Fig. 1. Body weight changes in male rats in the main and recovery groups and female rats in the recovery group in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test for perfluorododecanoic acid. *Significantly different from the 0 mg/kg/day group at $p \leq 0.05$, **Significantly different from the 0 mg/kg/day group at $p \leq 0.01$.

Fig. 2. Body weight changes in female rats in the main group in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test for perfluorododecanoic acid. *Significantly different from the 0 mg/kg/day group at $p \leq 0.05$, **Significantly different 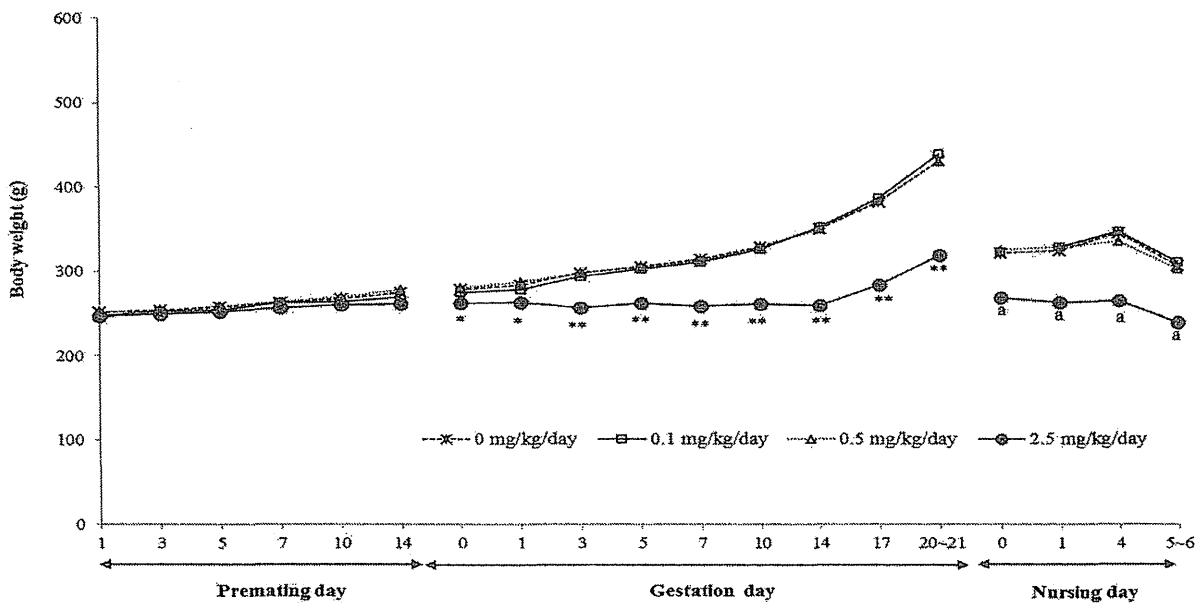from the 0 mg/kg/day group at $p \leq 0.01$. a: The data were exempt from statistical evaluation because only one female normally delivered pups.

PFDoA/kg/day than in the control group at the end of the administration period (Table II). In this dose group, significant decreases were noted in WBC, RBC, HGB, HCT, and lymphocyte, monocyte, and eosinophil counts on a differential counts of WBC, and a significant increase was observed in the reticulocyte counts at the end of the recovery period.

Many changes, including decreases in HCT, MCV, and the reticulocyte count and an increase in MCHC, were found in one female given 2.5 mg/kg/day, as shown in Table II. In the 0.1 and 0.5 mg/kg/day groups, no significant changes were found in any parameters. In the recovery group, HGB, HCT, MCV, and MCH were significantly lower, while the neutrophil differential count of WBC was significantly higher in females given 2.5 mg PFDoA/kg/day than in the control group.

Blood Biochemistry

Serum TP and albumin levels were significantly decreased in males at 2.5 mg/kg/day at the completion of the administration period (Table III). Significant increases were observed in the albumin and γ-globulin ratios in the protein fraction, as well as a significant decrease in the α_1-globulin ratio at 2.5 mg/kg/day and a significant decrease in the α_2-globulin ratio at 0.5 mg/kg/day and above. ALP activity was

TABLE II. Hematological findings in male and female rats administered PFDoA

Dose (mg/kg/day)	Main Group				Recovery Group	
	0 (control)	0.1	0.5	2.5	0 (control)	2.5
MALES						
Number of animals examined	5	5	5	5	5	5
Red blood cells ($10^4/\mu L$)	918 ± 37	914 ± 18	927 ± 31	943 ± 58	978 ± 56	859 ± 41**
Hemoglobin (g/dL)	16.3 ± 0.6	16.2 ± 0.4	17.0 ± 0.6	16.1 ± 0.9	16.4 ± 0.6	14.2 ± 0.6**
Hematocrit (%)	46.3 ± 1.4	46.2 ± 1.6	48.2 ± 2.2	44.0 ± 1.9	46.0 ± 1.3	40.3 ± 13.0**
MCV (fL)	50.5 ± 2.8	50.6 ± 2.2	52.0 ± 2.0	46.7 ± 1.6	47.1 ± 1.6	47.0 ± 1.4
MCH (pg)	17.8 ± 1.1	17.7 ± 0.7	18.3 ± 0.5	17.1 ± 0.4	16.8 ± 0.4	16.5 ± 0.4
MCHC (g/dL)	35.2 ± 0.3	35.0 ± 0.4	35.2 ± 0.5	36.7 ± 0.8**	35.7 ± 0.5	35.2 ± 0.5
Reticulocytes (%)	3.18 ± 0.47	3.26 ± 0.55	3.35 ± 0.63	1.17 ± 0.67**	3.15 ± 0.31	4.63 ± 0.56**
Platelets ($10^4/\mu L$)	124.8 ± 8.6	112.5 ± 11.8	118.0 ± 9.0	116.7 ± 33.2	127.6 ± 15.6	169.9 ± 41.2
White blood cells ($10^2/\mu L$)	113.4 ± 28.2	148.2 ± 33.9	108.4 ± 19.6	135.0 ± 36.9	139.8 ± 14.2	112.5 ± 6.9**
Neutrophils ($10^2/\mu L$)	16.8 ± 4.1	17.9 ± 6.2	13.8 ± 3.1	21.2 ± 8.6	19.3 ± 8.4	15.3 ± 5.0
Lymphocytes ($10^2/\mu L$)	90.6 ± 24.5	123.7 ± 30.6	89.8 ± 17.9	108.0 ± 32.4	112.5 ± 15.0	93.0 ± 6.0*
Monocytes ($10^2/\mu L$)	4.24 ± 1.22	4.28 ± 0.65	3.56 ± 1.18	4.76 ± 3.85	5.36 ± 1.90	2.54 ± 0.78*
Eosinophils ($10^2/\mu L$)	1.68 ± 0.78	2.26 ± 0.73	1.22 ± 0.55	1.00 ± 0.58	2.60 ± 0.62	1.56 ± 0.56*
Basophils ($10^2/\mu L$)	0.04 ± 0.09	0.04 ± 0.05	0.02 ± 0.04	0.06 ± 0.09	0.06 ± 0.05	0.00 ± 0.00
PT (sec)	21.3 ± 4.3	22.7 ± 3.9	20.7 ± 3.5	19.1 ± 0.9	20.1 ± 3.4	18.5 ± 1.0
APTT (sec)	26.0 ± 2.3	26.9 ± 2.2	25.9 ± 1.6	22.0 ± 3.5	25.5 ± 4.5	23.0 ± 1.2
FEMALES						
Number of animals examined	5	5	5	1	5	5
Red blood cells ($10^4/\mu L$)	812 ± 24	828 ± 44	837 ± 26	803[a]	868 ± 27	836 ± 43
Hemoglobin (g/dL)	15.3 ± 0.5	15.5 ± 0.5	15.6 ± 0.4	14.5[a]	15.8 ± 0.4	14.2 ± 0.7**
Hematocrit (%)	44.6 ± 1.9	45.6 ± 1.5	45.2 ± 1.2	40.5[a]	45.2 ± 1.1	41.1 ± 2.2**
MCV (fL)	54.9 ± 2.2	55.1 ± 1.6	54.1 ± 1.7	50.4[a]	52.1 ± 1.6	49.2 ± 2.1*
MCH (pg)	18.8 ± 0.5	18.8 ± 0.6	18.6 ± 0.3	18.1[a]	18.3 ± 0.5	17.0 ± 0.6**
MCHC (g/dL)	34.3 ± 0.5	34.0 ± 0.3	34.4 ± 0.6	35.8[a]	35.1 ± 0.5	34.6 ± 0.4
Reticulocytes (%)	9.79 ± 1.26	10.20 ± 1.14	8.67 ± 1.46	4.05[a]	3.00 ± 0.56	4.00 ± 0.90
Platelets ($10^4/\mu L$)	124.3 ± 11.6	144.5 ± 9.9*	136.7 ± 9.5	143.0[a]	112.8 ± 14.3	118.8 ± 35.4
White blood cells ($10^2/\mu L$)	116.5 ± 34.4	101.9 ± 37.0	102.0 ± 17.4	95.7[a]	82.7 ± 24.7	104.9 ± 31.8
Neutrophils ($10^2/\mu L$)	41.0 ± 28.6	24.6 ± 13.8	28.6 ± 7.8	14.2[a]	10.1 ± 4.0	17.5 ± 5.2*
Lymphocytes ($10^2/\mu L$)	67.8 ± 14.9	70.2 ± 21.5	64.6 ± 9.0	72.3[a]	68.5 ± 20.7	81.1 ± 32.2
Monocytes ($10^2/\mu L$)	5.60 ± 1.39	5.50 ± 2.09	6.86 ± 2.58	8.20[a]	2.82 ± 0.84	5.18 ± 2.28
Eosinophils ($10^2/\mu L$)	2.02 ± 0.66	1.54 ± 0.76	1.90 ± 0.32	0.90[a]	1.28 ± 0.38	1.16 ± 0.73
Basophils ($10^2/\mu L$)	0.04 ± 0.05	0.06 ± 0.05	0.04 ± 0.05	0.10[a]	0.00 ± 0.00	0.02 ± 0.04
PT (sec)	18.7 ± 1.0	18.0 ± 0.8	17.8 ± 1.1	17.2[a]	17.4 ± 0.9	18.9 ± 4.6
APTT (sec)	20.4 ± 1.0	20.2 ± 1.2	20.7 ± 1.9	21.8[a]	18.9 ± 1.6	25.3 ± 8.6

Values are given as the mean ± S.D.
*Significantly different from the control, $p \leq 0.05$.
**Significantly different from the control, $p \leq 0.01$.
[a]Data from only one animal. In this group, other females did not deliver pups normally or survive to the end of the study.

