0.7	0.5 ± 0.1	1.1 ± 0.2**
Calcium (mg/dL)	9.83 ± 0.41	9.65 ± 0.34	9.65 ± 0.24	10.21 ± 0.64	9.83 ± 0.17	10.32 ± 0.26**
L.phosphorus (mg/dL)	6.80 ± 0.49	6.11 ± 0.61	6.41 ± 0.20	6.28 ± 1.02	6.26 ± 0.50	6.58 ± 0.78
Sodium (mmol/L)	143.5 ± 1.8	143.7 ± 2.2	144.5 ± 1.4	143.0 ± 1.8	143.6 ± 1.4	142.5 ± 1.3
Potassium (mmol/L)	4.57 ± 0.30	4.51 ± 0.30	4.36 ± 0.19	3.92 ± 0.41*	4.39 ± 0.21	4.20 ± 0.44
Chloride (mmol/L)	107.4 ± 0.8	108.6 ± 1.4	108.9 ± 1.4	107.1 ± 4.0	108.1 ± 1.1	106.9 ± 1.9
<b>Female</b>						
No. of animals examined	6	5	5	5	5	5
T.protein (g/dL)	6.12 ± 0.34 (5)	6.16 ± 0.30	6.14 ± 0.26	6.56 ± 0.62	6.43 ± 0.31	6.71 ± 0.54
Albumin (g/dL)	3.32 ± 0.18 (5)	3.27 ± 0.13	3.36 ± 0.18	3.39 ± 0.40	3.72 ± 0.22	3.51 ± 0.68
A/G	1.19 ± 0.12 (5)	1.15 ± 0.13	1.21 ± 0.08	1.07 ± 0.11	1.38 ± 0.16	1.11 ± 0.25
Glucose (mg/dL)	134 ± 18 (5)	150 ± 10	134 ± 16	120 ± 8	138 ± 13	122 ± 16
Triglyceride (mg/dL)	48.2 ± 27.8 (5)	49.4 ± 23.2	49.3 ± 15.5	33.9 ± 6.6	22.0 ± 7.7	127.6 ± 238.9
T.cholesterol (mg/dL)	65 ± 10 (5)	63 ± 6	75 ± 3	90 ± 12**	93 ± 13	151 ± 130
BUN (mg/dL)	15.4 ± 2.1 (5)	17.3 ± 3.7	16.6 ± 3.5	19.3 ± 3.7	14.3 ± 1.7	16.0 ± 1.3
Creatinine (mg/dL)	0.31 ± 0.03 (5)	0.35 ± 0.05	0.39 ± 0.04	0.51 ± 0.11**	0.33 ± 0.07	0.58 ± 0.06**
T.bilirubin (mg/dL)	0.05 ± 0.02 (5)	0.04 ± 0.02	0.05 ± 0.03	0.04 ± 0.01	0.06 ± 0.02	0.03 ± 0.03
Total bile acid (μmol/L)	20.0 ± 12.0 (5)	11.4 ± 2.4	26.2 ± 13.8	24.3 ± 20.3	12.6 ± 2.2	13.8 ± 3.1
AST (U/L)	117 ± 32 (5)	98 ± 13	101 ± 35	97 ± 18	108 ± 41	100 ± 41
ALT (U/L)	53 ± 10 (5)	47 ± 13	51 ± 17	67 ± 13	25 ± 3	44 ± 12**
ALP (U/L)	231 ± 62 (5)	255 ± 51	224 ± 58	284 ± 48	187 ± 44	183 ± 55
Gamma-GTP (U/L)	0.6 ± 0.1 (5)	0.6 ± 0.2	0.7 ± 0.3	0.8 ± 0.1	0.7 ± 0.2	1.1 ± 0.5
Calcium (mg/dL)	10.13 ± 0.35 (5)	10.21 ± 0.17	10.29 ± 0.22	10.17 ± 0.48	9.92 ± 0.27	10.34 ± 0.20*
L.phosphorus (mg/dL)	8.39 ± 0.20 (5)	8.40 ± 1.46	7.65 ± 0.95	6.84 ± 0.70*	4.81 ± 0.81	5.40 ± 0.43
Sodium (mmol/L)	141.6 ± 1.6 (5)	142.3 ± 1.2	142.1 ± 1.7	139.2 ± 1.1*	142.2 ± 0.6	139.9 ± 1.0**
Potassium (mmol/L)	4.22 ± 0.26 (5)	4.45 ± 0.34	4.19 ± 0.43	3.63 ± 0.14*	4.15 ± 0.20	3.73 ± 0.30*
Chloride (mmol/L)	107.3 ± 2.0 (5)	108.9 ± 2.7	110.2 ± 2.6	108.4 ± 2.2	109.8 ± 1.3	107.9 ± 2.9

Values are expressed as Mean ± S.D.

