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SUBCUTANEOUS ADMINISTRATION OF PIROCTONE OLAMINE TO RATS FROM PRECONCEPTIONAL PERIOD THROUGH EARLY STAGE OF GESTATION

Ryuichi Sato, Masaaki Kajima
Nippon Experimental Medical Research Institute

Report No. NEMRI-H-82-15

March 22, 1983

Subcutaneous Administration of Piroctone Olamine to Rats
from Pre-Conceptional Period through Early Stage of Gestation

Summary

Piroctone olamine (PO) was administered subcutaneously to rats (Sprague-Dawley) at dose levels of 500, 100, 50 and 20 mg/kg/day (vehicle: 0.5 % CMC), and its effects on reproduction were investigated. Males were treated for 9 weeks before mating and through the mating period, and females for 2 weeks before mating and through day 6 of gestation. Cesarean sections were performed on all pregnant females from each group on day 18 of gestation. The following results were obtained:

1. The animals of both sexes in all the groups including the control showed signs of pain at each time of administration and nodes caused by the residual PO at the site of injection (dorsal area). The males treated with 500 and 100 mg/kg/day showed a markedly protrusive injection site with pus, induration, depilation and cracked appearence.

2. At 500 mg/kg/day, 8 males died supposedly due to inflammation caused by the large amount of PO which was poorly absorbable by the subcutaneous route.

3. At 500 mg/kg/day, body weight gains were depressed in the males and in the females during gestation.

4. The occurrence of copulation was delayed at 500 mg/kg/day. The cause of the delay was considered to be inflammation at the injection site due to the residual PO. However, the females and surviving males at this dose level showed reproductive ability; the copulation and conception rates were normal.

5. In the females, the heart and spleen weighed more at all the treatment levels and the uterus weighed less at 500 mg/kg/day. In the 19 weeks old males, weights of the following organs were increased: the thyroid, heart, liver and spleen at all the levels of treatment, the pancreas at 50 mg/kg/day or higher, and the kidney, adrenal and lung at 500 mg/kg/day.

6. At each treatment level, fetuses exhibited no changes in any parameters examined at cesarean section. There were no fetuses with external anomalies at any level and no difference occurred between each treated group and the control in the incidences of visceral anomalies, skeletal anomalies and variations, and delayed ossification.

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6. At each treatment level, fetuses exhibited no changes in any parameters examined at cesarean section. There were no fetuses with external anomalies at any level and no difference occurred between each treated group and the control in the incidences of visceral anomalies, skeletal anomalies and variations, and delayed ossification.

Subcutaneous Administration of Piroctone Olamine to Rats

from Pre-Conceptional Period through Early Stage of Gestation

I. Abstract

Piroctone Olamine was subcutaneously administered to rats at the daily doses of 500, 100 and 20 mg/kg/day from pre-conceptional period through early stage of gestation to study the effects on various reproductive parameters, and the following results were obtained.

1) Each administration group showed signs of pain at the time of administration and a node caused by the compound remnant at the site of administration was observed. In the 100 mg/kg group, the site remarkably swelled, and showed hardening, loss of fur, cracks and pus.

2) In the 500 mg/kg group, 8 males died possibly due to the inflammatory change caused by such a high dose of the compound that is less absorbable through the subcutaneous route.

3) In the 500 mg/kg group, the body weights of males were decreased, but those of females showed no change.

4) In the generative test, the confirmation of the generative capability in the 500 mg/kg group was delayed, but the cause was considered to come from the inflammatory change caused by a physical effect of the compound. In spite of delayed confirmation, the generative capability of all females and surviving males were confirmed.

5) In the 500 mg/kg group, the body-weights of dams decreased during the pregnancy period.

6) Regarding the organ weights of dams at laparotomy, the weight of the spleen increased in each administration group, and that of uterus decreased in the 500 mg/kg group.

7) Regarding the organ weight of 19 weeks old males, those of the heart, the thyroid gland, the liver and spleen in the over 20 mg/kg group, that of the pancreas in the over 50 mg/kg group and those of the kidney, the adrenal gland and the lung in the 500 mg/kg group respectively increased.

8) Regarding the findings in embryos, no change was observed in each administration group, and no apparent abnormality of embryos was found.

9) The incidences of abnormal skeletons, mutation and abnormal organs were not different between the control group and the administration groups. Also, no difference was observed in the skeleton ossification rates of both groups.

Summary

Subcutaneous Administration of Piroctone Olamine to Rats from Pre-Conceptional Period through Early Stage of Gestation

Piroctone olamine (PO) was subcutaneously given to rats at 500, 100, 50 and 20 mg/kg/day, and its effects on reproduction were investigated. Males were treated for 9 weeks before mating and through the mating period and females for 2 weeks before mating and through day 6 of gestation. Cesarean sections were performed on all pregnant females from each group on day 18 of gestation.

(1) The animals of both sexes in all the groups including the control showed a sign of pain at each time of administration and a node caused by the residual PO at the site of injection. The males at 500 and 200 mg/kg/day had a markedly protrusive injection site with pus which was indurated, depilated and cracked.

(2) At 500 mg/kg/day, 8 males died supposedly due to inflammation caused by such large amount of PO which was poorly absorbable by the subcutaneous route.

(3) At 500 mg/kg/day, body weight gains were depressed in the males and in the females during gestation.

(4) The occurrence of copulation was delayed at 500 mg/kg/day. The cause of the delay was considered to be inflammation at the injection site due to the residual PO. However, the females and surviving males at the level were shown to have reproductive ability; the copulation and conception rates were normal.

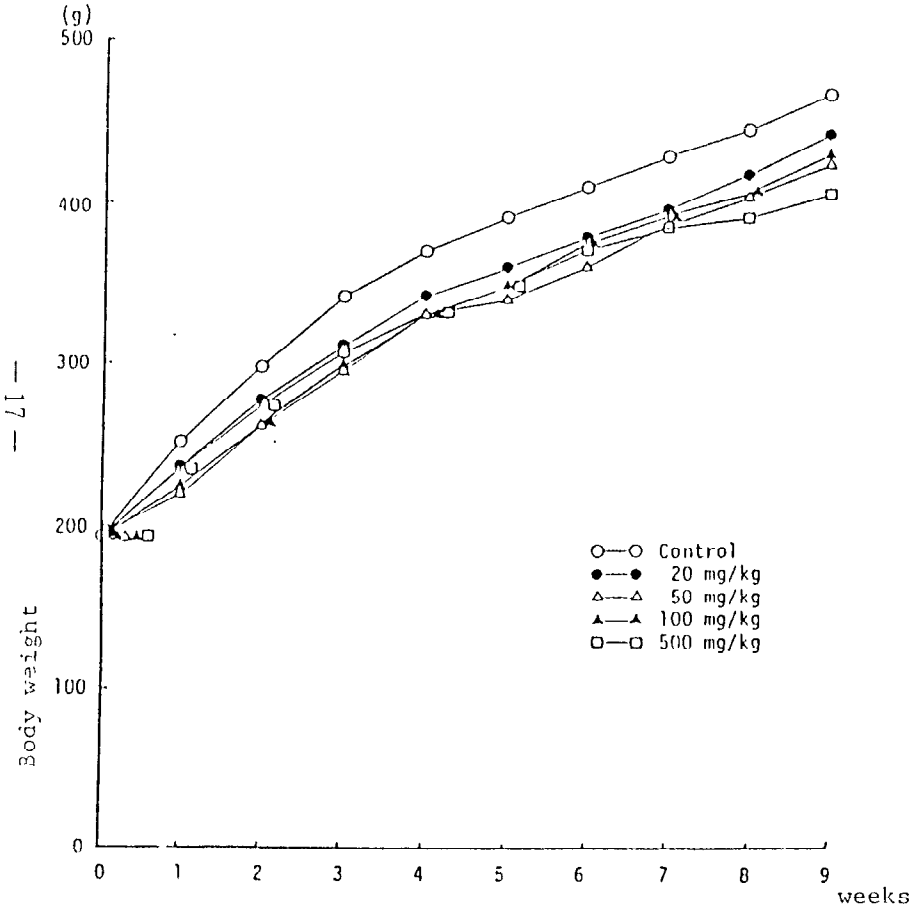
(5) In the females, the heart and spleen weighed more at all the treatment levels and the uterus weighed less at 500 mg/kg/day. In the males, weights of the following organs were increased: the thyroid, heart, liver and spleen at all the levels of treatment, the pancreas at 50 mg/kg/day or higher, and the kidney, adrenal and lung at 500 mg/kg/day.