TABLE III. Blood biochemical findings in male and female rats administered PFDoA

	Main Group				Recovery Group	
Dose (mg/kg/day)	0 (control)	0.1	0.5	2.5	0 (control)	2.5
MALES						
Number of animals examined	5	5	5	5	5	5
Total protein (g/dL)	5.62 ± 0.13	5.54 ± 0.24	5.50 ± 0.16	4.30 ± 0.43**	5.74 ± 0.11	4.70 ± 0.14**
Albumin (g/dL)	2.82 ± 0.12	2.85 ± 0.18	2.85 ± 0.06	2.32 ± 0.30**	2.78 ± 0.14	2.62 ± 0.12
A/G (ratio)	1.01 ± 0.09	1.06 ± 0.06	1.08 ± 0.08	1.17 ± 0.08*	0.95 ± 0.10	1.26 ± 0.12**
Protein fraction (%)						
Albumin	50.2 ± 2.2	51.4 ± 1.4	51.9 ± 1.8	53.8 ± 1.8*	48.5 ± 2.5	55.8 ± 2.5**
Globulin α_1	19.9 ± 3.9	19.9 ± 1.7	21.2 ± 2.3	14.3 ± 2.5*	23.7 ± 2.2	14.4 ± 2.3**
Globulin α_2	8.04 ± 0.80	7.14 ± 0.43	6.92 ± 0.40*	5.82 ± 0.86**	6.70 ± 0.20	6.60 ± 0.22
Globulin β	16.6 ± 1.9	16.3 ± 1.1	15.0 ± 0.8	16.9 ± 0.7	16.2 ± 0.8	16.4 ± 1.5
Globulin γ	5.28 ± 0.88	5.28 ± 0.96	4.92 ± 0.57	9.18 ± 2.40**	4.86 ± 0.59	6.90 ± 0.91**
AST (IU/L)	74.6 ± 11.1	83.0 ± 22.1	103.6 ± 84.8	125.4 ± 56.1	65.6 ± 12.0	69.0 ± 5.7
ALT (IU/L)	31.2 ± 3.1	34.0 ± 5.7	48.2 ± 41.0	53.2 ± 28.0	26.0 ± 6.2	28.0 ± 6.4
ALP (IU/L)	357.2 ± 28.6	366.6 ± 88.4	551.6 ± 95.2**	630.0 ± 72.0**	251.0 ± 30.2	534.4 ± 78.0**
γ-GTP (IU/L)	0.52 ± 0.25	0.60 ± 0.20	0.54 ± 0.15	2.56 ± 2.20	0.58 ± 0.19	1.08 ± 1.70
Total bilirubin (mg/dL)	0.066 ± 0.018	0.044 ± 0.011	0.052 ± 0.022	0.390 ± 0.260**	0.054 ± 0.009	0.080 ± 0.027
Glucose (mg/dL)	166.2 ± 6.9	173.2 ± 19.0	156.8 ± 3.4	122.0 ± 4.6**	180.0 ± 17.0	154.6 ± 28.0
Total cholesterol (mg/dL)	67.0 ± 9.7	44.6 ± 7.6*	40.6 ± 7.8*	53.4 ± 20.0	56.8 ± 8.6	58.2 ± 8.6
Triglycerides (mg/dL)	37.4 ± 14.3	45.2 ± 12.8	33.6 ± 12.0	27.2 ± 5.40	56.6 ± 21.7	19.0 ± 5.50**
BUN (mg/dL)	15.7 ± 0.8	15.6 ± 0.9	16.2 ± 3.5	21.9 ± 1.2**	14.9 ± 1.1	19.7 ± 2.3**
Crea (mg/dL)	0.57 ± 0.03	0.56 ± 0.03	0.54 ± 0.04	0.48 ± 0.05**	0.54 ± 0.04	0.49 ± 0.05
Na (mEq/L)	143.8 ± 0.8	143.8 ± 0.8	143.8 ± 1.3	144.4 ± 2.4	140.6 ± 0.5	140.2 ± 0.8
K (mEq/L)	4.68 ± 0.32	4.86 ± 0.28	4.98 ± 0.22	4.83 ± 0.75	4.74 ± 0.62	5.32 ± 0.38
CI (mEq/L)	109.0 ± 0.7	108.2 ± 1.1	108.8 ± 1.8	110.2 ± 0.8	103.6 ± 1.1	105.4 ± 1.7
Ca (mg/dL)	9.52 ± 0.38	9.62 ± 0.36	9.40 ± 0.32	8.38 ± 0.26**	10.0± 0.05	9.04 ± 0.37**
IP (mg/dL)	6.60 ± 0.27	7.06 ± 0.74	6.64 ± 0.74	7.28 ± 0.36	6.92 ± 0.59	8.12 ± 0.73*
FEMALES						
Number of animals examined	5	5	5	1	5	5
Total protein (g/dL)	6.20 ± 0.23	6.50 ± 0.31	6.16 ± 0.19	5.30[a]	6.20 ± 0.23	4.56 ± 0.77**
Albumin (g/dL)	2.96 ± 0.23	3.34 ± 0.25*	3.26 ± 0.13	2.38[a]	3.53 ± 0.26	2.68 ± 0.43**
A/G (ratio)	0.92 ± 0.09	1.05 ± 0.09	1.13 ± 0.08**	0.81[a]	1.32 ± 0.11	1.45 ± 0.12
Protein fraction (%)						
Albumin	47.7 ± 2.6	51.3 ± 2.2*	52.9 ± 1.7**	44.8[a]	56.9 ± 2.1	59.1 ± 1.9
Globulin α_1	19.9 ± 2.1	18.8 ± 1.6	17.6 ± 1.9	16.3[a]	15.7± 1.5	10.6 ± 3.5*
Globulin α_2	7.98 ± 0.61	6.82 ± 0.69*	7.22 ± 0.73	7.80[a]	5.72 ± 0.64	6.54 ± 1.41
Globulin β	19.0 ± 1.5	17.7± 1.6	16.9 ± 0.7	17.2[a]	14.7± 1.0	14.8 ± 2.2
Globulin γ	5.46 ± 1.30	5.32 ± 0.88	5.36 ± 0.89	13.90[a]	7.06 ± 1.60	9.00 ± 3.80
AST (IU/L)	79.4 ± 12.8	66.8 ± 4.7	87.0 ± 48.1	147.0[a]	66.2 ± 8.7	143.6 ± 104.0**
ALT (IU/L)	26.2 ± 4.0	24.4 ± 2.3	28.2 ± 6.2	32.0[a]	27.2 ± 7.5	53.4 ± 61.0
ALP (IU/L)	199.6± 21.6	180.0 ± 62.0	174.6 ± 40.7	499.0[a]	144.4± 44.7	657.4 ± 439.0**
y-GTP (IU/L)	0.62 ± 0.28	0.62 ± 0.36	0.54 ± 0.11	1.50[a]	0.50 ± 0.20	15.4 ± 17.0**
Total bilirubin (mg/dL)	0.048 ± 0.008	0.046 ± 0.009	0.038 ± 0.008	0.070[a]	0.074± 0.011	1.240 ± 2.300
Glucose (mg/dL)	145.0± 23.0	150.4 ± 8.7	160.8 ± 13.0	121.0[a]	140.4± 19.0	115.4 ± 25.0
Total cholesterol (mg/dL)	67.2 ± 19.0	55.6 ± 16.0	45.8 ± 12.0	54.0[a]	69.2 ± 15.0	55.2 ± 22.0
Triglycerides (mg/dL)	26.8 ± 8.9	67.4 ± 86.9	33.0 ± 15.2	19.0[a]	17.4 ± 4.5	21.2 ± 10.0
BUN (mg/dL)	26.52 ± 4.52	22.74 ± 3.84	23.34 ± 2.77	29.70[a]	15.98± 1.04	17.06 ± 4.14
Crea (mg/dL)	0.62 ± 0.03	0.60 ± 0.01	0.59 ± 0.03	0.48[a]	0.60 ± 0.02	0.52 ± 0.03**
Na (mEq/L)	139.0± 2.0	139.2 ± 1.9	138.2 ± 2.0	139.0[a]	140.2± 1.3	139.6 ± 0.9
K (mEq/L)	4.97 ± 0.17	5.23 ± 0.20	5.11 ± 0.28	5.44[a]	4.50 ± 0.56	4.97 ± 0.30
CI (mEq/L)	103.6± 1.3	104.4 ± 1.5	103.4 ± 1.1	106.0[a]	104.8± 0.8	104.8 ± 0.8
Ca (mg/dL)	10.5 ± 0.5	10.6 ± 0.5	10.4 ± 0.3	9.70[a]	10.3 ± 0.2	9.2 ± 0.8**
IP (mg/dL)	8.96 ± 0.71	8.42 ± 0.40	8.76 ± 0.86	7.80[a]	6.62 ± 0.51	7.82 ± 1.40

Values are given as the mean ± S.D.

*Significantly different from the control, $p \leq 0.05$.