Values in parentheses are expressed no. of animals examined

Significant difference from the control group; \* $p \leq 0.05$ , \*\* $p \leq 0.01$ .

Table 3 Absolute and relative organ weights of rats treated orally with C.I. fluorescent brightner 271 in the combined repeated dose and reproductive/developmental toxicity screening test

Dose level	End of administration period				End of recovery period	
	0 mg/kg	20 mg/kg	60 mg/kg	200 mg/kg	0 mg/kg	200 mg/kg
<b>Male</b>						
No. of animals examined	12	12	12	12	5	5
Body weight (g)	452 ± 29	446 ± 31	456 ± 31	391 ± 30	480 ± 32	401 ± 52
Absolute organ weight						
Brain (g)	2.19 ± 0.11	2.2 ± 0.09	2.21 ± 0.09	2.19 ± 0.07	2.22 ± 0.07	2.21 ± 0.09
Thymus (mg)	289 ± 89	266 ± 63	298 ± 79	270 ± 60	258 ± 51	280 ± 76
Heart (g)	1.43 ± 0.13	1.47 ± 0.19	1.42 ± 0.14	1.33 ± 0.12	1.47 ± 0.16	1.26 ± 0.2
Liver (g)	11.14 ± 1.06	10.86 ± 1.34	11.15 ± 1.41	9.88 ± 1.20*	11.47 ± 1.07	11.61 ± 0.72
Spleen (g)	0.66 ± 0.1	0.73 ± 0.16	0.64 ± 0.07	0.76 ± 0.18	0.68 ± 0.07	0.76 ± 0.1
Kidneys (g)	2.94 ± 0.35	2.93 ± 0.26	2.88 ± 0.17	3.21 ± 0.34	2.99 ± 0.19	3.07 ± 0.25
Adrenals (mg)	59 ± 7	51 ± 6*	55 ± 9	59 ± 7	57 ± 9	63 ± 11
Testes (g)	3.25 ± 0.31	3.3 ± 0.32	3.4 ± 0.19	3.39 ± 0.29	3.29 ± 0.2	3.53 ± 0.25
Epididymides (mg)	1218 ± 106	1220 ± 138	1206 ± 101	1184 ± 87	1229 ± 134	1161 ± 104
Relative organ weight						
Brain (g%)	0.485 ± 0.031	0.496 ± 0.025	0.485 ± 0.025	0.562 ± 0.035**	0.463 ± 0.032	0.556 ± 0.054*
Thymus (mg%)	64.17 ± 21.063	60.034 ± 14.605	65.229 ± 16.542	68.536 ± 12.103	53.364 ± 7.804	69.149 ± 12.953*
Heart (g%)	0.318 ± 0.029	0.328 ± 0.023	0.312 ± 0.024	0.341 ± 0.023	0.306 ± 0.035	0.314 ± 0.024
Liver (g%)	2.463 ± 0.139	2.429 ± 0.138	2.438 ± 0.157	2.523 ± 0.188	2.387 ± 0.159	2.929 ± 0.414*
Spleen (g%)	0.147 ± 0.019	0.164 ± 0.028	0.14 ± 0.016	0.194 ± 0.043**	0.141 ± 0.02	0.193 ± 0.035*
Kidneys (g%)	0.649 ± 0.062	0.657 ± 0.045	0.633 ± 0.037	0.82 ± 0.067**	0.625 ± 0.053	0.773 ± 0.097*
Adrenals (mg%)	13.073 ± 1.618	11.327 ± 1.045*	12.181 ± 1.838	15.07 ± 2.085*	11.957 ± 2.324	15.923 ± 3.13
Testes (g%)	0.723 ± 0.092	0.741 ± 0.068	0.748 ± 0.039	0.871 ± 0.083**	0.686 ± 0.053	0.891 ± 0.130*
Epididymides (mg%)	270.526 ± 30.507	273.713 ± 22.323	264.847 ± 16.929	303.824 ± 23.115**	255.992 ± 23.132	293.073 ± 44.486
<b>Female</b>						
No. of animals examined	12	12	9	12	5	5
Body weight (g)	299 ± 23	295 ± 17	300 ± 22	251 ± 13	281 ± 16	228 ± 11
Absolute organ weight						
Brain (g)	2.05 ± 0.08	2.08 ± 0.06	2.1 ± 0.06	2.09 ± 0.1	2.09 ± 0.05	2.06 ± 0.08
Thymus (mg)	245 ± 69	223 ± 80	242 ± 69	174 ± 79	255 ± 117	244 ± 53
Heart (g)	1.06 ± 0.17	1 ± 0.06	0.99 ± 0.06	0.94 ± 0.08	0.94 ± 0.09	0.83 ± 0.03*
Liver (g)	9.71 ± 0.92	10.35 ± 1.59	9.57 ± 0.69	8.97 ± 1.07	7.16 ± 0.36	6.58 ± 0.87
Spleen (g)	0.63 ± 0.08	0.64 ± 0.12	0.6 ± 0.07	0.58 ± 0.08	0.48 ± 0.05	0.54 ± 0.07
Kidneys (g)	1.96 ± 0.19	1.94 ± 0.15	1.92 ± 0.14	2.57 ± 0.34**	1.95 ± 0.19	2.17 ± 0.09
Adrenals (mg)	75 ± 11	81 ± 8	76 ± 9	73 ± 11	69 ± 11	62 ± 4
Ovaries (mg)	99 ± 15	107 ± 15	93 ± 11	93 ± 10	85 ± 14	85 ± 9
Relative organ weight						
Brain (g%)	0.687 ± 0.048	0.708 ± 0.036	0.702 ± 0.041	0.836 ± 0.064**	0.746 ± 0.042	0.903 ± 0.041**
Thymus (mg%)	81.833 ± 21.652	75.247 ± 26.178	79.84 ± 18.091	69.469 ± 31.524	91.255 ± 42.894	106.87 ± 20.904
Heart (g%)	0.355 ± 0.049	0.338 ± 0.027	0.332 ± 0.024	0.378 ± 0.042	0.335 ± 0.020	0.365 ± 0.028
Liver (g%)	3.249 ± 0.296	3.505 ± 0.471	3.195 ± 0.128	3.574 ± 0.394*	2.550 ± 0.077	2.891 ± 0.431**
Spleen (g%)	0.209 ± 0.017	0.216 ± 0.04	0.2 ± 0.023	0.231 ± 0.032	0.170 ± 0.016	0.237 ± 0.031**
Kidneys (g%)	0.657 ± 0.049	0.659 ± 0.034	0.64 ± 0.029	1.033 ± 0.172**	0.694 ± 0.029	0.951 ± 0.035**
Adrenals (mg%)	25.012 ± 3.092	27.494 ± 2.751	25.306 ± 2.792	29.294 ± 4.815*	24.557 ± 3.102	27.262 ± 1.287
Ovaries (mg%)	33.193 ± 4.809	36.578 ± 5.825	31.14 ± 4.202	37.016 ± 4.863	30.424 ± 5.140	37.366 ± 3.803*