(6) At each treatment level, fetuses exhibited no changes in any parameters examined at cesarean section. There were no fetuses with external anomalies at any levels and no difference occurred between each treated group and the control in the incidences of visceral anomalies, skeletal anomalies and variations, and delayed ossification.

SEGMENT 1

SEGMENT I

図 1　Growth curve and food consumption of male rats



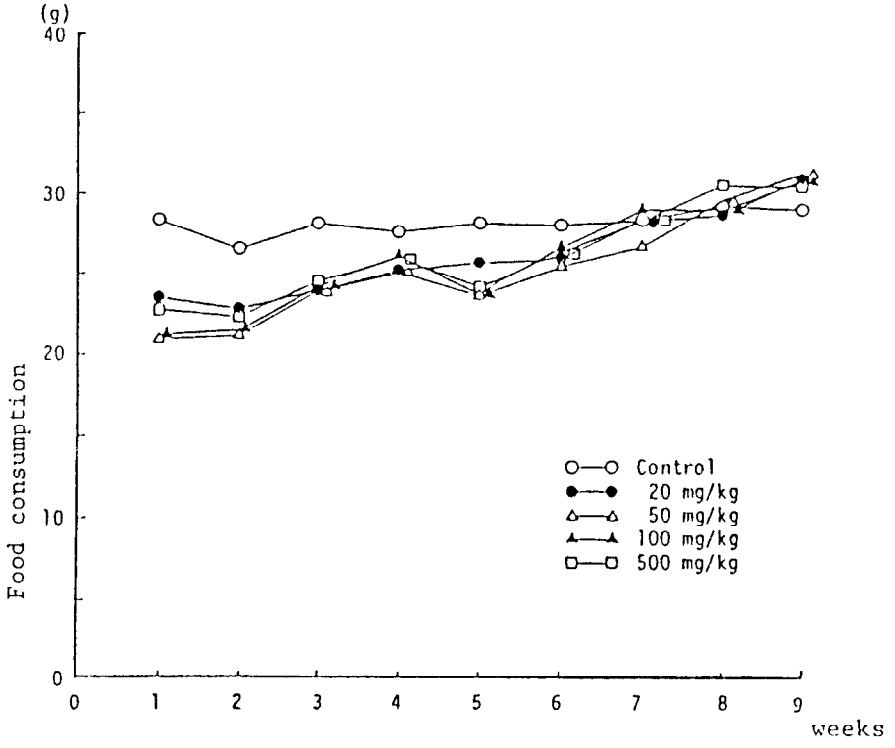
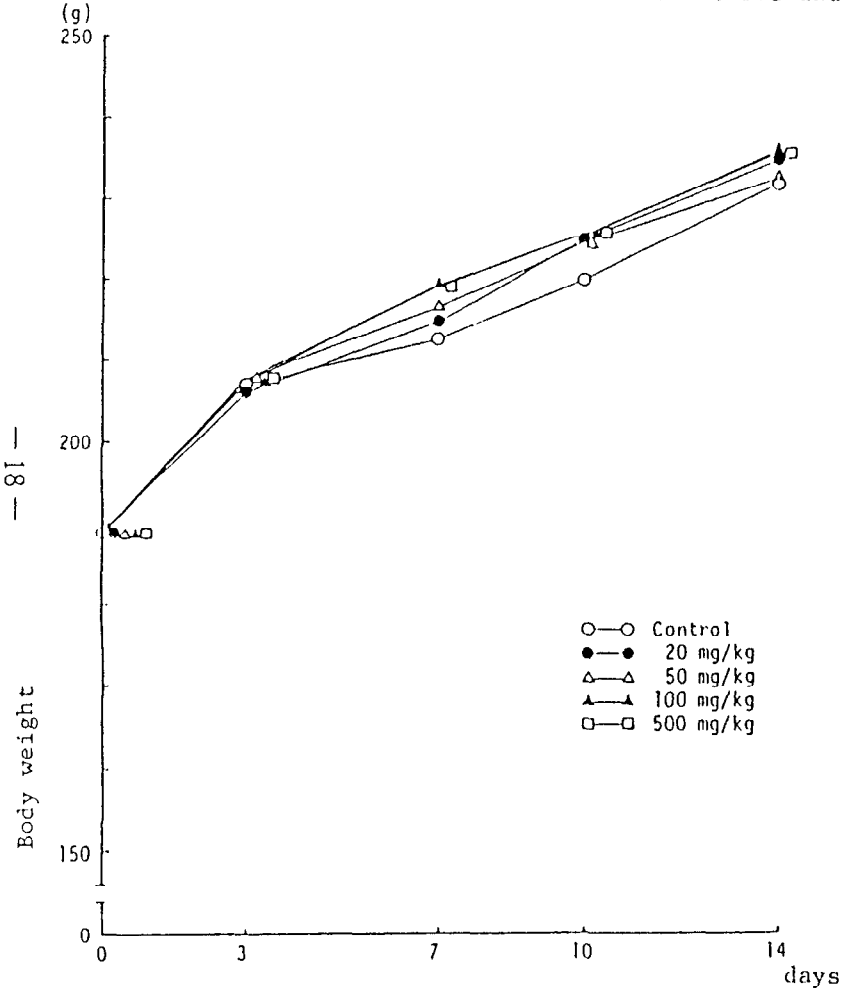