**Significantly different from the control, $p \leq 0.01$.

[a]Data from only one animal. In this group, other females did not deliver pups normally or survive to the end of the study.

significantly increased at 0.5 and 2.5 mg/kg/day in males. Significant increases in T-Bil and BUN and decreases in glucose, Crea, and Ca were noted in the 2.5 mg/kg/day group. Furthermore, T-Cho was significantly decreased at 0.1 and 0.5 mg/kg/day in males. Significant decreases in TP, the α_1-globulin fraction ratio, TG, and Ca, and significant increases in albumin and the γ-globulin fraction ratios, ALP, BUN, and IP were observed in males of the 2.5 mg/kg/day group at the end of recovery periods.

In females in the main group, many changes, including decreases in TP, albumin, and Crea, and increases in AST, ALP, γ-GTP, and T-Bil, were found in one female in the 2.5 mg/kg/day group (Table III). The albumin fraction ratio was significantly increased in females in the 0.1 and 0.5 mg/kg/day groups. Significant decreases in TP, albumin, α_1-globulin fraction ratio, Crea, and Ca, and significant increases in AST, ALP, and γ-GTP were observed in females in the recovery group at 2.5 mg/kg/day.

Necropsy Findings

In the main group, gross observations at necropsy revealed atrophy of the thymus in 3/7 males and 10/12 females, atrophy of the spleen in 3/7 males and 5/12 females, yellowish brown discoloration of the liver in 7/7 males and 6/12 females, black patches on the glandular stomach mucosa in 4/12 females, pancreas edema in 2/12 females, small-sized epididymis in 4/7 males, small-sized seminal vesicle in 4/7 males, pale yellow discoloration of the subcutis of general skin in 1/12 females, and atrophy of the lateral great muscle in 3/7 males at 2.5 mg/kg/day. In addition, opacity of the eye ball, granular surface on the forestomach mucosa, thickening of the forestomach mucosa, diverticulum of the ileum, yellow mass of the epididymis cauda, and yellow patches on the epididymis corpus were each observed in 1 male given 2.5 mg PFDoA/kg/day. Blood retention was identified in the uterus in five of seven females that died or were euthanized due to a moribund condition at the end of the gestation period in the 2.5 mg/kg/day group.

Yellowish brown or yellow discoloration in the liver (5/5 males and 4/5 females) and enlarged liver (4/5 males and 2/5 females), atrophy of the thymus (1/5 males and 1/5 females), spleen atrophy (1/5 females), edema of the submandibular gland and sublingual gland (1/5 females), atrophy of the lateral great muscle (1 female), pale yellow discoloration of the subcutis of the general skin (1 female), and small-sized seminal vesicle (1 male) were observed in the 2.5 mg/kg/day recovery group.

Organ Weight

The relative weight of the liver in males was significantly higher at 0.5 mg/kg/day and 2.5 mg/kg/day at the end of the administration period (Table IV). The absolute and relative weights of the thymus and absolute weights of the kidney,

spleen, heart, pituitary gland, thyroid, adrenal gland, and epididymis in males given 2.5 mg PFDoA/kg/day were significantly decreased while the relative weights of the kidney and brain were significantly increased.

In females in the main group, many changes, including an increase in the relative liver weight and decreases in the absolute and relative weights of the spleen and thymus, were found in one surviving female given 2.5 mg/kg/day (Table IV). A significant increase in the relative liver weight and significant decreases in absolute weights of spleen, heart, pituitary gland, and thymus were observed at the end of the administration period in females given 0.5 mg PFDoA/kg/day.

Most changes observed in males given 2.5 mg PFDoA/kg/day at the end of the administration period remained after the 14-day recovery period (Table IV). In addition, the relative weights of testes and epididymides were significantly increased in the male recovery group. Significant increases in the relative weights of the brain, liver, and kidney and significant decreases in the absolute and relative weights of the ovary and absolute weights of the heart, pituitary gland, and adrenal gland were observed in females in the 2.5 mg/kg/day recovery group.

Histopathological Findings

Upon completion of the administration period, hepatic changes were observed in all males and females given 2.5 mg PFDoA/kg/day (Table V). They included diffuse hepatocyte hypertrophy, peribiliary inflammatory cellular infiltration, single cell necrosis of hepatocytes, focal necrosis, bilirubin deposition, and bile duct proliferation. The incidence of diffuse hepatocyte hypertrophy in both sexes and single cell necrosis of hepatocytes in females was significantly higher in the 2.5 mg/kg/day group. Focal necrosis was also detected in two females given 0.5 mg PFDoA/kg/day.

Histopathological changes were observed not only in the liver, but also in various organs in the 2.5 mg/kg/day group (Table V). A reduction in zymogen granules was seen in the pancreas in both sexes, and the frequency of the reduction in males was significantly increased. The incidence of edema of the interstitium in the pancreas was significantly higher in females in the 2.5 mg/kg/day group than in the controls. Atrophic changes were observed in the spleen, thymus, adrenal gland, muscle fibers, and male reproductive organs. The incidences of adrenal cortex atrophy in males and thymus cortex atrophy in females were significantly increased. Furthermore, a decrease in hematopoiesis in the bone marrow, ulcers in the glandular stomach, and erosion, hyperkeratosis, squamous cell hyperplasia, and inflammatory cellular infiltration, edema, and fibrosis of submucosa in the forestomach were noted.

As for male reproductive organs, atrophy of the glandular epithelium was observed in the prostate, seminal vesicle, and coagulating gland (Table V). In addition, cell debris at

TABLE IV. Organ weights of male and female rats administered PFDoA

Dose (mg/kg/day)		Main Group				Recovery Group	
		0 (control)	0.1	0.5	2.5	0 (control)	2.5
MALES							
Number of animals examined		5	5	5	5	5	5
Liver	(g)	12.0 ± 1.3	13.5 ±2.1	14.7 ±2.6	13.6 ±3.1	13.1 ± 1.2	15.2 ±2.8
	(%)ᵃ	2.51 ±0.14	2.67 ±0.21	3.00 ± 0.30*	4.30 ±0.27**	2.56 ±0.18	3.94 ±0.61**
Kidneyᵇ	(g)	3.01 ±0.27	3.28 ±0.22	3.26 ± 0.43	2.39 ±0.45*	3.15 ±0.14	2.76 ±0.21**
	(%)ᵃ	0.628 ±0.032	0.656 ±0.052	0.670 ± 0.058	0.760 ± 0.060**	0.614 ±0.029	0.718 ± 0.050**
Spleen	(g)	0.784 ±0.102	0.802 ±0.091	0.724 ± 0.123	0.488 ±0.155**	0.862 ±0.074	0.650 ± 0.025**
	(%)ᵃ	0.166 ±0.015	0.158 ±0.015	0.148 ± 0.013	0.152 ±0.029	0.170 ±0.019	0.170 ±0.010
Heart	(g)	1.480 ±0.180	1.520 ±0.150	1.390 ± 0.160	0.864 ±0.180**	1.480 ±0.094	1.110 ±0.110**
	(%)ᵃ	0.312 ±0.041	0.300 ±0.016	0.288 ± 0.029	0.274 ±0.013	0.292 ±0.023	0.286 ±0.011
Brain	(g)	2.21 ±0.13	2.14 ±0.10	2.16 ± 0.03	2.10 ±0.12	2.21 ±0.01	2.15 ±0.08
	(%)ᵃ	0.460 ±0.020	0.428 ±0.033	0.446 ± 0.034	0.688 ±0.160**	0.430 ±0.032	0.562 ±0.049**
Pituitary gland	(mg)	12.3 ±0.8	12.3 ±0.6	13.0 ±2.1	7.5 ± 1.6**	12.2 ±2.2	9.4 ± 1.5*
	(10⁻³%)ᵃ	2.57 ±0.05	2.46 ±0.27	2.68 ± 0.51	2.37 ±0.30	2.40 ±0.43	2.44 ±0.33
Thymus	(mg)	325 ±97	442 ±75	328 ± 86	133 ±79**	382 ± 114	239 ±62*
	(10⁻³%)ᵃ	67.6 ± 19.9	87.8 ± 12.7	67.0 ± 15.3	39.1 ± 18.9*	75.3 ±24.2	62.5 ± 19.0
Thyroid	(mg)	23.3 ±3.7	23.7 ± 1.5	22.3 ± 4.3	14.4 ±4.1**	22.4 ±3.7	16.2 ±3.8*
	(10⁻³%)ᵃ	4.87 ±0.81	4.73 ±0.30	4.61 ± 1.00	4.53 ±0.77	4.38 ±0.61	4.22 ±0.96
Adrenal gland	(mg)	65.0 ±8.5	68.4 ±9.8	63.4 ± 17.0	38.4 ±5.9**	62.4 ±8.6	40.4 ±6.2**
	(10⁻³%)ᵃ	13.5 ± 1.3	13.6 ± 1.9	13.2 ±3.9	12.5 ±2.9	12.3 ± 1.9	10.5 ± 1.5
Testisᵇ,ᶜ	(g)	3.36 ±0.37	3.34 ±0.23	3.39 ± 0.30	2.81 ±0.65	3.43 ±0.28	3.39 ±0.42
	(%)ᵃ	0.671 ±0.054	0.647 ±0.049	0.677 ± 0.080	0.839 ±0.160	0.672 ±0.077	0.882 ±0.110**
Epididymisᵇ,ᶜ	(g)	1.33 ±0.17	1.35 ±0.12	1.32 ± 0.10	0.90 ±0.31**	1.43 ±0.090	1.23 ±0.13*
	(%)ᵃ	0.267 ±0.033	0.263 ±0.024	0.263 ± 0.020	0.266 ±0.073	0.280 ±0.025	0.316 ±0.017*
FEMALES							
Number of animals examined		5	5	5	1	5	5
Liver	(g)	9.9 ±0.6	10.8 ±0.9	10.9 ± 0.9	10.9ᵈ	7.6 ±0.4	8.7 ± 1.8
	(%)ᵃ	3.23 ±0.19	3.43 ±0.11	3.70 ± 0.22**	4.52ᵈ	2.45 ±0.11	3.61 ±0.35**
Kidneyᵇ	(g)	2.19 ±0.17	2.06 ±0.22	1.97 ± 0.09	1.86ᵈ	2.02 ±0.08	1.99 ±0.29
	(%)ᵃ	0.720 ±0.093	0.656 ±0.038	0.668 ± 0.022	0.780ᵈ	0.656 ±0.022	0.836 ± 0.063**
Spleen	(g)	0.780 ±0.052	0.750 ±0.046	0.674 ± 0.081*	0.400ᵈ	0.560 ±0.100	0.522 ±0.110
	(%)ᵃ	0.256 ±0.040	0.240 ±0.019	0.232 ± 0.023	0.170ᵈ	0.182 ±0.035	0.218 ±0.035
Heart	(g)	1.040 ±0.090	1.080 ±0.054	0.928 ± 0.048*	0.770ᵈ	0.994 ±0.036	0.796 ±0.160*
	(%)ᵃ	0.342 ±0.022	0.346 ±0.018	0.316 ± 0.017	0.320ᵈ	0.324 ±0.011	0.334 ±0.034
Brain	(g)	2.02 ±0.11	2.00 ±0.06	2.00 ± 0.02	2.14ᵈ	2.10 ±0.09	1.99 ±0.09
	(%)ᵃ	0.662 ±0.057	0.640 ±0.041	0.680 ± 0.025	0.890ᵈ	0.682 ±0.028	0.852 ±0.150*
Pituitary gland	(mg)	17.3 ± 1.7	16.0 ±2.3	14.1 ± 1.9*	12.0ᵈ	17.7 ±2.2	10.4 ±4.5*
	(10⁻³%)ᵃ	5.67 ±0.71	5.13 ±0.87	4.80 ± 0.60	5.00ᵈ	5.75 ±0.75	4.27 ± 1.3
Thymus	(mg)	310 ±27	297 ± 102	264 ± 16**	66.0ᵈ	298 ±70	241 ± 137
	(10⁻³%)ᵃ	101.0 ± 11.2	93.7 ±27.8	89.5 ±3.8	27.5ᵈ	96.9 ±23.9	95.9 ±50.3
Thyroid	(mg)	17.8 ±4.7	18.8 ±3.7	18.3 ±4.2	11.0ᵈ	15.5 ± 1.5	13.1 ±5.6
	(10⁻³%)ᵃ	5.74 ± 1.10	6.01 ± 1.20	6.21 ± 1.30	4.58ᵈ	5.02 ±0.47	5.32 ± 1.70
Adrenal gland	(mg)	84.2 ± 13.0	77.2 ± 8.6	79.4 ± 5.0	53.0ᵈ	70.8 ±9.2	47.4 ± 12.0**
	(10⁻³%)ᵃ	27.5 ± 4.3	24.7 ± 3.3	27.0 ± 1.5	22.1ᵈ	23.0 ±3.1	19.9 ± 3.3
Ovaryᵇ	(mg)	115.4 ± 14.3	114.0 ± 21.1	109.6 ± 10.0	119.0ᵈ	100.4 ±17.4	56.0 ± 7.3**
	(10⁻³%)ᵃ	37.6 ± 3.3	36.2 ± 4.7	37.2 ± 3.1	49.6ᵈ	32.6 ±6.0	23.7 ± 3.1*