Values are expressed as Mean ± S.D.

(%)(Organ weight / body weight) × 100

Significant difference from the control group; \*p ≤ 0.05; \*\*p ≤ 0.01.

Table 4 Summary of histological findings with statistical analysis treated orally with C. I. fluorescent brightner 271 in the combined repeated dose and reproductive/developmental toxicity screening test

Dose level(mg / kg)	Administration period												Recovery period																							
	Sacriced												Non-pregnancy				Sacriced																			
	0			20			60			200			60				0				200															
Organ Findings	1	2	3	T	1	2	3	T	1	2	3	T	1	2	3	T	1	2	3	T	1	2	3	T	1	2	3	T								
Male	12				12				9				12				2					5					5									
No. of animals necropsied																																				
<b>CARDIOVASCULAR SYSTEM</b>																																				
heart	(5)				(0)				(0)				(5)				(0)					(5)					(5)									
cellular infiltration, mononuclear fibrosis	1	0	0	1	-	-	-	-	-	-	-	-	-	-	-	-	2	0	0	2	-	-	-	-	-	-	-	-	1	0	0	1	2	0	0	2
<b>HEMATOPOIETIC SYSTEM</b>																																				
bone marrow	(5)				(5)				(5)				(5)				(0)					(5)					(5)									
hematopoiesis, decreased, erythrocytic series	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	-	-	-	-	-	-	-	-	0	0	0	0	1	0	0	1				
spleen	(5)				(5)				(5)				(5)				(0)					(5)					(5)									
hyperplasia, follicle increase, pigment deposition	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	3	-	-	-	-	-	-	-	-	0	0	0	0	0	0	0	0				
<b>RESPIRATORY SYSTEM</b>																																				
lung	(6)				(1)				(0)				(6)				(0)					(5)					(5)									
congestion	0	0	0	0	0	0	0	0	-	-	-	-	1	0	0	1	-	-	-	-	-	-	-	-	0	0	0	0	0	0	0	0				
hemorrhage	1	0	0	1	0	0	0	0	-	-	-	-	0	0	0	0	-	-	-	-	-	-	-	-	0	0	0	0	0	0	0	0				
accumulation of foamy cells	2	0	0	2	1	0	0	1	-	-	-	-	0	0	0	0	-	-	-	-	1	0	0	1	2	0	0	2								
deposit, pigment	0	0	0	0	0	0	0	0	-	-	-	-	0	0	0	0	-	-	-	-	1	0	0	1	0	0	0	0								
hypertrophy, media, artery	0	0	0	0	0	0	0	0	-	-	-	-	0	0	0	0	-	-	-	-	0	0	0	0	1	0	0	1								
<b>DIGESTIVE SYSTEM</b>																																				
glandular stomach	(5)				(0)				(1)				(5)				(0)					(5)					(5)									
squamous cyst	0	0	0	0	-	-	-	-	1	0	0	1	0	0	0	0	-	-	-	-	0	0	0	0	0	0	0	0								
exocrine pancreas	(5)				(0)				(0)				(5)				(0)					(5)					(5)									
atrophy, acinus	1	0	0	1	-	-	-	-	-	-	-	-	0	0	0	0	-	-	-	-	0	0	0	0	0	0	0	0								
cellular infiltration, lymphocyte	0	0	0	0	-	-	-	-	-	-	-	-	1	0	0	1	-	-	-	-	0	0	0	0	0	0	0	0								
hyperplasia, duct	0	0	0	0	-	-	-	-	-	-	-	-	1	0	0	1	-	-	-	-	0	0	0	0	0	0	0	0								
decrease, zymogen granules, acinar cell	0	0	0	0	-	-	-	-	-	-	-	-	0	0	0	0	-	-	-	-	0	0	0	0	1	0	0	1								
duodenum	(5)				(0)				(0)				(5)				(0)					(5)					(5)									
erosion	0	0	0	0	-	-	-	-	-	-	-	-	0	0	0	0	-	-	-	-	0	0	0	0	1	0	0	1								
liver	(5)				(5)				(5)				(5)				(0)					(5)					(5)									
necrosis, hepatocyte	1	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	-	-	-	-	0	0	0	0	0	0	0	0								
microgranuloma	5	0	0	5	5	0	0	5	1	1	0	2	4	1	0	5	-	-	-	-	4	0	0	4	3	0	0	3								
hyperplasia, bile duct	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	-	-	-	-	0	0	0	0	0	0	0	0								