図 2　Growth curve and food consumption of female rats



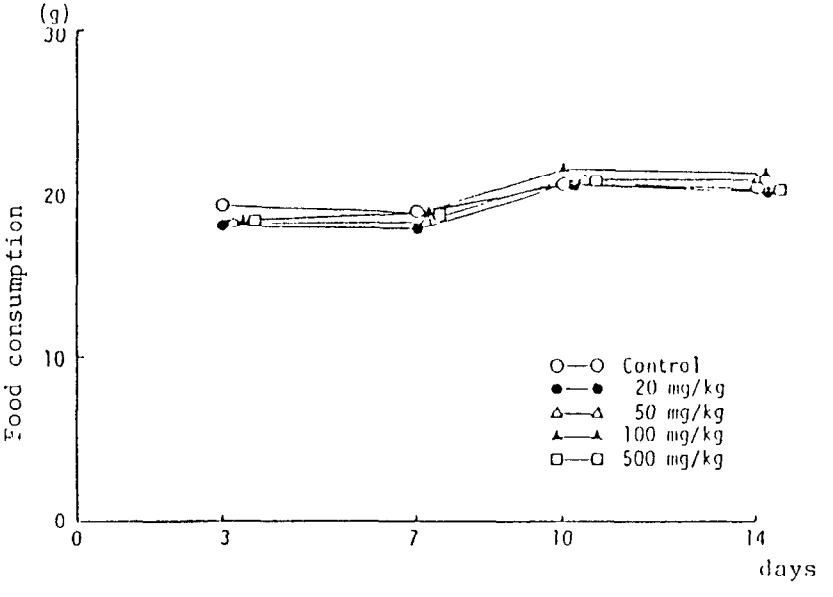


図 3　Growth curve of pregnant rats

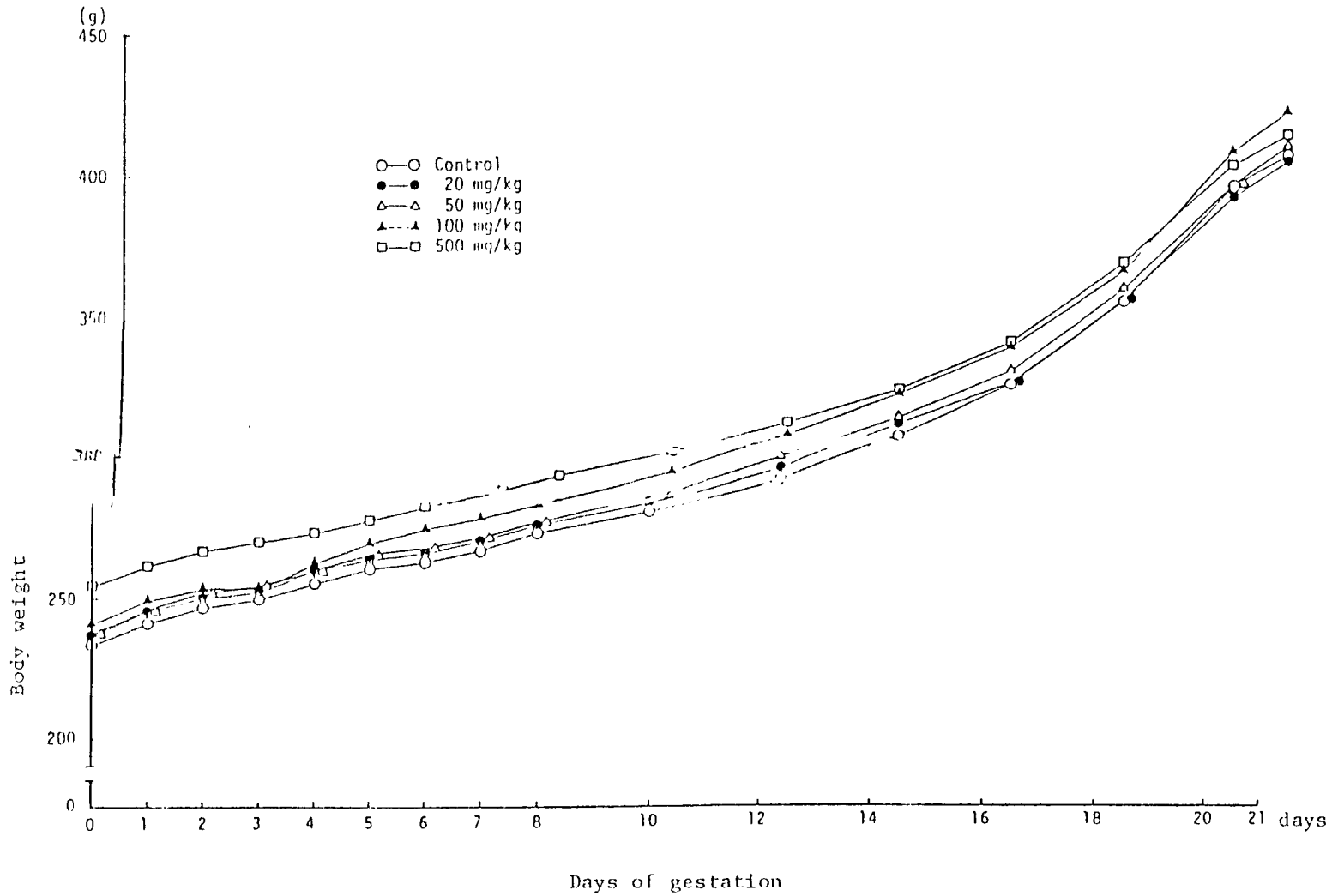


図 4　Food consumption of pregnant rats

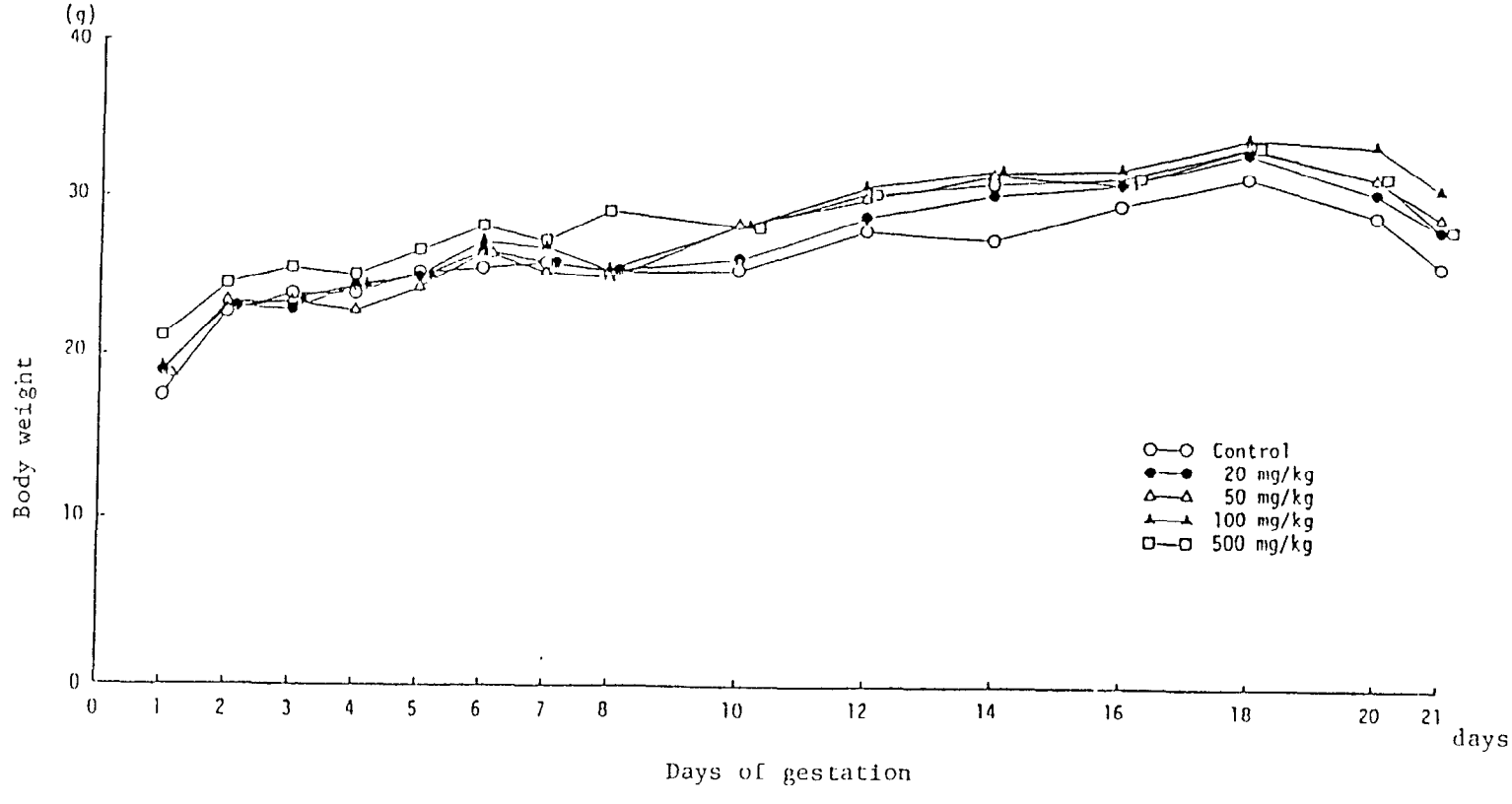


表 1　Body weight of male rats

Group	No. of animals	0	1	2	3	4	5	6	7	8	9 weeks	Gain of body weight
Control	24	24 193.6 ±1.59	24 251.4 ±2.10	24 298.0 ±3.07	24 342.3 ±4.60	24 370.8 ±5.93	24 393.1 ±6.99	24 412.6 ±7.92	24 431.1 ±8.56	24 446.7 ±8.82	24 467.0 ±8.79	24 273.4 ±8.28
20 mg/kg	24	24 193.6 ±1.59	24*** 235.6 ±2.61	24*** 277.3 ±3.65	24*** 311.9 ±4.26	24*** 343.5 ±4.50	24*** 360.6 ±4.98	24 ** 379.6 ±5.06	24 ** 397.2 ±5.72	24 * 419.0 ±6.05	24 * 443.3 ±6.66	24 * 249.6 ±6.11
50 mg/kg	24	24 193.6 ±1.59	24*** 219.3 ±3.04	24*** 260.4 ±3.96	24*** 295.8 ±5.41	24*** 331.0 ±5.92	24*** 340.3 ±7.61	24*** 361.0 ±7.09	24*** 387.5 ±8.55	24 ** 405.1 ±7.81	24 ** 425.2 ±7.78	24*** 231.6 ±6.94
100 mg/kg	24	24 193.6 ±1.59	24*** 223.8 ±2.42	24*** 262.5 ±3.11	24*** 299.2 ±3.82	24*** 330.7 ±4.20	24*** 349.3 ±5.41	24*** 375.6 ±6.52	24 ** 393.0 ±6.56	24 ** 409.2 ±7.11	24 ** 432.2 ±7.76	24 ** 238.6 ±7.47
500 mg/kg	24	24 193.6 ±1.59	24*** 233.5 ±2.34	24*** 273.3 ±2.89	24*** 306.6 ±4.42	24*** 332.9 ±4.84	24*** 349.2 ±5.31	22*** 371.6 ±6.82	19*** 385.5 ±9.33	19*** 392.3 ±9.79	19*** 406.7 ±9.34	19*** 212.4 ±8.90

Mean(g)
±S.E.