Values are given as the mean ± S.D.

*Significantly different from the control, at $p \leq 0.05$.

**Significantly different from the control, at $p \leq 0.01$.

ᵃRatio of absolute organ weight to body weight on the necropsy day (relative organ weight).

ᵇValues are represented as the total weights of the organs on both sides.

ᶜOrgan weight was measured for all animals (number of examined animals: 7 at 0 and 2.5 mg/kg/day and 12 at 0.1 and 0.5 mg/kg/day in the main group, and 5 at 0 and 2.5 mg/kg/day in the recovery group).

ᵈData from only one animal. In this group, other females did not deliver pups normally or survive to the end of the study.

TABLE V. Histopathological findings in male and female rats administered PFDoA

Dose (mg/kg/day)	Grade	Main Group				Recovery Group	
		0 (control)	0.1	0.5	2.5	0 (control)	2.5
MALES							
Number of animals examined		7	12	12	7	5	5
Forestomach							
Erosion	+	0	0	0	1	0	0
Hyperkeratosis	+	0	0	0	2	0	0
Hyperplasia in squamous cells	+	0	0	0	2	0	0
Infiltration of inflammatory cells in the submucosa	+	0	0	0	1	0	0
Fibrosis of the submucosa	+	0	0	0	2	0	0
Pancreas							
Decrease in zymogen granules	+	0	0	0	5⎤[**]	0	1
	++	0	0	0	1⎦	0	0
Liver							
Deposition of bilirubin	+	0	0	0	1	0	2
Peribiliary infiltration of inflammatory cells	+	0	0	0	4	0	4[*]
Single cell necrosis of hepatocytes	+	0	0	0	1	0	0
	++	0	0	0	1	0	0
Focal necrosis	+	0	0	0	3	0	0
	++	0	0	0	1	?0	0
Diffuse hepatocyte hypertrophy	+	0	0	0	5[*]	0	3
Centrolobular hepatocyte hypertrophy	+	0	0	0	0	0	2
Periportal fatty changes	+	0	0	0	1	0	0
Fatty changes in midzonal	+	0	0	0	1	0	0
Fatty changes in diffuse	+	0	0	0	1	0	0
Testis							
Cell debris (Stage VII–VIII)	+	0	0	0	1	0	0
Decrease in elongated spermatids (Stage XII–XIV)	+	0	0	0	2	0	0
Epididymis							
Decrease in spermatozoa	+	0	0	0	2	0	1
	++	0	0	0	1	0	0
	+++	0	0	0	1	0	0
Cell debris in the lumen	+	0	0	0	2	0	0
	++	0	0	0	1	0	0
Spermatic granuloma	+	0	0	0	2	0	0
Prostate							
Glandular epithelium atrophy	+	0	0	0	3	0	1
Fibrosis in the interstitium	+	0	0	0	1	0	0
Seminal vesicles							
Glandular epithelium atrophy	+	0	0	0	3	0	1
	++	0	0	0	1	0	0
Coagulating gland							
Glandular epithelium atrophy	+	0	0	0	3	0	1
	++	0	0	0	1	0	0
Spleen							
White pulp atrophy	+	0	0	0	1	0	0
Red pulp atrophy	+	0	0	0	3	0	0
Thymus							
Atrophy of the cortex	+	0	0	0	0	0	1
	++	0	0	0	1	0	0
	+++	0	0	0	2	0	0
Bone marrow							
Decrease in hematopoiesis	+	0	0	0	2	0	0
	++	0	0	0	2	0	0
Adrenal glands							

TABLE V. *Continued*

Dose (mg/kg/day)	Grade	Main Group 0 (control)	0.1	0.5	2.5	Recovery Group 0 (control)	2.5
Atrophy of the cortex	+	0	0	0	5*	0	0
Skeletal muscle							
Muscle fiber atrophy	+	—	—	—	2(3)	—	—
	++	—	—	—	1(3)	—	—
FEMALES							
Number of animals examined		12	12	12	12[a]	5	5
Forestomach							
Edema of the submucosa	+	0	0	0	1	0	0
Glandular stomach							
Ulcer	+	0	0	0	4	0	0
Pancreas							
Edema of the interstitium	+	0	0	0	5 ⎤*	0	0
	++	0	0	0	1 ⎦	0	0
Decrease in zymogen granuled	+	0	0	0	2	0	2
	++	0	1	0	1	0	0
Liver							
Deposition of bilirubin	+	0	0	0	1	0	5**
Single cell necrosis of hepatocytes	+	0	0	0	2 ⎤*	0	3
	++	0	0	0	5 ⎦	0	0
Focal necrosis	+	0	0	2	4	0	0
Centrilobular hepatocyte necrosis	++	0	0	0	1	0	0
Bile duct proliferation	+	0	0	0	1	0	2
	++	0	0	0	1	0	2 ⎤**
Diffuse hepatocyte hypertrophy	+	0	0	0	6 ⎤**	0	2 ⎦
	++	0	0	0	6 ⎦	0	3
Inflammatory cell infiltration in peribiliary	+	0	0	0	0	0	3
Increase in mitosis in hepatocytes	+	0	0	0	1	0	0
Fatty changes in periportal	+	1	0	0	0	0	0
Fatty changes in diffuse	++	0	0	0	0	0	1
Uterus							
Hemorrhage at the implantation site	+	0	0	0	7 ⎤**	0	0
	++	0	0	0	1 ⎦	0	0
Congestion of the endometrium	+	0	0	0	4	0	0
	++	0	0	0	1	0	0
Atrophy of endometrium and myometrium	+	0	0	0	0	0	2
Spleen							
White pulp atrophy	+	0	0	0	1(9)	0	1
	++	0	0	0	2 (9)	0	0
Red pulp atrophy	+	0	0	0	2(9)	0	0
	++	0	0	0	1(9)	0	0
Thymus							
Atrophy of the cortex	+	0	0	0	3 (10) ⎤**	0	1
	++	0	0	0	4 (10) ⎥	0	0
	+++	0	0	0	2 (10) ⎦	0	0
Bone marrow							
Decrease in hematopoiesis	+	0	0	0	2 (10)	0	1
Adrenal glands							
Atrophy of the cortex	+	0	0	0	0	0	5**
Skeletal muscle							
Muscle fiber atrophy	+	—	—	—	—	—	1(1)

Values are the number of animals with findings.
Values in parentheses are the number of animals examined.
—, Not examined; Grade +, slight change; ++, moderate change; +++, severe change.
[a]Including animals euthanized and found dead.
*Significantly different from the control, at $p \le 0.05$.
**Significantly different from the control, at $p \le 0.01$.