proliferation, sinusoidal cell	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	-	-	-	-	0	0	0	0	0	0	0	0								
hemorrhage	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	-	-	-	0	0	0	0	1	0	0	1								
hepatodiaphragmatic nodule	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	-	-	-	0	0	0	0	1	0	0	1								
<b>URINARY SYSTEM</b>																																				
kidney	(6)				(8)				(5)				(12)				(0)					(5)					(5)									
basophilic tubules	1	0	0	1	4	0	0	4	2	0	0	2	0	0	0	0	-	-	-	-	0	0	0	0	0	0	0	0								
cast, hyaline	0	0	0	0	0	0	0	0	0	0	0	0	7	2	1	10 <sup>****</sup>	-	-	-	-	0	0	0	0	0	2	3	5 <sup>****</sup>								
cyst	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	-	-	-	-	0	0	0	0	0	0	0	0								
degeneration, vacuolar, proximal tubule	0	0	0	0	1	0	0	1	4	1	0	5 <sup>****</sup>	0	0	12	12 <sup>****</sup>	-	-	-	-	0	0	0	0	0	1	4	5 <sup>****</sup>								
dilatation, tubules	0	0	0	0	0	0	0	0	0	0	0	0	6	1	0	7 <sup>**</sup>	-	-	-	-	0	0	0	0	4	1	0	5 <sup>****</sup>								
hyaline droplet, glomerulus	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	-	-	-	-	0	0	0	0	0	0	0	0								
mineralization	0	0	0	0	1	0	0	1	1	0	0	1	2	0	0	2	-	-	-	-	1	0	0	1	0	0	0	0								
necrosis, tubular epithelium, proximal tubule	0	0	0	0	0	0	0	0	0	0	0	0	1	3	8	12 <sup>****</sup>	-	-	-	-	0	0	0	0	0	5	0	5 <sup>****</sup>								
regeneration, tubule	0	0	0	0	0	0	0	0	0	0	0	0	5	7	0	12 <sup>****</sup>	-	-	-	-	0	0	0	0	0	0	5	5 <sup>****</sup>								
cellular infiltration, lymphocyte	0	0	0	0	0	0	0	0	1	0	0	1	7	0	0	7 <sup>**</sup>	-	-	-	-	0	0	0	0	5	0	0	5 <sup>**</sup>								
dilatation, renal pelvis	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	-	-	-	-	0	0	0	0	0	0	0	0								
fibrosis, diffuse	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	-	-	-	0	0	0	0	2	0	0	2								
fibrosis, focal	1	0	0	1	3	0	0	3	0	0	0	0	1	0	0	1	-	-	-	-	1	0	0	1	0	0	0	0								
hypertrophy, tubular epithelium, collecting tubule	0	0	0	0	0	0	0	0	2	0	0	2	11	0	0	11 <sup>**</sup>	-	-	-	-	0	0	0	0	5	0	0	5 <sup>**</sup>								
hypertrophy, tubular epithelium, Henle's loop	0	0	0	0	0	0	0	0	4	1	0	5 <sup>****</sup>	0	1	11	12 <sup>****</sup>	-	-	-	-	0	0	0	0	0	0	5	5 <sup>****</sup>								
<b>REPRODUCTIVE SYSTEM</b>																																				
epididymis	(5)				(1)				(0)				(5)				(2)					(5)					(5)									
spermatic granuloma	0	0	0	0	1	0	0	1	-	-	-	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0								
prostate	(5)				(0)				(0)				(5)				(2)					(5)					(5)									
cellular infiltration, lymphocyte	4	0	0	4	-	-	-	-	-	-	-	-	2	0	0	2	1	0	0	1	2	0	0	2	1	0	0	1								
inflammation	1	0	0	1	-	-	-	-	-	-	-	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0								
<b>ENDOCRINE SYSTEM</b>																																				
adrenal gland	(5)				(0)				(0)				(5)				(0)					(5)					(5)									
vacuolation, cortex	0	0	0	0	-	-	-	-	-	-	-	-	1	0	0	1	-	-	-	-	1	0	0	1	0	0	0	0								
<b>SPECIAL SENSE SYSTEM</b>																																				
eye	(5)				(0)				(0)				(5)				(0)					(5)					(5)									
dysplasia, retina	1	0	0	1	-	-	-	-	-	-	-	-	1	0	0	1	-	-	-	-	0	0	0	0	0	0	0	0								
Harderian gland	(5)				(0)				(0)				(5)				(0)					(5)					(5)									
cellular infiltration, lymphocyte	1	0	0	1	-	-	-	-	-	-	-	-	1	0	0	1	-	-	-	-	0	0	0	0	1	0	0	1								