* P<0.05　** P<0.01　*** P<0.001

表 2　Food consumption of male rats

Group	No. of animals*1)	1	2	3	4	5	6	7	8	9 weeks
Control	12	28.40 ±0.47	26.51 ±0.95	28.14 ±0.56	27.53 ±0.81	28.13 ±0.60	27.91 ±0.80	28.26 ±0.74	28.13 ±0.73	28.88 ±0.57
20 mg/kg	12	*** 23.50 ±0.36	** 22.73 ±0.53	*** 23.98 ±0.48	* 25.13 ±0.35	* 25.59 ±1.03	* 25.78 ±0.40	28.05 ±0.36	28.55 ±0.23	** 30.83 ±0.38
50 mg/kg	12	*** 20.92 ±0.41	*** 21.25 ±0.43	*** 23.76 ±0.50	* 24.98 ±0.53	*** 23.58 ±0.70	* 25.36 ±0.43	26.53 ±0.73	29.43 ±0.65	* 31.12 ±0.77
100 mg/kg	12	*** 21.23 ±0.40	*** 21.53 ±0.33	*** 24.20 ±0.41	26.02 ±0.54	*** 23.69 ±0.39	26.63 ±0.38	28.97 ±0.41	28.92 ±0.41	* 30.59 ±0.46
500 mg/kg	12	*** 22.73 ±0.45	*** 22.37 ±0.31	*** 24.52 ±0.56	25.88 ±0.88	*** 24.23 ±0.71	26.17 ±0.93	28.28 ±0.99	30.45 ±1.24	30.36 ±0.79

Mean(g/day) ±S.E.　　* P<0.05　　** P<0.01　　*** P<0.001

*1) '12' is the number of cage which contains 2 rats in each.

表 3　Body weight and food consumption of female rats

Group	No. of animals	0	3	7	10	14 days	Gain of body weight	3	7	10	14 days
Control	24	188.8 ±1.65	207.1 ±1.94	212.6 ±1.92	219.8 ±2.55	231.5 ±2.92	42.7 ±1.89	19.25 ±0.27	18.80 ±0.34	20.43 0.77	20.21 0.48
20 mg/kg	24	188.8 ±1.65	206.1 ±1.89	214.8 ±1.76	224.9 ±2.17	234.7 ±2.15	45.8 ±1.07	** 17.88 ±0.27	* 17.64 ±0.30	20.28 ±0.18	19.88 ±0.34
50 mg/kg	24	188.8 ±1.65	207.8 ±2.07	216.8 ±2.18	224.2 ±2.50	232.6 ±2.95	43.8 ±1.85	** 18.05 ±0.28	18.12 ±0.32	20.68 ±0.47	20.73 ±0.29
100 mg/kg	24	188.8 ±1.65	207.2 ±2.08	* 219.2 ±2.36	225.3 ±2.45	235.8 ±2.48	47.0 ±2.11	* 18.23 ±0.36	18.68 ±0.35	21.43 ±0.43	21.14 ±0.33
500 mg/kg	24	188.8 ±1.65	207.8 ±2.14	* 219.0 ±2.36	225.6 ±2.45	235.3 ±2.60	46.6 ±1.54	* 18.24 ±0.39	18.53 ±0.16	20.63 ±0.37	20.12 ±0.35

Mean(g) ±S.E.　　* P<0.05　　** P<0.01　　Mean(g/day) S.E.

表4 Fertility findings

Group		Control	20 mg/kg	50 mg/kg	100 mg/kg	500 mg/kg
No. of animals						
At time of starting dosing before mating						
	male	24	24	24	24	24
	female	24	24	24	24	24
At time of starting mating						
	male	24	24	24	24	19
	female	24	24	24	24	24
At time of finishing mating						
	male	24	24	24	24	16
	female	24	24	24	24	24
1st mating						
Animals with verified fertility	male	23	24	24	23	13*
	female	23	24	24	23	15*
Animals with unverified fertility	male	1	0	0	1	5 ⊕1
	female	1	0	0	1	9 ⊕2
Copulation rate		24/24(100%)	24/24(100%)	24/24(100%)	24/24(100%)	20/24(83.3%)
Conception rate		23/24(95.8%)	24/24(100%)	24/24(100%)	23/24(95.8%)	15/20(75.0%)
2nd mating						
Animals with verified fertility	male	1	–	–	1	3
	female	1	–	–	1	9
Animals with unverified fertility	male	0	0	0	0	2
	female	0	0	0	0	0
Total No. of animals with verified fertility	male	24	24	24	24	16
	female	24	24	24	24	24
Copulation rate		1/1(100%)	–	–	1/1(100%)	9/9(100%)
Conception rate		1/1(100%)	–	–	1/1(100%)	9/9(100%)
3rd mating						
Animals with verified fertility	male	–	–	–	–	1
	female	–	–	–	–	–
Animals with unverified fertility	male	0	0	0	0	0 ⊕3
	female	0	0	0	0	0
Total No. of animals with verified fertility	male	24	24	24	24	17 ⊕4
	female	24	24	24	24	24
Frequency of estrus cycles from start of mating till pregnancy						
	Mean ± S.E.	1.0 ±0.04	1.0 ±0.04	1.0 ±0.04	1.1 ±0.09	1.8** ±3.28

* P<0.05 ** P<0.01

⊕1 : During mating Nos. 1503 and 1516 died before and after fertility was verified, respectively.

⊕2 : Two of 5 females with unverified copulation lost their matches, Nos. 1503 and 1516 during mating.

⊕3 : During mating No. 1519 died before fertility was verified.

⊕4 : Including No.1516 which died.

表 5 Gain of body weights of female rats during pregnancy

Group	No. of females	0	1	2	3	4	5	6	7	8	10	12	14	16	18	20	21 days
Control	24	233.1 ±2.63	241.3 ±2.83	246.9 ±2.71	249.9 ±3.14	255.3 ±3.15	260.0 ±3.21	262.3 ±3.17	265.9 ±3.21	272.3 ±3.26	280.3 ±3.27	290.0 ±3.38	306.0 ±3.60	324.5 ±4.03	354.0 ±4.18	393.8 ±5.16	404.8 ±5.34
20 mg/kg	24	237.1 ±2.01	245.3 ±2.12	250.0 ±2.21	252.5 ±2.30	259.5 ±2.11	263.0 ±1.99	265.7 ±2.02	268.6 ±2.20	275.1 ±2.34	283.2 ±2.52	294.0 ±2.86	310.0 ±3.07	325.4 ±3.17	354.8 ±3.97	390.6 ±5.21	402.5 ±5.96
50 mg/kg	24	237.4 ±3.02	245.7 ±3.12	251.8 ±3.03	254.4 ±2.82	259.3 ±3.05	264.3 ±3.19	267.3 ±3.50	270.9 ±3.80	276.3 ±3.68	286.7 ±3.71	298.0 ±3.94	312.8 ±3.86	329.3 ±4.18	358.2 ±4.45	394.9 ±5.19	408.3 ±5.12
100 mg/kg	24	241.2 ±3.27	249.0 ±3.42	253.3 ±3.32	257.6 ±3.56	* 265.0 ±3.24	268.7 ±3.40	* 273.9 ±3.51	* 277.7 ±3.48	* 282.6 ±3.73	* 293.3 ±4.04	** 306.8 ±4.22	** 321.7 ±4.41	* 338.2 ±4.39	365.0 ±4.76	406.9 ±5.33	420.3 ±5.86
500 mg/kg	24	*** 254.3 ±4.70	** 261.5 ±5.00	** 266.3 ±5.20	** 269.8 ±5.41	** 272.8 ±5.47	** 277.4 ±5.29	** 282.4 ±5.67	** 287.3 ±5.68	** 292.2 ±6.01	** 300.3 ±6.23	** 311.6 ±6.35	* 323.0 ±6.42	340.5 ±7.00	368.2 ±6.93	401.7 ±7.28	412.1 ±7.61

Mean(g) ±S.E.