TABLE VI. Reproductive performance and developmental findings in rats administered PFDoA

Dose (mg/kg/day)		0 (control)	0.1	0.5	2.5
MAIN GROUP					
Premating period					
Estrous cycle normality		12/12	12/12	11/12	12/12
Length (days)[a]		4.13 ±0.30	4.29 ±0.54	4.18 ±0.43	4.23 ±0.41
Number of pairs		12	12	12	12
Copulation index (%)	Male	100	100	100	100
	Female	100	100	100	100
Fertility index (%)		100	91.7	100	100
Gestation index (%)		100	100	100	8.33**
Gestation length (days)[a]		22.1 ±0.3	22.2 ±0.4	22.1 ±0.3	23.0[b]
Number of pregnant animals		12	11	12	12
Number of corpora lutea[a]		17.1 ± 1.6	16.9 ± 1.3	15.8 ± 1.5	15.4 ±2.2
Number of implantation sites[a]		16.0 ± 1.3	16.6 ± 1.3	15.5 ± 1.4	14.5 ±2.1
Implantation index (%)[a]		93.9 ±4.8	98.4 ±2.7[d]	98.5 ±3.7[d]	94.4 ±8.9
Delivery index (%)[a]		94.3 ±6.3	91.7 ±6.5	89.7 ±9.7	31.4 ±54.0[c]
Number of litters		12	11	12	1
Number of pups delivered[a]	Total	15.1 ± 1.5	15.3 ± 1.7	13.9 ±2.0	16.0[b]
	Alive	15.1 ± 1.5	14.9 ± 1.8	13.8 ±2.1	14.0[b]
	Dead	0.0 ±0.0	0.4 ±0.7	0.1 ±0.3	2.0[b]
Sex ratio of live pups[a]		0.55 ±0.10	0.61 ±0.10	0.57 ±0.13	0.43[b]
Live birth index (%)[a]		100.0 ±0.0	97.6 ±4.6	99.3 ±2.4	87.5[b]
Number of live pups[a]	on nursing day 4	14.8 ± 1.3	14.6 ± 1.7	13.6 ±2.0	14.0[b]
Viability index (%)[a]		98.5 ±2.8	98.3 ±3.0	98.3 ±3.0	100[b]
Male pups					
Body weight (g)[a]	PND 0	6.48 ±0.30	6.52 ±0.64	6.70 ±0.52	4.70[b]
	PND 1	7.08 ±0.33	7.12 ±0.79	7.33 ±0.71	4.90[b]
	PND 4	10.50 ±0.63	10.70 ± 1.20	10.70 ± 1.40	6.20[b]
Female pups					
Body weight (g)[a]	PND 0	6.19 ±0.28	6.17 ±0.51	6.26 ±0.65	4.70[b]
	PND 1	6.81 ±0.32	6.75 ±0.62	7.01 ±0.68	5.00[b]
	PND 4	10.10 ±0.50	10.00 ± 1.10	10.20 ± 1.40	6.50[b]
RECOVERY GROUP					
Administration period					
Estrous cycle normality		5/5			0/5[e]
Length (days)[a]		4.24 ±0.43			-[d]
Recovery period					
Estrous cycle normality		5/5			1/5*
Length (days)[a]		4.10 ±0.22			4.00[e]

Estrous cycle normality, number of females with a normal estrous cycle / number of females examined; Copulation index, (number of animals with successful copulation / number of animals mated) × 100; Fertility index, (number of pregnant females/number of pairs with successful copulation) × 100; Gestation index, (number of females with live pups/number of pregnant females) × 100; Implantation index, (number of implantation sites/number of corpora lutea) × 100; Delivery index, (number of pups born/number of implantation sites) × 100; Sex ratio, (number of live male pups/number of live pups); Live birth index, (number of live pups on nursing day 0/number of pups born) × 100; Viability index, (number of live pups on nursing day 4/number of live pups on nursing day 0) × 100.

[a]Values are means and S.D.

[b]The number of dams or litters examined was one because only one dam normally delivered pups. The data were excluded from statistical evaluation.

[c]The number of litters examined was three because seven animals were found dead or moribund at the end of pregnancy and two females did not deliver pups normally. The data were excluded from statistical evaluation.

[d]Since continuous diestrous was observed in all five females, the length of the estrous cycle could not be calculated.

[e]The length of the estrous cycle was only calculated for one female because continuous diestrous was observed in the four other females. The data were excluded from statistical evaluation.

*Significantly different from the control, at $p \leq 0.05$.

**Significantly different from the control, at $p \leq 0.01$.

stages VII–VIII and a decrease in the number of elongate spermatids at stages XII–XIV in the testis, decrease in spermatozoa, cell debris in the lumen and spermatic granuloma in the epididymis, and fibrosis of the interstitium in the prostate were found. In female reproductive organs, hemorrhage on the implantation site and/or congestion on the endometrium were detected in the uterus of all 7 females found dead or moribund at the end of the gestation period. Hemorrhage at the implantation site was also found in one female that did not deliver live pups (all pups were stillborn).

Most hepatic changes remained after the 14-day recovery period (Table V). The incidences of peribiliary inflammatory cellular infiltration in males and bilirubin deposition and diffuse hepatocyte hypertrophy in females were significantly higher in the 2.5 mg/kg/day recovery group. Atrophy of the adrenal cortex was observed in all females in the 2.5 mg/kg/day recovery group with a significantly higher incidence. Endometrium and myometrium atrophy was noted in the uterus in 2 of 5 females given 2.5 mg PFDoA/kg/day after the 14-day recovery period. Although histopathological changes were also observed in the pancreas, thymus, spleen, bone marrow, skeletal fibers, and male reproductive organs after the 14-day recovery period, their incidences or degree was generally lower than those at the end of the administration period.

Reproductive and Developmental Findings

All females in the main group exhibited a normal estrous cycles during the premating period, except for one female in the 0.5 mg/kg/day group in which persistent diestrous was noted (Table VI). No significant deviations were observed in the incidence of a normal estrous cycle and length of the estrous cycle during the premating period. On the other hand, continuous diestrous was observed in the recovery group from day 27 of the administration period in all females given 2.5 mg/kg/day. A normal estrous cycle could not be recovered in four of the five females, even after termination of the administration period.

All males and females in the main groups were successfully copulated (Table VI). Although one female was not impregnated in the 0.1 mg/kg/day group, all other females became pregnant. No significant changes were found in the fertility index, the number of corpora lutea, or the number of implantation sites between the control and PFDoA-treated groups. In the 2.5 mg/kg/day group, 7 of 12 females given 2.5 mg PFDoA/kg/day were found dead or fell into a moribund state at the end of pregnancy, as mentioned above. Two of five surviving pregnant females did not deliver any pups, and 2 other females did not deliver live pups (all pups were stillborn). Consequently, only one female delivered live pups in the 2.5 mg/kg/day group; therefore, the gestation and delivery indices in this group were markedly lower than those of the control group. The gestation length of this one

female in the 2.5 mg/kg/day group did not differ from that in the other groups.

The number of normally delivered pups in the 2.5 mg/kg/day group was 16 in one litter; however, two of them were found dead on nursing day 0 (Table VI). Although the other 14 pups survived to the end of the study, their body weights on PNDs 0, 1, and 4 were markedly lower than those of the control group. Necropsy of dead pups revealed renal pelvis dilatation and ascites in one pup in the 0.5 mg/kg/day group, while no other gross external or internal alterations were found in pups that survived until PND 4 or pups found dead during the postnatal period. No significant changes were observed in any reproductive/developmental parameters in the 0.1 and 0.5 mg/kg/day groups.

DISCUSSION

In this study, 7/12 females receiving 2.5 mg PFDoA/kg/day were found dead or moribund at the end of pregnancy. In contrast, no clear dose-related clinical signs of toxicity were observed in females of the recovery group or in males, which suggested that the cause of death involved factors that associated with pregnancy or delivery. Vaginal hemorrhage and/or blood retention in the uterus were observed in the dead and moribund females. Histopathological examinations of the uterus revealed hemorrhage in the implantation sites and congestion of the endometrium. These findings demonstrated that these females could not maintain a pregnancy, and excessive bleeding after placental separation may worsen their general condition.

Food consumption and body weight gain were markedly decreased in both sexes in the 2.5 mg/kg/day group. The effects on body weight are typically observed in rodents given PFCAs at relatively high doses, but they were not accompanied with reduced food intake necessarily (ATSDR, 2009; Hirata-Koizumi et al., 2012). Interestingly, Yang et al. (2002) reported that a 7-day dietary treatment with PFOA lowered the body weight of mice and this effect disappeared when peroxisome proliferator activated receptor (PPAR) α, a nuclear receptor important in regulating fatty acid metabolism in tissues such as liver, kidney, heart, and intestinal mucosa (Corton et al., 2000), was knocked out. PFDoA was recently shown to activate mouse PPARα in transiently transfected COS-1 cells (Wolf et al., 2012). Although no data are currently available on the interaction between PFDoA and rat PPARα, the significant induction of the mRNA levels of important PPARα target genes, acyl CoA oxidase and CYP4A1, was demonstrated in male rats orally dosed with PFDoA at 1 mg/kg and higher for 14 days (Zhang et al., 2008) and at 0.2 mg/kg/day and higher for 110 days (Ding et al., 2009). Taken together, these findings suggest that PFDoA may inhibit body weight gain via the activation of PPARα. In our studies for PFDoA and PFOdA, hepatic necrosis was observed at a dose affecting the body

weight (Hirata-Koizumi et al., 2012); therefore, there is also the possibility that hepatic necrosis is one factor for inhibition of body weight gain.