Grade of histopathological finding; 1:slight, 2:moderate, 3:marked T:Total -:Not examined  
 Numbers in parentheses indicate number of animals examined microscopically at this site  
 Significant difference from control group; Fisher \*:p<0.05 \*\*:p<0.01, Mann-Whitney #:p<0.05 ##:p<0.01

Table 4 (Continued)

Dose level(mg / kg)	Administration period												Recovery period																			
	Sacrificed												Non-pregnancy																			
	0				20				60				200				60				Sacrificed											
Organ Findings	1	2	3	T	1	2	3	T	1	2	3	T	1	2	3	T	1	2	3	T	1	2	3	T	1	2	3	T				
Female	12				12				9				12				2				5				5							
No. of animals necropsied																																
<b>CARDIOVASCULAR SYSTEM</b>																																
heart	(5)				(0)				(0)				(5)				(0)				(5)				(5)							
cellular infiltration, mononuclear	0	0	0	0	-	-	-	-	-	-	-	-	0	0	0	0	-	-	-	-	2	0	0	2	0	0	0	0				
<b>HEMATOPOIETIC SYSTEM</b>																																
bone marrow	(5)				(5)				(5)				(5)				(0)				(5)				(5)							
hematopoiesis, increased, granulocytic series	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	2	-	-	-	-	0	0	0	0	0	0	0	0	0	0	0	0
hematopoiesis, decreased, erythrocytic series	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	-	-	-	0	0	0	0	2+	0	0	2+				
thymus	(5)				(1)				(0)				(5)				(0)				(5)				(5)							
atrophy, cortex	0	0	0	0	1	0	0	1	-	-	-	-	1	0	0	1	-	-	-	-	0	0	0	0	0	0	0	0	0	0	0	0
Kuersteiner's duct/cyst	1	0	0	1	0	0	0	0	-	-	-	-	1	0	0	1	-	-	-	-	2	0	0	2	0	0	0	0	0	0	0	0
<b>RESPIRATORY SYSTEM</b>																																
lung	(5)				(0)				(1)				(5)				(0)				(5)				(5)							
lymphangiectasis	0	0	0	0	-	-	-	-	0	0	0	0	1	0	0	1	-	-	-	-	0	0	0	0	0	0	0	0	0	0	0	0
accumulation of foamy cells	2	0	0	2	-	-	-	-	0	0	0	0	1	0	0	1	-	-	-	-	2	0	0	2	1	0	0	1				
cellular infiltration, mixed	0	0	0	0	-	-	-	-	1	0	0	1	0	0	0	0	-	-	-	-	0	0	0	0	0	0	0	0	0	0	0	0
osseous metaplasia	0	0	0	0	-	-	-	-	0	0	0	0	0	0	0	0	-	-	-	-	1	0	0	1	1	0	0	1				
<b>DIGESTIVE SYSTEM</b>																																
forestomach	(5)				(0)				(0)				(5)				(0)				(5)				(5)							
squamous hyperplasia	1	0	0	1	-	-	-	-	-	-	-	-	0	0	0	0	-	-	-	-	0	0	0	0	0	0	0	0	0	0	0	0
glandular stomach	(5)				(1)				(1)				(5)				(0)				(5)				(5)							
erosion	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	-	-	-	-	0	0	0	0	0	0	0	0	0	0	0	0
ulcer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	-	-	-	-	0	0	0	0	0	0	0	0	0	0	0	0
fibrosis	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	-	-	-	-	0	0	0	0	0	0	0	0	0	0	0	0
exocrine pancreas	(5)				(0)				(0)				(5)				(0)				(5)				(5)							
decrease, zymogen granules, acinar cell	0	0	0	0	-	-	-	-	-	-	-	-	0	0	0	0	-	-	-	-	1	0	0	1	2	0	0	2				
cellular infiltration, lymphocyte	0	0	0	0	-	-	-	-	-	-	-	-	0	0	0	0	-	-	-	-	1	0	0	1	0	0	0	0	0	0	0	0
focus, hypertrophic, basophilic	0	0	0	0	-	-	-	-	-	-	-	-	0	0	0	0	-	-	-	-	0	0	0	0	1	0	0	1				
hyperplasia, duct	0	0	0	0	-	-	-	-	-	-	-	-	0	0	0	0	-	-	-	-	1	0	0	1	0	0	0	0	0	0	0	0
liver	(5)				(5)				(5)				(5)				(0)				(5)				(5)							
fatty change, hepatocyte	1	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	-	-	-	-	1	0	0	1	0	0	0	0	0	0	0	0