* P<0.05 ** P<0.01 *** P<0.001

Gain of body weight

21-0	21-8	8-0 days
171.8 ±3.77	132.6 ±3.15	39.2 ±1.32
165.4 ±5.00	127.4 ±4.95	38.0 ±1.04
170.8 ±3.74	131.9 ±3.60	38.9 ±1.92
179.2 ±3.83	137.8 ±3.75	41.4 ±1.30
* 157.8 ±5.15	* 119.9 ±5.11	37.9 ±2.25

表 6　Food consumption of female rats during pregnancy

Group	No. of females	1	2	3	4	5	6	7	8	10	12	14	16	18	20	21 days
Control	24	17.5 ±0.82	22.7 ±0.56	23.8 ±0.54	23.8 ±0.61	25.3 ±0.82	25.5 ±0.65	25.8 ±0.62	25.3 ±0.73	25.38 ±0.43	28.00 ±0.57	27.42 ±0.91	29.67 ±0.67	31.46 ±0.93	29.08 ±0.76	25.8 ±0.88
20 mg/kg	24	19.0 ±0.97	23.0 ±0.38	22.8 ±0.70	24.3 ±0.76	24.9 ±0.64	26.6 ±0.61	25.8 ±0.49	25.4 ±0.70	26.17 ±0.67	28.83 ±0.76	* 30.33 ±0.63	31.00 ±0.62	33.00 ±0.60	30.50 ±0.69	* 28.2 ±0.70
50 mg/kg	24	18.8 ±0.85	23.4 ±0.50	23.4 ±0.66	22.8 ±0.65	24.3 ±0.67	26.5 ±0.75	25.3 ±0.68	24.9 ±0.61	*** 28.38 ±0.66	* 30.00 ±0.53	*** 31.63 ±0.47	31.08 ±0.55	33.50 ±0.47	* 31.42 ±0.73	* 29.0 ±0.90
100 mg/kg	24	19.3 ±1.23	22.8 ±0.69	23.5 ±0.77	24.4 ±0.59	25.0 ±0.92	27.3 ±0.60	26.8 ±0.66	25.5 ±0.45	*** 28.33 ±0.57	** 30.75 ±0.67	*** 31.88 ±0.62	* 32.00 ±0.76	* 34.08 ±0.86	33.63 ±0.73	*** 30.8 ±1.02
500 mg/kg	24	** 21.3 ±0.80	24.5 ±0.75	25.5 ±0.67	25.1 ±0.80	26.7 ±0.84	** 28.3 ±0.68	27.3 ±0.78	29.2 ±1.68	*** 28.17 ±0.61	** 30.33 ±0.54	** 31.08 ±0.89	31.46 ±0.73	33.54 ±0.77	* 31.58 ±0.71	28.3 ±1.27

* P<0.05　　** P<0.01　　*** P<0.001

表 7　Weight of organs - female rats

Group	No. of females	Final body weight (g)	Brain (g)	Pituitary (mg)	Thyroid (mg)	Thymus (mg)	Heart (g)	Lung (g)	Liver (g)	Spleen (g)	Pancreas (g)	Kidney (g) right	Kidney (g) left	Adrenal (mg) right	Adrenal (mg) left	Ovary (mg) right	Ovary (mg) left	Uterus (g)
Control	24	404.8 ±5.34	1.86 ±0.02	13.6 ±0.68	17.8 ±0.85	257.0 ±10.94	0.93 ±0.02	1.28 ±0.03	14.43 ±0.33	0.67 ±0.02	0.71 ±0.05	1.01 ±0.02	1.00 ±0.02	36.3 ±1.18	38.2 ±0.87	74.0 ±4.60	75.0 ±3.07	5.58 ±0.24
20 mg/kg	24	402.5 ±5.96	1.84 ±0.02	* 16.0 ±0.88	20.2 ±0.85	288.4 ±15.32	** 1.00 ±0.02	1.29 ±0.02	15.15 ±0.32	* 0.77 ±0.03	0.71 ±0.03	1.04 ±0.02	1.02 ±0.02	* 39.8 ±1.04	41.5 ±1.41	71.2 ±3.81	75.3 ±3.49	5.16 ±0.27
50 mg/kg	24	408.3 ±5.49	1.82 ±0.02	14.3 ±0.67	19.5 ±1.22	260.0 ±14.84	* 1.00 ±0.02	1.34 ±0.03	15.01 ±0.35	* 0.76 ±0.03	0.74 ±0.04	1.02 ±0.02	1.02 ±0.02	36.5 ±1.31	40.2 ±1.23	71.2 ±4.11	79.8 ±2.99	5.37 ±0.21
100 mg/kg	24	420.3 ±5.86	1.83 ±0.03	15.3 ±0.71	17.7 ±0.89	258.1 ±12.35	** 1.02 ±0.03	1.28 ±0.02	15.17 ±0.52	** 0.78 ±0.03	0.71 ±0.02	* 1.08 ±0.02	1.08 ±0.04	38.0 ±1.09	39.8 ±1.25	76.1 ±3.83	71.9 ±3.35	5.46 ±0.22
500 mg/kg	24	412.1 ±7.61	1.82 ±0.02	14.3 ±0.88	17.1 ±0.81	227.2 ±12.07	* 1.01 ±0.03	1.33 ±0.03	14.96 ±0.41	0.74 ±0.04	0.67 ±0.03	1.06 ±0.02	1.05 ±0.02	37.8 ±1.61	40.6 ±1.54	66.4 ±3.79	70.4 ±3.75	* 4.88 ±0.23

Mean ±S.E.　　* P<0.05　　** P<0.01

[illegible] weeks' male rats

		[illegible]	[illegible]	[illegible]	Thyroid (mg)	Thymus (mg)	Heart (g)	Lung (g)	Liver (g)	Spleen (g)	Pancreas (g)	Testes (g) right	Testes (g) left	Adrenal (mg) right	Adrenal (mg) left	Epididymis (g) right	Epididymis (g) left	Prostate (g)
[illegible]	24	512.3 ±8.45	2.03 ±0.03	13.0 ±0.70	23.9 ±1.33	277.8 ±11.56	1.37 ±0.03	1.70 ±0.03	19.64 ±0.37	0.91 ±0.02	0.67 ±0.02	1.72 ±0.03	1.71 ±0.03	27.9 ±1.22	30.7 ±1.16	1.87 ±0.02	1.83 ±0.03	0.73 ±0.05
20 mg/kg	24	523.3 ±8.90	2.05 ±0.02	14.1 ±0.51	* 29.2 ±1.47	290.6 ±17.88	* 1.47 ±0.03	1.72 ±0.03	*** 21.67 ±0.38	*** 1.15 ±0.02	0.74 ±0.04	1.77 ±0.04	1.74 ±0.04	29.9 ±0.82	33.0 ±1.06	1.85 ±0.02	1.84 ±0.02	0.70 ±0.04
50 mg/kg	24	514.1 ±8.43	2.03 ±0.02	14.5 ±0.43	** 29.7 ±1.21	* 320.4 ±13.40	* 1.46 ±0.03	1.75 ±0.04	*** 21.94 ±0.53	*** 1.15 ±0.03	* 0.76 ±0.03	1.76 ±0.03	1.73 ±0.04	30.2 ±1.14	33.3 ±1.11	1.82 ±0.03	1.83 ±0.03	0.64 ±0.03
100 mg/kg	24	504.3 ±8.03	2.00 ±0.01	13.6 ±0.43	* 28.0 ±1.39	** 337.3 ±15.73	** 1.53 ±0.04	1.76 ±0.03	* 21.16 ±0.50	*** 1.17 ±0.04	** 0.79 ±0.04	1.79 ±0.04	1.70 ±0.05	31.3 ±1.21	32.8 ±1.40	1.83 ±0.02	1.79 ±0.04	0.73 ±0.06
500 mg/kg	16	** 468.8 ±10.02	2.00 ±0.02	13.6 ±0.58	27.5 ±1.84	274.6 ±21.54	*** 1.58 ±0.05	* 1.86 ±0.05	19.56 ±0.96	*** 1.41 ±0.09	** 0.99 ±0.11	1.82 ±0.05	1.79 ±0.05	31.5 ±1.67	** 37.1 ±1.91	*** 1.70 ±0.04	1.76 ±0.04	0.87 ±0.11
			(g%)	(mg%)	(mg%)	(mg%)	(g%)	(g%)	(g%)	(g%)	(g%)	(g%)	(g%)	(mg%)	(mg%)	(g%)	(g%)	(g%)
			0.40 ±0.01	2.54 ±0.14	4.66 ±0.24	54.59 ±2.45	0.27 ±0.005	0.33 ±0.01	3.84 ±0.05	0.18 ±0.004	0.13 ±0.004	0.34 ±0.004	0.34 ±0.004	5.46 ±0.23	5.98 ±0.20	0.37 ±0.01	0.36 ±0.01	0.14 ±0.01
			0.39 ±0.01	2.69 ±0.08	* 5.57 ±0.26	55.74 ±3.42	0.28 ±0.01	0.33 ±0.01	*** 4.14 ±0.04	*** 0.22 ±0.005	0.14 ±0.01	0.34 ±0.01	0.33 ±0.01	5.74 ±0.17	6.34 ±0.21	0.36 ±0.01	0.35 ±0.005	0.14 ±0.01
			0.40 ±0.01	2.84 ±0.08	*** 5.82 ±0.27	* 62.53 ±2.54	* 0.28 ±0.004	0.34 ±0.01	*** 4.28 ±0.09	*** 0.22 ±0.01	* 0.15 ±0.01	0.34 ±0.01	0.34 ±0.01	5.89 ±0.23	6.52 ±0.23	0.36 ±0.01	0.36 ±0.01	0.12 ±0.01
			0.40 ±0.01	2.70 ±0.09	* 5.54 ±0.26	** 67.27 ±3.19	*** 0.31 ±0.01	0.35 ±0.01	** 4.21 ±0.10	*** 0.23 ±0.01	** 0.16 ±0.01	* 0.36 ±0.01	0.34 ±0.01	* 6.19 ±0.21	6.50 ±0.25	0.37 ±0.01	0.36 ±0.01	0.14 ±0.01
			* 0.43 ±0.01	2.91 ±0.10	** 5.84 ±0.33	57.91 ±3.67	*** 0.34 ±0.01	*** 0.40 ±0.01	* 4.16 ±0.15	*** 0.30 ±0.02	*** 0.21 ±0.02	*** 0.39 ±0.01	*** 0.38 ±0.01	** 6.71 ±0.33	*** 7.93 ±0.39	0.36 ±0.01	0.38 ±0.01	0.18 ±0.02