As with PFOA and the other PFCAs, the primary target of PFDoA was the liver. Relative liver weights increased in both sexes in the 0.5 and 2.5 mg/kg/day groups. Various histopathological changes, including hepatocyte hypertrophy and necrosis, were observed in the liver in both sexes given 2.5 mg PFDoA/kg/day, and focal necrosis was also found in the liver of 2/12 females receiving 0.5 mg PFDoA/kg/day. These changes have been attributed, at least in part, to PPARα activation by PFDoA because PPARα is considered to mediate the biological effects of peroxisome proliferators, such as increases in liver weight due to hepatocyte hypertrophy and hyperplasia, transcriptional increases in enzymes involved in the metabolism of fatty acids, and hepatocarcinogenesis (Green, 1995; Holden et al., 1999; Corton et al., 2000). On the other hand, the following findings suggest that a different mechanism from PPARα activation is involved in the hepatotoxicity of PFDoA. The peribiliary infiltration of inflammatory cells, bilirubin deposition, and proliferation of the bile duct were observed in the 2.5 mg/kg/day group, and blood biochemical examinations revealed an increased level of T-Bil and γ-GTP activity at 2.5 mg/kg/day and increased ALP activity at 0.5 and 2.5 mg/kg/day. These changes indicate inflammatory cholestasis. Yellow brown discoloration of the liver and subcutis, and yellow mass and patch on the epididymis observed in some animals given 2.5 mg PFDoA/kg/day may have resulted from the accumulation of yellow bilirubin pigment. The dose-independent changes in serum T-Cho observed in males suggest that the hypocholesterolemic action of PFDoA via PPARα activation may have been countervailed by impaired cholesterol excretion associated with cholestasis in the high dose group.

Most of the other changes observed in the 2.5 mg/kg/day group may be secondary effects that occur with the pronounced reduction in body weight gain and food consumption. A reduction in motor activity and grip strength may reflect muscle weakness accompanying decreases in body weight rather than neurotoxicity. Atrophy of the lateral great muscle in the 2.5 mg/kg/day group supports this hypothesis. Histopathological changes observed in the stomach, thymus, pancreas, and bone marrow are known to be associated with nutrient deficiencies and/or stress. The prolonged administration of PFDoA, which had a marked influence on food consumption and body weight, must have been stressful for animals. On the other hand, atrophy of the adrenal gland cannot only be explained by changes in body weight and food consumption because previous food restriction studies demonstrated that the adrenal gland was hypertrophied (Moriyama et al., 2008; Shallie et al., 2012). Such atrophic changes in the adrenal gland were shown to be induced by adrenal steroidogenesis inhibitors such as 1-(o-chlorophenyl)−1-(p-chlorophenyl)−2,2-dichloroethane (o,p'-DDD), and α-[1,4-dioxido-3-methylquinoxalin-2-yl]-N-methylnitrone (DMNM)

(Hamid et al., 1974; Rosol et al., 2001). Because PFDoA was demonstrated to inhibit steroidogenesis in the testis and ovary (Shi et al., 2007; Shi et al., 2009a,b; 2010a,b), it may also alter adrenal steroidogenesis to cause atrophy of the adrenal cortex.

PFDoA affected the male and female reproductive systems. In males, cell debris and a reduction in the number of spermatid or spermatozoa were observed in the testis and epididymis, and atrophic changes were identified in the prostate, seminal vesicle, and coagulating gland in the 2.5 mg/kg/day group. Although these changes may have been due to the inhibition of body weight gain, a previous study demonstrated that the oral administration of PFDoA to rats for 110 days at a dose as low as 0.2 mg/kg/day decreased serum testosterone levels without affecting body weight (Shi et al., 2009a). An in vitro study reported the dose-dependent inhibition of steroidogenesis in mouse Leydig tumor cells and primary rat Leydig cells (Shi et al., 2010a), which indicated that PFDoA directly affected testicular testosterone synthesis, and not via the hypothalamic-pituitary-testicular axis. Since decreased testosterone biosynthesis is known to result in the degeneration and reduction in the number of germ cells as well as decreased size of accessory sex glands (O'Connor et al., 2002; OECD, 2009), the histopathological changes observed in the male reproductive organs in this study were attributed, at least in part, to the disruption of steroidogenesis. Shi et al. (2007, 2009a) reported that levels of the steroidogenic acute regulatory protein (StAR), which is responsible for cholesterol transport to the inner mitochondrial membrane, and StAR mRNA were markedly decreased in the testes of rats exposed to PFDoA, and treatment with the hydrosoluble form of cholesterol, which readily enters the inner mitochondrial membrane without the help of StAR, to mouse Leydig tumor cells prevented the inhibitory effect of PFDoA on steroidogenesis (Shi et al., 2010a). These results suggest that StAR is one of the target proteins for PFDoA activity in Leydig cells. A recently conducted proteomic analysis on the testis of rats exposed to PFDoA indicated that alterations in multiple pathways, including mitochondrial disruption and oxidative stress, may be associated with the testicular toxicity of PFDoA in rats (Shi et al., 2010b). Decreased testosterone levels in the testes and/or blood was also caused by PFOA, perfluorononanoic acid (PFNA, C9) and perfluorodecanoic acid (PFDeA, C10) (Bookstaff et al., 1990; Biegel et al., 1995; Jensen et al., 2008; Feng et al., 2009; Feng et al., 2010), which may involve the same mechanism as PFDoA. Recent study on PFOA-induced disruption of testosterone biosynthesis suggests the involvement of PPARα (Li et al., 2011).

A previous study demonstrated that PFDoA decreased serum estradiol levels in female rats following a 28-day oral administration period at a dose that affected body weight (Shi et al., 2009b). Alterations in the ovarian expression of genes responsible for cholesterol transport and steroidogenesis (StAR protein, cholesterol side-chain cleavage enzyme, and 17-beta-hydroxysteroid dehydrogenase) were also found

in this previous study. Such effects on the ovarian steroidogenesis may explain why continuous diestrous was observed in the recovery group in this study because estrogen and progesterone, which are steroid hormones synthesized from cholesterol in the ovary, play an important role in controlling the estrous cycle (OECD, 2009). Continuous diestrous indicates at least the temporary and possibly permanent cessation of follicular development and ovulation, and thus temporary infertility (Parker, 2006). In this study, the lack of an effect on the copulation and fertility indices was consistent with the findings that the abnormal estrous cycle was observed after the 27th day of the administration period in the recovery group and not found during the 14-day premating period in the main group. Considering that continuous diestrous was induced around the same time as changes in body weight and food consumption became apparent, the disruption of energy homeostasis could be a factor in the abnormal estrous cycles observed in this study. Food restriction in rats has been shown to result in weight loss and constant diestrous (Kotsuji et al., 1986; Narita et al., 2011). Recent evidence has suggested that many of the central and peripheral endocrine signals that govern energy homeostasis are involved in the control of reproductive function by acting at different levels of the hypothalamic-pituitary-gonadal axis (Narita et al., 2011). Effects on estrous cyclicity have not been reported for the other PFCAs, which may be because the reproductive toxicity of the other PFCAs were not examined at doses causing severe inhibition of body weight gain as observed in the 2.5 mg/kg/day PFDoA group.

PFDoA exerted no effects on the copulation and fertility indices or on the number of corpora lutea and implantation; however, only one of twelve pregnant females delivered live pups in the 2.5 mg/kg/day group. As mentioned above, PFDoA has been reported to disrupt ovarian steroidogenesis (Shi et al., 2009b). Since pregnancy is maintained under the control of estradiol and progesterone (Ogle et al., 1990; Bartholomeusz et al., 1999), PFDoA may affect pregnancy by disrupting steroidogenesis. Another possible factor is impaired fetal development, which could affect the maintenance of pregnancy and normal delivery. Live pups delivered from one pregnant female in the 2.5 mg/kg/day group had markedly lower body weights than those of the controls. The effects of PFDoA on fetal development could be attributed to secondary effects due to maternal toxicity; however, the lipophilic properties of PFDoA (Inoue et al., 2012) also indicate the possibility that it was transferred via the placenta and directly affected the fetuses.

In this study, some of the changes observed during and at the end of the administration period were detected even after the 14-day recovery period, including reductions in body weight, hypertrophy of hepatocytes, bilirubin disposition, peribiliary infiltration of inflammatory cells and bile duct proliferation in the liver, and atrophy of the adrenal cortex. Although no data are currently available on the toxicokinetics of PFDoA, previous studies demonstrated that PFCAs with a

longer carbon chain were eliminated more slowly from the body; the elimination half-life was shown to be 6.38 h for perfluorobutanoic acid (C4), 2.4 h for perfluoroheptanoic acid (C7), 135–185 h for PFOA (C8), 710 hours for PFNA (C9), and 958 h for PFDeA (C10) in male rats intravenously administered PFCAs (Kudo et al., 2002; Kemper, 2003; Ohmori et al., 2003; Chang et al., 2008). Therefore, incomplete recovery of the toxic effects caused by PFDoA may be attributed to its slow elimination from the body.

In summary, 42- to 47-day oral gavage administration of PFDoA mainly affected the liver, causing hypertrophy, necrosis, and inflammatory cholestasis, at 0.5 and 2.5 mg/kg/day. In the 2.5 mg/kg/day group, body weight gain was markedly inhibited, and various changes, mostly viewed as secondary effects, were observed in the bone marrow, spleen, thymus, and adrenal gland. These toxic effects did not recover completely during the 14-day recovery period. Regarding reproductive/developmental toxicity, various histopathological changes, including decreased spermatid and spermatozoa counts, were observed in the male reproductive organs, and continuous diestrous was found in females in the 2.5 mg/kg/day group. Seven of twelve females receiving 2.5 mg/kg/day died during late pregnancy while four other females in this group did not deliver live pups. Based on these findings, the NOAELs of PFDoA were concluded to be 0.1 mg/kg/day for repeated dose toxicity and 0.5 mg/kg/day for the reproductive/developmental toxicity.