microgranuloma	1	0	0	1	1	0	0	1	2	0	0	2	3	1	0	4	-	-	-	-	5	0	0	5	4	1	0	5				
extramedullary hematopoiesis	2	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	-	-	-	-	0	0	0	0	0	0	0	0	0	0	0	0
proliferation, sinusoidal cell	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	-	-	-	-	0	0	0	0	1	0	0	1				
<b>URINARY SYSTEM</b>																																
kidney	(6)				(6)				(7)				(12)				(0)				(5)				(5)							
angiectasis, glomerulus	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	-	-	-	-	0	0	0	0	0	0	0	0	0	0	0	0
basophilic tubules	1	0	0	1	3	1	0	4	0	0	0	0	0	0	0	0	-	-	-	-	1	0	0	1	0	0	0	0	0	0	0	0
cast, hyaline	0	0	0	0	0	0	0	0	0	0	0	0	5	1	1	7 <sup>++</sup>	-	-	-	-	0	0	0	0	2	1	1	4 <sup>++</sup>				
degeneration, vacuolar, proximal tubule	0	0	0	0	2	0	0	2	0	2	4	6 <sup>***</sup>	0	0	12	12 <sup>***</sup>	-	-	-	-	0	0	0	0	0	0	5	5 <sup>***</sup>				
dilatation, tubules	0	0	0	0	0	0	0	0	0	0	0	0	7	1	0	8 <sup>++</sup>	-	-	-	-	0	0	0	0	1	0	0	1				
mineralization	0	0	0	0	3	0	0	3	1	0	0	1	3	0	0	3	-	-	-	-	2	0	0	2	0	0	0	0				
necrosis, tubular epithelium, proximal tubule	0	0	0	0	0	0	0	0	0	0	0	0	0	3	9	12 <sup>***</sup>	-	-	-	-	0	0	0	0	0	5	0	5 <sup>***</sup>				
regeneration, tubule	0	0	0	0	0	0	0	0	0	0	0	0	4	8	0	12 <sup>***</sup>	-	-	-	-	0	0	0	0	0	0	5	5 <sup>***</sup>				
cellular infiltration, lymphocyte	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	7 <sup>+</sup>	-	-	-	-	0	0	0	0	5	0	0	5 <sup>**</sup>				
fibrosis, focal	3	0	0	3	1	0	0	1	3	0	0	3	0	0	0	0 <sup>+</sup>	-	-	-	-	2	0	0	2	0	0	0	0				
hypertrophy, tubular epithelium, collecting tubule	0	0	0	0	0	0	0	0	4	0	0	4	12	0	0	12 <sup>**</sup>	-	-	-	-	0	0	0	0	5	0	0	5 <sup>**</sup>				
hypertrophy, tubular epithelium, Henle's loop	0	0	0	0	0	0	0	0	0	3	3	6 <sup>***</sup>	0	1	11	12 <sup>***</sup>	-	-	-	-	0	0	0	0	0	1	4	5 <sup>***</sup>				
<b>REPRODUCTIVE SYSTEM</b>																																
ovary	(5)				(0)				(0)				(5)				(2)				(5)				(5)							
decreased, mature follicle	0	0	0	0	-	-	-	-	-	-	-	-	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
uterus	(5)				(0)				(0)				(5)				(2)				(5)				(5)							
post delivery lesion	5	0	0	5	-	-	-	-	-	-	-	-	5	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<b>ENDOCRINE SYSTEM</b>																																
pituitary gland	(5)				(0)				(0)				(5)				(0)				(5)				(5)							
cyst	0	0	0	0	-	-	-	-	-	-	-	-	1	0	0	1	-	-	-	-	0	0	0	0	0	0	0	0	0	0	0	0
thyroid gland	(5)				(0)				(0)				(5)				(0)				(5)				(5)							
ultimobranchial remnant	1	0	0	1	-	-	-	-	-	-	-	-	1	0	0	1	-	-	-	-	3	0	0	3	1	0	0	1				
parathyroid gland	(5)				(0)				(0)				(5)				(0)				(4)				(4)							
hyperplasia	0	0	0	0	-	-	-	-	-	-	-	-	0	0	0	0	-	-	-	-	0	0	0	0	1	0	0	1				
adrenal gland	(5)				(0)				(0)				(5)				(0)				(5)				(5)							
extramedullary hematopoiesis	0	0	0	0	-	-	-	-	-	-	-	-	0	0	0	0	-	-	-	-	1	0	0	1	0	0	0	0				
<b>SPECIAL SENSE SYSTEM</b>																																
Harderian gland	(5)				(0)				(0)				(5)				(0)				(5)				(5)							
cellular infiltration, lymphocyte	2	0	0	2	-	-	-	-	-	-	-	-	0	0	0	0	-	-	-	-	0	0	0	0	2	0	0	2				