Mean ±S.E.

* P<0.05 ** P<0.01 *** P<0.001

表9 Summary

Group		Control	20 mg/kg	50 mg/kg	100 mg/kg	500 mg/kg
No. of dams		24	24	24	24	24
No. of corpora lutea		365	337	333	369	358
	Mean ± S.E.	15.2 ±0.29	14.0* ±0.36	13.9** ±0.32	15.4 ±0.25	14.9 ±0.32
Total No. of implantation		355(97.3%)	303(89.9%)***	313(94.0%)*	344(93.2%)*	329(91.9%)**
	Mean ± S.E.	14.8 ±0.34	12.6** ±0.66	13.0** ±0.40	14.3 ±0.42	13.7 ±0.53
No. of living fetuses		349(98.3%)	297(98.0%)	310(99.0%)	337(98.0%)	310(94.2%)**
	Mean ± S.E.	14.5 ±0.35	12.4** ±0.68	12.9** ±0.38	14.0 ±0.44	12.9* ±0.70
No. of male living fetuses		174(49.9%)	145(48.8%)	150(48.4%)	171(50.7%)	148(47.7%)
	Mean ± S.E.	7.3 ±0.45	6.3 ±0.40	6.3 ±0.36	7.1 ±0.36	6.2 ±0.50
No. of female living fetuses		175(50.1%)	152(51.2%)	160(51.6%)	166(49.3%)	162(52.3%)
	Mean ± S.E.	7.3 ±0.38	6.3 ±0.47	6.7 ±0.45	6.9 ±0.36	6.8 ±0.52
No. of unnaitured fetuses		1(0.3%)	1(0.3%)	0	2(0.6%)	0
No. of dead fetuses		0	0	0	0	0
No. of resorption						
Implantation		1(0.3%)	0	0	1(0.3%)	1(0.3%)
Placental remnant		5(1.4%)	6(2.0%)	3(1.0%)	5(1.5%)	18(5.5%)**
Early resorption		0	0	0	0	0
Late resorption		0	0	0	1(0.3%)	0
Macerated fetuse		0	0	0	0	0
No. of fetuses with external abnormalities		0	0	0	0	0
Body weight of living fetuses male	Mean ± S.E.	5.51 ±0.05	5.60 ±0.06	5.70 * ±0.06	5.66 ±0.07	5.70 * ±0.05
female	Mean ± S.E.	5.22 ±0.05	5.46 ±0.13	5.35 ±0.05	5.33 ±0.08	5.39 * ±0.05

* P<0.05 ** P<0.01 *** P<0.001

表10 Skeletal abnormalities observed in fetuses

Group	Control	20 mg/kg	50 mg/kg	100 mg/kg	500 mg/kg
No. of dams	24	24	24	24	24
No. of pups	179	154	159	173	162
No. of dams with skeletal abnormalities	0	0	0	1(4.2%)	0
No. of pups with ditto	0	0	0	1(0.6%)	0
Fusion of sternbrae	0	0	0	1(0.6%)	0
No. of dams with skeletal variation	19((79.2%)	17(70.8%)	21(87.5%)	18(75.0%)	12(50.0%)
No. of pups with ditto	66(36.9%)	48(31.2%)	53(33.3%)	55(31.8%)	39(24.1%)
Cervical rib	0	0	1(0.6%)	0	0
Unilateral lumbar rib	36(20.1%)	21(13.6%)	28(17.6%)	32(18.5%)	19(11.7%)
Bilateral lumbar rib	29(16.2%)	27(17.5)	24(15.1%)	22(12.7%)	20(12.3%)
Asymmetry of sternbrae	1(0.6%)	0	0	1(0.6%)	0
Lumbar vertebral body, arch	2(1.1%)	1(0.6%)	1(0.6%)	1(0.6%)	1(0.6%)
Nasal bone	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
Frontal bone	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
Parietal bone	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
Occipital bone	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
Basioccipital bone	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
Occipitotemporal bone	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
Interparietal bone	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
Supraoccipital bone	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
Lacrimal bone	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
Maxilla	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
Mandible	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
Hyoid bone	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
Palatine bone	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
Odontoid process	162(90.5%)	123(79.9%)	144(90.6%)	144(83.2%)	145(89.5%)
Vertebrae					
Cervical vertebral body 1	25(14.0%)	15(9.7%)	19(11.9%)	15(8.7%)	25(15.4%)
2	25(14.0%)	15(9.7%)	18(11.3%)	11(6.4%)	19(11.7%)
3	19(10.6%)	22(14.3%)	24(15.1%)	19(11.0%)	28(17.3%)
4	27(15.1%)	27(17.5%)	24(15.1%)	24(13.9%)	37(22.8%)
5	33(18.4%)	41(26.6%)	41(25.8%)	25(14.5%)	45(27.8%)
6	48(26.8%)	47(30.5%)	42(26.4%)	41(23.7%)	53(32.7%)
7	119(66.5%)	148(96.1%)	104(65.4%)	88(50.9%)	107(66.0%)
Cervical vertebral arches 1-7	179(100%)	154(100%)	159(100%)	172(99.4%)	162(100%)

Thoracic vertebral body	1-13	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
Delayed ossification	1	0	1(0.6%)	0	0	0
	9	0	0	0	1(0.6%)	0
	10	0	0	0	0	0
	11	0	0	0	2(1.2%)	0
	12	1(0.6%)	1(0.6%)	1(0.6%)	4(2.3%)	1(0.6%)
	13	1(0.6%)	0	1(0.6%)	2(1.2%)	0
Splitting	8	0	0	0	1(0.6%)	0
	10	0	0	0	1(0.6%)	0
	11	0	0	1(0.6%)	0	2(1.2%)
	12	0	1(0.6%)	0	0	1(0.6%)
	13	1(0.6%)	0	0	0	3(1.9%)
Thoracic vertebral arch	1-13	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
Lumbar vertebral body, arch	1-6	177(98.9%)	153(99.4%)	158(99.4%)	172(99.4%)	161(99.4%)
	1-7 (変)	2(1.1%)	1(0.6%)	1(0.6%)	1(0.6%)	1(0.6%)
Sacral vertebral body, arch	1-4	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
Caudal vertebral body	1	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
	2	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
	3	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
	4	179(100%)	154(100%)	158(99.4%)	173(100%)	162(100%)
	5	178(99.4%)	154(100%)	158(99.4%)	171(98.8%)	162(100%)
	6	154(86.0%)	142(92.8%)	139(87.4%)	141(81.5%)	151(93.2%)
	7	94(52.5%)	86(55.8%)	81(50.9%)	66(38.2%)	95(58.6%)
	8	30(16.8%)	27(17.5%)	24(15.1%)	15(8.7%)	27(16.7%)
	9	7(3.9%)	10(6.5%)	2(1.3%)	2(1.2%)	6(3.7%)
	10	1(0.6%)	1(0.6%)	0	0	5(3.1%)
	11	0	1(0.6%)	0	0	0
	12	0	1(0.6%)	0	0	0
Calcified caudal vertebral body		6.60	6.96	6.60	6.32	6.74
Mean ± S.E.		±0.15	±0.26	±0.14	±0.13	±0.16
Caudal vertebral arch	1	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
	2	179(100%)	154(100%)	158(99.4%)	173(100%)	161(99.4%)
	3	143(79.9%)	127(82.5%)	128(80.5%)	141(81.5%)	129(79.6%)
	4	13(7.3%)	12(7.8%)	5(3.1%)	10(5.8%)	13(8.0%)
	5	4(2.2%)	0	0	0	0
	6	0	0	0	0	0
	7	0	0	0	0	0