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ORIGINAL ARTICLE

Historical control data on developmental toxicity studies in rodents

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A Study Group for Historical Control Data on Prenatal Developmental Toxicity Studies in Rodents

ABSTRACT   Historical control data on rodent developmental toxicity studies, performed between 1994 and 2010, were obtained from 19 laboratories in Japan, including 10 pharmaceutical and chemical companies and nine contract research organizations. Rats, mice, and hamsters were used for developmental toxicity studies. Data included maternal reproductive findings at terminal cesarean sections and fetal findings including the spontaneous incidences of external, visceral, and skeletal anomalies. No noticeable differences were observed in maternal reproductive data between laboratories. Inter-laboratory variations in the incidences of fetuses with anomalies appeared to be due to differences in the selection of observation parameters, observation criteria, classification of the findings, and terminology of fetal alterations. Historical control data are useful for the appropriate interpretation of experimental results and evaluation of the effects of chemical on reproductive and developmental toxicities.

Key Words: developmental toxicity, fetal malformation, historical control data, reproductive toxicity, rodent

INTRODUCTION

The availability of comprehensive historical control data is of importance because a comparison of data from study controls with historical control data may be beneficial to evaluate toxicity. Historical control data on reproductive and developmental toxicity studies may be useful for the adequate interpretation of experimental results and evaluation of reproductive and developmental toxicity. Historical control data may help to distinguish treatment-induced changes from spontaneously occurring background changes specific to the species/strains.

Rodents have been widely used in toxicological studies of pharmaceuticals, crop protection compounds, and industrial chemicals, while rats, mice, and rabbits are the more universally accepted laboratory animal species for standardized developmental toxicity testing (Wilson 1973; Schardein 2000; Barrow 2009). Historical control data on reproductive and developmental toxicity studies in laboratory animals have been previously reported in Japan by

Kameyama et al. (1980), Morita et al. (1987) (Japanese Pharmaceutical Manufacturer's Association [JPMA] survey, data between 1980 and 1985), and Nakatsuka et al. (1997) (JPMA survey, data between 1986 and 1993). Historical control data on reproductive and developmental toxicity studies using rodents have been extensively reported in abstracts; however, detailed information can not be obtained from these abstracts. Reproductive data can be obtained from a website for rats (CLEA Japan, Inc. 2007) and mice (Giknis and Clifford 2007). Detailed information on reproductive and developmental toxicity studies including spontaneous fetal malformations is available from a website for rats (CD[SD]IGS Study Group 1998, 1999, 2000, 2001, 2003) and a chapter of a book for rats and mice (Kimmel and Price 1990; Tyl and Marr 2006). Only a few peer-reviewed studies are available for Wistar Hannover rats (Aoyama et al. 2002; Liberati et al. 2002; Takeuchi et al. 2011). A retrospective analysis of multi-generation studies using rats has also been performed (Marty et al. 2009).

However, no historical control data have been published on reproductive and developmental toxicity studies of rodents, except for Wistar Hannover rats, over the last decade in Japan. Subtle changes may occur with time due to genetic alterations in the strain or stock of the species used and changes in environmental conditions both in the breeding colony of the supplier and in the laboratory (Kimmel and Price 1990). Therefore, examining changes in data over time within historical control data and comparing study control data with recent as well as cumulative historical control data are of importance. We previously reported historical control data between 1994 and 2010 for developmental toxicity studies of rabbits (Ema et al. 2012). Recent historical control data for rodents between 1994 and 2010 were collected and summarized in this paper.

MATERIALS AND METHODS

The participating laboratories in pharmaceutical and chemical companies and contract research organizations are shown in Table 1. Data were obtained from 19 laboratories in Japan, including 10 pharmaceutical and chemical companies and nine contract research organizations. Data regarding terminal cesarean sections, fetal external anomalies, and visceral and skeletal anomalies and variations in rodents were collected from developmental toxicity studies conducted between 1994 and 2010. Data from range-finding studies that utilized a small number of dams per group (less than 16 litters) were not included in this dataset. Data were summarized separately between 1994 and 2000 and between 2001 and 2010. The animal strain was expressed as a brand name. Data were incorporated from

Table 1 Participating laboratories and researchers

Laboratory	Researcher
Astellas Pharma Inc., Drug Safety Research Laboratories	Seiki Matsuo
	Hiroko Noyori
Public Interest Incorporated Foundation Biosafety Research Center, Foods, Drugs and Pesticides (BSRC)	Keiichi Itoh
	Ryota Tanaka
Bozo Research Center Inc.	Yoshihiro Katsumata
Chiba Institute of Science, Faculty of Risk and Crisis Management	Masao Horimoto
Daiichi Sankyo Co., Ltd., Medicinal Safety Research Laboratories	Toshiki Matsuoka
	Kazuhiro Shimomura
Dainippon Sumitomo Pharma Co., Ltd., Preclinical Research Laboratories	Akihito Yamashita
	Hiroshi Inada
Eisai Co., Ltd., Tsukuba Drug Safety/Sunplanet Co., Ltd., Preclinical Safety Research Laboratories	Maki Maeda
	Hiroshi Mineshima
Ina Research Inc.	Hiroaki Hara
	Tatsuya Shimizu
Institute of Environmental Toxicology, Toxicology Division	Hitoshi Hojo
	Chizuru Urakawa
Kissei Pharmaceutical Co., Ltd.	Ikuro Takakura
	Ryohei Yokoi
Mitsubishi Chemical Medience Corporation, Kashima Laboratory	Ikuo Matsuura
Mitsubishi Chemical Medience Corporation, Kumamoto Laboratory	Nobuhito Hoshino
	Hiroyuki Izumi
	Takafumi Ohta
National Institute of Advanced Industrial Science and Technology (AIST), Research Institute of Science for Safety and Sustainability	Makoto Ema
	Masato Naya
National Institute of Health Sciences, Division of Risk Assessment	Akihiko Hirose
	Mutsuko Hirata-Koizumi
	Atsushi Ono
Nihon Bioresearch Inc.	Katsumi Endoh
	Yoji Miwa
Ono Pharmaceutical Co., Ltd., Safety Research Laboratories, Department of Biology & Pharmacology	Yukari Imai
	Harutaka Oku
Otsuka Pharmaceutical Co., Ltd., Tokushima Research Institute	Yuko Tominaga
	Tohru Uesugi
Safety Research Institute for Chemical Compounds Co., Ltd.	Sakiko Fujii
	Kaoru Yabe
Shin Nippon Biomedical Laboratories (SNBL), Ltd., Drug Safety Research Laboratories	Hirohito Kato
	Taishi Tateishi
Shionogi & Co., Ltd., Drug Developmental Research Laboratories	Nao Nakano
	Ryou Fukushima
Sumitomo Chemical Co., Ltd., Environmental Health Science Laboratory	Yoshinori Hosokawa
	Kunifumi Inawaka
Takeda Pharmaceutical Co. Ltd., Drug Safety Research Laboratories	Kiyoshi Matsumoto
	Toshiaki Yamauchi

a laboratory if the information was based on four studies or more for Crlj:CD(SD) (former name: Crj:CD[SD]) rats, and three studies or more for Crl:CD(SD) (former name: Crj:CD[SD]IGS) rats between 1994 and 2000, and 10 studies or more for Crl:CD(SD) rats between 2001 and 2010. Data were incorporated if there was one study or more for SD rats from other breeders, other strains of rats, mice, and hamsters between 1994 and 2000 and between 2001 and 2010; however, these data were not sufficient for a definitive analysis.

The day of detection of copulation was designated as gestational day (GD) 0. The category of fetal mortality included early resorptions and late fetal deaths. Incidence data for fetal alterations were based on the number of alterations observed in each category as a percentage of the total number of live fetuses examined. If more than one alteration was observed in a fetus, each was reported individually. The incidence of fetuses with malformations or variations was expressed as a proportion of the total number of fetuses with malformations or variations to the total number of fetuses examined. The terminology used for fetal external, visceral, and skeletal alterations was principally based on Horimoto et al. (1998) and Makris et al. (2009).

RESULTS AND DISCUSSION

Mating and cesarean section data

All pregnant dams were prepared by natural mating in rats, mice, and hamsters. Cesarean sections were performed on GD 20 or GD 21 in rat dams, on GD 17 or GD 18 in mouse dams, or on GD 14 in hamster dams.

Mating and cesarean section data from Crlj:CD(SD) rats between 1994 and 2000 and from Crl:CD(SD) rats between 1994 and 2000 and between 2001 and 2010 are shown in Tables 2, 3 and 4, respectively. The average pregnancy rate, number of corpora lutea, number of implantations, number of live fetuses, and fetal mortality were summarized in Table 5. Whereas the average values of fetal mortality in Crlj:CD(SD) rats and Crl:CD(SD) rats were similar, the average numbers of corpora lutea, implantations, and live fetuses in Crl:CD(SD) rats were slightly smaller than those in Crlj:CD(SD) rats. These phenomena were also observed in some surveys of the CD(SD)IGS Study Group (1998, 2000). The values of reproductive parameters of Crlj:CD(SD) were not clearly different from those of the same rat strain previously surveyed in Japan (Morita et al. 1987; Nakatsuka et al. 1997). No noticeable variation was observed in the reproductive parameters of Crl:CD(SD) rats between the two intervals evaluated (1994–2000 and 2001–2010).