Grade of histopathological finding; 1:slight, 2:moderate, 3:marked T:Total -:Not examined

Numbers in parentheses indicate number of animals examined microscopically at this site

Significant difference from control group: Fisher \*:p&lt;0.05 \*\*:p&lt;0.01, Mann-Whitney #:p&lt;0.05 ##:p&lt;0.01

+:Including one animal which showed decreased number both of erythrocytic and granulocytic series.

Table 5 Summary of reproductive performance and estrous cycle in rats treated orally with C.I. fluorescent brightner 271 in the combined repeated dose and reproductive/developmental toxicity screening test

Dose level	0 mg/kg	20 mg/kg	60 mg/kg	200 mg/kg
No. of pairs mated	12	12	12	12
No. of pairs copulated	12	12	11	12
No. of pregnant females	12	12	9	12
Copulation index (%) <sup>a)</sup>	100	100	91.7	100
Fertility index (%) <sup>b)</sup>	100	100	81.8	100
Estrous cycle				
No. of animals examined	12	12	12	12
Mean estrous cycle (Days, Mean ± S.D.)	4.2 ± 0.4	4.1 ± 0.3	4.2 ± 0.7	4.4 ± 0.5
Irregular estrous cycle <sup>c)</sup> (%) <sup>d)</sup>	2(16.7)	0(0.0)	2(16.7)	3(25.0)

a) (No. of animals with successful copulation/no. of animals mated)×100

b) (No. of pregnant animals/no. of animals with successful copulation)×100

c) No. of animals having irregular estrous cycle

d) (No. of animals having irregular estrous cycle/no. of animals examined)×100

Significant difference from the control group; \* $p \leq 0.05$ , \*\* $p \leq 0.01$ .