Rib						
Normal		114(63.7%)	106(68.8%)	107(67.2%)	119(68.8%)	123(75.9%)
Unilateral lumbar rib		36(20.1%)	21(13.6%)	28(17.6%)	32(18.5%)	19(11.7%)
Bilateral lumbar rib		29(16.2%)	27(17.5)	24(15.1%)	22(12.7%)	20(12.3%)
Cervical rib		0	0	1(0.6%)	0	0
Sternebrae	1	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
	2	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
	3	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
	4	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
	5	174(97.2%)	153(99.4)	153(96.2%)	172(99.4%)	162(100%)
	6	179(100%)	154(100%)	159(100%)	172(99.4%)	162(100%)
Delayed ossification of sternebrae						
No. of fetuses		18(10.1%)	10(6.5%)	8(5.0%)	14(8.1%)	23(14.2%)
	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0
	5	18(10.1%)	10(6.5%)	8(5.0%)	14(8.1%)	23(14.2%)
	6	0	0	0	0	0
Splitting of sternebra						
No. of fetuses		0	0	1(0.6%)	0	0
	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0
	5	0	0	1(0.6%)	0	0
	6	0	0	0	0	0
Half calcified sternbra						
No. of fetuses		9(5.0%)	6(3.9%)	11(6.9%)	5(2.9%)	11(6.8%)
	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0
	5	9(5.0%)	6(3.9%)	11(6.9%)	5(2.9%)	11(6.8%)
	6	0	0	0	0	0
Accessory sternebra						
No. of fetuses		0	0	0	0	0
Asymmetry of sternebra						
No. of fetuses		1(0.6%)	0	0	1(0.6%)	0
	1	0	0	0	0	0
	2	1(0.6%)	0	0	0	0
	3	1(0.6%)	0	0	1(0.6%)	0
	4	1(0.6%)	0	0	1(0.6%)	0
	5	1(0.6%)	0	0	1(0.6%)	0
	6	1(0.6%)	0	0	1(0.6%)	0
Fused sternebrae		0	0	0	1(0.6%)	0

Clavicle	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
Scapula	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
Humerus	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
Radius	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
Ulna	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
Metacarpus					
1	0	0	0	0	0
2	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
3	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
4	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
5	178(99.4%)	154(100%)	159(100%)	172(99.4%)	162(100%)
Proximal phalanges					
1	0	0	0	0	0
2	161(89.9%)	142(92.2%)	128(80.5%)	151(87.3%)	149(92.0%)
3	178(99.4%)	154(100%)	155(97.5%)	171(98.8%)	162(100%)
4	178(99.4%)	154(100%)	152(95.6%)	169(97.7%)	160(98.8%)
5	144(80.4%)	131(85.1%)	103(64.8%)	136(78.6%)	131(80.9%)
Middle phalanges					
2-5	0	0	0	0	0
Distal phalanges					
1	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
2	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
3	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
4	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
5	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
Iliac bone	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
Ischium	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
Pubis	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
Femur	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
Tibia	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
Fibura	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
Talus	0	0	0	0	0
Calcaneus	3(1.7%)	7(4.5%)	10(6.3%)	10(5.8%)	8(4.9%)
Metatarsus					
1	147(82.1%)	125(81.2%)	123(74.4%)	145(83.8%)	145(89.5%)
2	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
3	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
4	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
5	177(98.9%)	154(100%)	159(100%)	173(100%)	162(100%)
Hind paw					
Proximal phalanges 1	0	1(0.6%)	0	0	0
2	91(50.8%)	101(65.6%)	100(62.9%)	107(61.8%)	118(72.8%)
3	119(66.5%)	112(72.7%)	111(69.8%)	118(68.2%)	129(79.6%)
4	116(64.8%)	112(72.7%)	110(69.2%)	117(67.6%)	129(79.6%)
5	46(25.7%)	51(33.1%)	52(32.7%)	46(26.6%)	54(33.3%)
Middle phalanges					
1-5	0	0	0	0	0
Distal phalanges					
1	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
2	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
3	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
4	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)
5	179(100%)	154(100%)	159(100%)	173(100%)	162(100%)

表11 Abnormalities in internal organs of fetuses

Group (mg/kg)	Control	20	50	100	500
No. of dams examined	24	24	24	24	24
No. of fetuses examined	169	142	151	162	148
No. of dams with abnormal fetuses	5	3	3	3	4
No. of abnormal fetuses	6	4	6	3	4
Dilatation of the renal pelnis	6	4	6	3	1
Hypoplastic lung	0	0	0	0	1
Hemorrhage of intra-pericardium	0	0	0	0	2

付表　Abnormalities in internal organs of fetuses

Group

No.	No. of examined fetuses	No. of abnormal fetuses	Dilatations of the renal pelvis	Hypoplastic lung	Hemorrhage of intrapericardium
2101	7				
2102	7	1	1		
2103	9				
2104	6				
2105	7				
2106	7				
2107	7				
2108	8				
2109	6				
2110	6				
2111	8	1	1		
2112	7				
2113	9				
2114	6	1	1		
2115	7				
2116	7				
2117	5				
2118	6				
2119	7				
2120	8	1	1		
2121	8				
2122	7				
2123	7				
2124	7	2	2		
Total	169	6	6		
%		3.6	3.6		

付表 Abnormalities in internal organs of fetuses

Group
20mg/kg

No.	No. of examined fetuses	No. of abnormal fetuses	Dilatations of the renal pelnis	Hypoplastic lung	Hemorrhage of intrapericardium
2201	7				
2202	7				
2203	5				
2204	5				
2205	7				
2206	3				
2207	7				
2208	6				
2209	7	1	1		
2210	6				
2211	4				
2212	1	1	1		
2213	7				
2214	6				
2215	6				
2216	7	2	2		
2217	5				
2218	7				
2219	5				
2220	8				
2221	7				
2222	7				
2223	6				
2224	6				
Total	142	4	4		
%		2.8	2.8		

Group
50mg/kg

付表 Abnormalities in internal organs of fetuses

No.	No. of examined fetuses	No. of abnormal fetuses	Dilatations of the renal pelnis	Hypoplastic lung	Hemorrhage of intrapericardium
2301	5				
2302	6				
2303	8				
2304	5	1	1		
2305	7				
2306	6				
2307	5				
2308	5				
2309	7				
2310	5				
2311	7				
2312	6				
2313	7				
2314	6				
2315	6	2	2		
2316	6				
2317	7				
2318	6	3	3		
2319	6				
2320	7				
2321	8				
2322	7				
2323	6				
2324	7				
Total	151	6	6		
%		4.0	4.0		

Group 付表 Abnormalities in internal organs of fetuses

100mg/kg

No.	No. of examined fetuses	No. of abnormal fetuses	Dilatations of the renal pelnis	Hypoplastic lung	Hemorrhage of intrapericardium
2401	8				
2402	6				
2403	7	1	1		
2404	7				
2405	6				
2406	7				
2407	8				
2408	6				
2409	6				
2410	5				
2411	7	1	1		
2412	4				
2413	8				
2414	5				
2415	7				
2416	7				
2417	6				
2418	8				
2419	7	1	1		
2420	7				
2421	8				
2422	8				
2423	8				
2424	6				
Total	102	3	3		
%		1.9	1.9		