Table 6 Findings of delivery in dams treated orally with C.I. fluorescent brightner 271 and observations on their pups in the combined repeated dose and reproductive/developmental toxicity screening test

Dose level	0 mg/kg	20 mg/kg	60 mg/kg	200 mg/kg
No. of pregnant females	12	12	9	12
No. of dams delivered live pups	12	12	9	12
Duration of gestation (Day, Mean ± S.D.)	22.4 ± 0.5	22.3 ± 0.5	22.1 ± 0.3	21.9 ± 0.5*
No. of corpora lutea (Mean ± S.D.)	226(18.8 ± 4.3)	225(18.8 ± 4.5)	144(16.0 ± 2.1)	204(17.0 ± 3.1)
No. of implantation sites (Mean ± S.D.)	185(15.4 ± 1.6)	181(15.1 ± 2.1)	129(14.3 ± 1.0)	177(14.8 ± 1.5)
No. of pups born (Mean ± S.D.)	167(13.9 ± 1.3)	164(13.7 ± 2.0)	122(13.6 ± 1.3)	165(13.8 ± 2.2)
No. of live pups born (Mean ± S.D.)				
Total	167(13.9 ± 1.3)	159(13.3 ± 2.2)	120(13.3 ± 1.2)	163(13.6 ± 2.2)
Male	84(7.0 ± 2.1)	88(7.3 ± 2.1)	53(5.9 ± 1.9)	79(6.6 ± 2.2)
Female	83(6.9 ± 2.2)	71(5.9 ± 2.2)	67(7.4 ± 2.1)	84(7.0 ± 1.9)
Sex ratio (Male/Female, Mean ± S.D.)	1.19 ± 0.70	1.48 ± 0.85	0.91 ± 0.52	1.08 ± 0.71
No. of live pups on day 4 (Mean ± S.D.)				
Total	166(13.8 ± 1.3)	151(12.6 ± 2.7)	118(13.1 ± 1.4)	158(13.2 ± 2.2)
Male	84(7.0 ± 2.1)	81(6.8 ± 2.3)	52(5.8 ± 1.9)	74(6.2 ± 2.3)
Female	82(6.8 ± 2.2)	70(5.8 ± 2.0)	66(7.3 ± 2.3)	84(7.0 ± 1.9)
No. of dead pups (Mean ± S.D.)	0(0.0 ± 0.0)	5(0.4 ± 0.7)	2(0.2 ± 0.4)	2(0.2 ± 0.4)
No. of cannibalism (Mean ± S.D.)	0(0.0 ± 0.0)	0(0.0 ± 0.0)	0(0.0 ± 0.0)	0(0.0 ± 0.0)
Gestation index <sup>a)</sup> (%)	100.0	100.0	100.0	100.0
Implantation index <sup>b)</sup> (% Mean ± S.D.)	84.4 ± 13.6	83.4 ± 15.7	90.3 ± 8.0	88.3 ± 11.2
Delivery index <sup>c)</sup> (% Mean ± S.D.)	90.4 ± 4.3	90.8 ± 6.6	94.5 ± 5.0	92.9 ± 8.9
Live birth index <sup>d)</sup> (% Mean ± S.D.)	100.0 ± 0.0	96.8 ± 5.2	98.5 ± 3.0	98.8 ± 2.8
Viability index on day 4 <sup>e)</sup> (% Mean ± S.D.)				
Total	99.4 ± 1.9	94.7 ± 11.9	98.3 ± 3.4	96.9 ± 3.9
Male	100.0 ± 0.0	92.9 ± 16.3	98.4 ± 4.8	93.0 ± 9.4
Female	99.0 ± 3.6	99.2 ± 2.9	97.2 ± 8.3	100.0 ± 0.0

a) (No. of females with live pups/no. of pregnant females)×100

b) (No. of implantation sites/no. of corpora lutea)×100

c) (No. of pups born/no. of implantation sites)×100

d) (No. of live pups born/no. of pups born)×100

e) (No. of live pups on day 4 after birth/no. of live pups born)×100

Significant difference from the control group; \* $p \leq 0.05$ , \*\* $p \leq 0.01$ .