付表 Abnormalities in internal organs of fetuses

Group
500mg/kg

No.	No. of examined fetuses	No. of abnormal fetuses	Dilatations of the renal pelnis	Hypoplastic lung	Hemorrhage of intrapericardium
2501	6				
2502	6				
2503	8				
2504	6				
2505	4	1			1
2506	7				
2507	7				
2508	5				
2509	8				
2510	7				
2511	7	1		1	
2512	6				
2513	7				
2514	7	1			1
2515	7	1	1		
2516	3				
2517	6				
2518	5				
2519	7				
2520	7				
2521	9				
2522	6				
2523	1				
2524	6				
Total	148	4	1	1	2
%		2.7	0.7	0.7	1.4

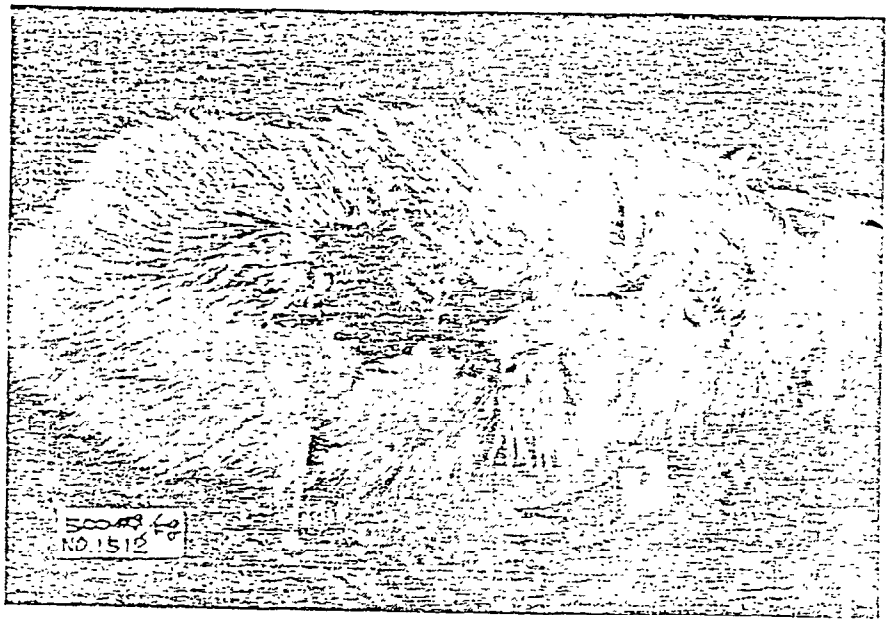


Photo 1. 500 mg/kg : External observation of administration site

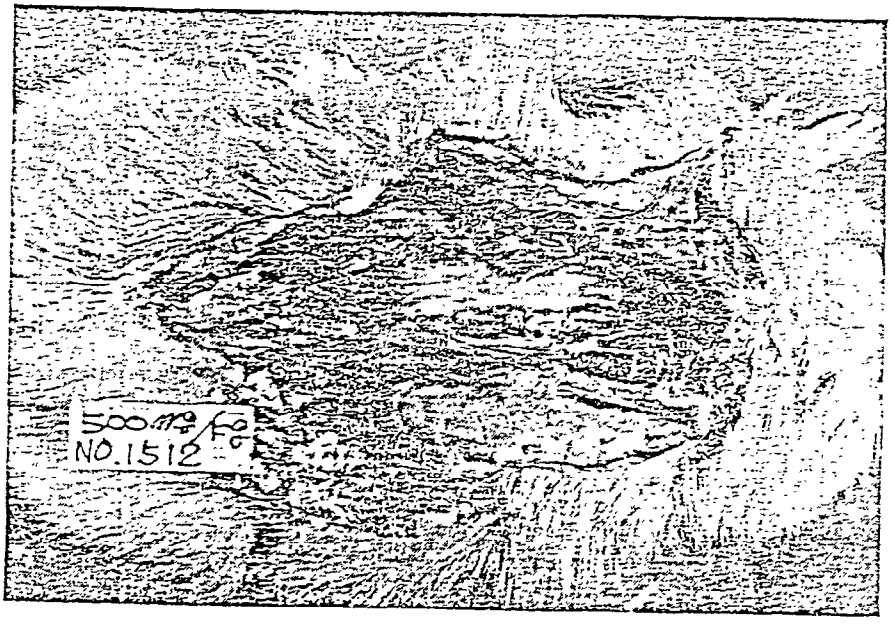


Photo 2. 500 mg/kg : Subcutaneous observation of administration site

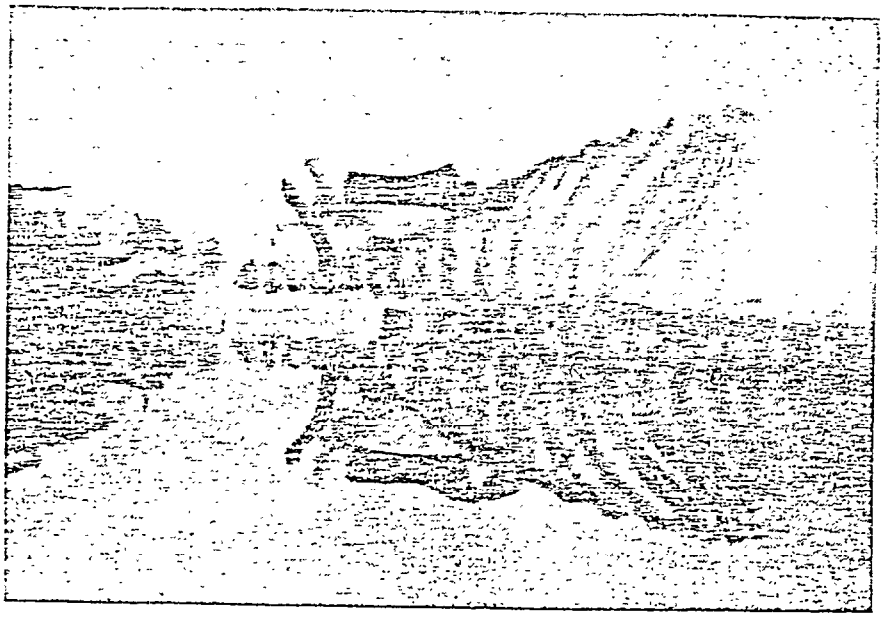


Photo 3. Control: Asymmetry of sternebrae

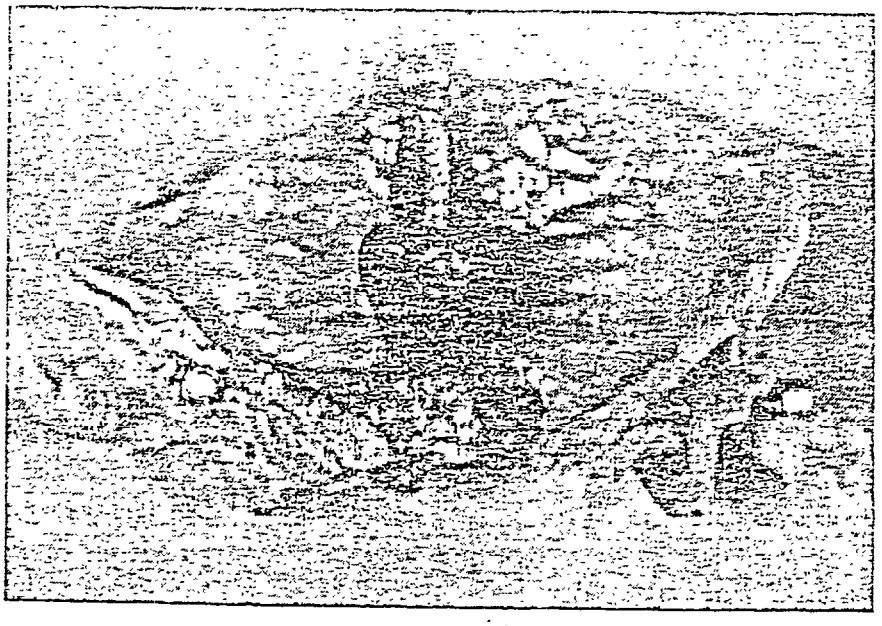


Photo 4. 500 mg/kg: Hemorrhage of intrapericardium