The data from Jcl:SD, Slc:SD, and Wistar rats between 1994 and 2000 and between 2001 and 2010 are presented in Tables 6 and 7, respectively. The data from mice and hamsters between 1994 and 2000 and between 2001 and 2010 are shown in Table 8. Although the pregnancy rates of SD rats from other breeders (Jcl:SD and Slc:SD), other strains of rats (Wistar rats), and mice were similar to those of Crlj:SD(CD) and Crl:CD(SD) rats in most laboratories, a relatively low pregnancy rate was noted in Wistar Hannover rats, mice, and hamsters in remaining laboratories. The average numbers of corpora lutea, implantations, and live fetuses of SD rats from other breeders and Wistar rats (Crlj:WI and Jcl:Wistar) were similar to those in Crlj:CD(SD) and/or Crl:CD(SD) rats. The value of fetal mortality in SD rats from other breeders and Wistar rats, including Wistar Hannover rats, was similar to that in Crlj:CD(SD) and/or Crl:CD(SD) rats. The numbers of corpora lutea, implantations, and live fetuses in Hannover Wistar rats were slightly smaller than those in Crlj:SD(CD) and Crl:CD(SD) rats. These findings were consistent with previous surveys, in which reproductive parameters, such as the numbers of corpora lutea, implantations, and live fetuses, were similar among three stocks of Wistar Hannover rats (Takeuchi et al. 2011) and were smaller than those in SD rats (Aoyama et al. 2002; Liberati et al. 2002). More data are required for a definitive analysis of historical control data in these animals.

One laboratory determined fetal body weight with males and females combined, and the remaining laboratories evaluated fetal body weight for each sex separately. Male and female fetal weights

should be determined separately because males are heavier than females. The fetal weight varied with each laboratory and in general, roughly related the time and GD of cesarean sections of the dams. The rearing environment may have also had an impact on fetal weight.

External anomalies

Table S1 shows data on external anomalies in Crlj:CD(SD) rats between 1994 and 2000. The incidence of fetuses with external malformations ranged from 0.04 to 0.53% between 1994 and 2000, which was comparable to that of Crlj:CD(SD) rats in previous surveys (0–1.33% in Morita et al. 1987; 0–0.51% in Nakatsuka et al. 1997). In the previous survey (Nakatsuka et al. 1997), a few cases of conjoined twins, but not conjoined triplets, were reported in this rat strain. In the present survey, one case of conjoined triplets was observed in one laboratory. However, no noticeable difference was observed in the types of external anomalies reported between the previous (Morita et al. 1987; Nakatsuka et al. 1997) and present surveys.

Data for Crl:CD(SD) rats between 1994 and 2000 and between 2001 and 2010 are presented in Tables S2 and S4, respectively. The incidence of fetuses with external malformations ranged from 0 to 0.36% between 1994 and 2000 and 0.05 to 0.18% between 2001 and 2010, which was comparable to that of Crl:CD(SD) rats in previous surveys (0–0.34%) (CD(SD)IGS Study Group 1998, 1999, 2000, 2001, 2003). The incidence of fetuses with external malformations in Crl:CD(SD) rats was slightly lower than that in Crlj:CD(SD) rats. No noticeable variability was observed in the types of external anomalies between Crl:CD(SD) and Crlj:CD(SD) rats or in the incidence of fetuses with external malformations in Crl:CD(SD) rats between the two intervals evaluated (1994–2000 and 2001–2010).

Visceral anomalies

Data on visceral anomalies in Crlj:CD(SD) rats between 1994 and 2000 are presented in Table S7. The incidence of fetuses with visceral malformations ranged from 0.45 to 16.57% between 1994 and 2000. This incidence was within the range of previous surveys of this rat strain (0–17.59% in Morita et al. 1987; 0.24–34.83% in Nakatsuka et al. 1997). No noticeable difference was found in types of anomalies between the previous (Morita et al. 1987; Nakatsuka et al. 1997) and present surveys.

Data for Crl:CD(SD) rats between 1994 and 2000 and between 2001 and 2010 are presented in Tables S8 and S10, respectively. The incidence of fetuses with visceral malformations ranged from 0 to 11.09% between 1994 and 2000 and 0.32 to 8.27% between 2001 and 2010. These incidences were within the ranges of those in the previous surveys on this rat strain (0–26.3%) (CD(SD)IGS Study Group 1998, 1999, 2000, 2001, 2003). No clear difference was noted in the types of anomalies between the previous and present surveys.No noticeable difference was found in the types of visceral anomalies between the two intervals evaluated (1994–2000 and 2001–2010).

Large variations were noted in the incidences of visceral malformations among laboratories between the previous (Morita et al. 1987; Nakatsuka et al. 1997; CD(SD)IGS Study Group 1998, 1999, 2000, 2001, 2003) and present surveys. This phenomenon appeared to be due to differences in the classification of visceral anomalies among laboratories. Visceral anomalies such as thymic cord and some anomalies of the vessels were classified as malformations by some laboratories, but as variations by other laboratories.

Table 2 Mating and cesarean section data from Crlj:CD(SD) [former Crj:CD(SD)] rats between 1994 and 2000

Year	1994–2000	1994–2000	1994–2000	1995–2000	1995–2000	1994–1996	1994–2000	1994–2000
Treatment†	V	V	V	V	V	V	V	V
Feed	NMF	CRF-1	CRF-1	NMF	NMF	MF	CRF-1	CRF-1
No. dams	721	302	201	264	180	120	78	73
No. experiments	35	14	11	12	8	5	4	4
No. dams/experiment	16–24	19–25	17–20	20–24	21–25	24	19–20	17–19
Pregnancy rate (%)	96.2 (80.0–100)	98.0 (95.0–100)	92.2 (81.8–100)	98.5 (90.9–100)	98.3 (95.5–100)	95.8 (91.7–100)	97.5 (95–100)	91.3 (85.0–95.0)
Gestation day (hour) of the cesarean section	20 (13:30–16:00)	20 (9:00–11:00)	20 (10:00–12:00)	20 (13:00–16:00)	20 (9:00–12:00)	20 (9:00–12:00)	20 (13:00–16:00)	20 (9:00–12:00)
No. corpora lutea	17.0 (15.7–18.7)	16.4 (14.4–17.6)	17.0 (15.2–19.4)	18.7 (17.7–19.6)	18.0 (16.6–19.9)	17.0 (16.4–17.2)	17.4 (16.7–17.9)	17.4 (16.7–18.1)
No. implantations	15.9 (13.2–18.1)	15.5 (13.1–16.8)	14.7 (12.9–16.8)	16.8 (15.7–17.7)	15.5 (15.0–16.5)	15.8 (14.8–16.8)	16.5 (15.6–17.3)	15.4 (14.9–15.7)
No. live fetuses	15.0 (12.4–17.2)	14.6 (12.6–13.8)	13.9 (11.8–15.8)	15.8 (14.9–16.6)	14.6 (14.2–15.4)	14.4 (13.7–15.0)	15.8 (15.2–16.4)	14.7 (14.3–15.2)
Fetal mortality (%)‡	6.0 (2.5–12.0)	6.1 (2.2–13.8)	5.5 (3.1–9.1)	6.5 (4.4–8.5)	6.0 (3.3–8.9)	8.1 (5.9–9.9)	4.3 (2.7–5.3)	4.3 (2.6–7.0)
Body weight (g)								
All fetuses						3.44 (3.23–3.58)	3.46 (3.45–3.48)	3.44 (3.36–3.48)
Male	3.73 (3.41–4.04)	3.63 (3.48–3.87)	3.35 (3.14–3.78)	3.76 (3.59–3.88)	3.40 (3.35–3.48)	3.53 (3.42–3.58)	3.54 (3.51–3.57)	3.52 (3.45–3.55)
Female	3.55 (3.32–3.83)	3.45 (3.31–3.70)	3.19 (2.97–3.58)	3.56 (3.44–3.66)	3.23 (3.17–3.31)	3.34 (3.23–3.42)	3.38 (3.35–3.42)	3.38 (3.29–3.45)

†V, Vehicle-treated.

‡(Number of early resorptions and late fetal deaths/number of implantations) × 100.

Minimum and maximum values from independent experiments are given in parentheses.

Table 3 Mating and cesarean section data from Crl:CD(SD) [former Crj:CD(SD)IGS] rats between 1994 and 2000

Year	1994–2000	1996–2000	1997–2000	1999–2000	1997–2000	1998–2000	1996–2000	1999–2000	2000	1994–2000	1997	1994–2000
Treatment†	V	V	V	V	V	V	V	V	V	V	N/V	V
Feed	CR-LPF	CRF-1/CR-LPF	CRF-1	NMF	CRF-1	CRF-1	NMF	NMF	CRF-LPF	CRF-1	CRF-1	CRF-1
No. dams	393	217	147	125	113	94	99	90	80	77	60	58
No. experiments	20	11	7	6	6	5	4	4	4	4	3	3
No. dams/experiment	19–20	19–22	19–24	20–22	16–20	18–20	19–36	21–25	18–20	18–20	20	18–21
Pregnancy rate (%)	98.3	97.3 (95.0–100)	97.5 (95.0–100)	99.2 (95.5–100)	97.1 (94.2–100)	93.0 (90.0–100)	98.8 (95.0–100)	98.9 (95.5–100)	93.8 (90.6–98.4)	96.3 (90.0–100)	100	93.5 (90.0–95.5)
Gestation day (hour) of the cesarean section	20 (9:00–12:00)	20 (8:00–11:00)	20 (9:00–12:00)	20 (13:00–16:00)	20 (9:00–12:00)	20 (9:00–12:00)	20 (13:30–16:00)	20 (9:00–12:00)	21 (9:00–12:00)	20 (13:00–16:00)	20 (9:00–11:00)	20 (9:00–12:00)
No. corpora lutea	15.7 (14.9–16.4)	16.1 (15.1–17.3)	16.0 (15.5–16.9)	17.4 (16.6–18.0-)	16.2 (15.6–16.5)	15.3 (14.9–15.7)	16.1 (15.7–16.3)	16.5 (15.6–17.9)	14.6 (13.7–15.7)	15.9 (15.6–16.1)	15.5 (15.1–15.9)	15.9 (15.7–16.2)
No. implantations	14.8 (13.3–15.8)	14.8 (13.7–16.0)	14.6 (13.7–15.1)	15.6 (14.7–16.2)	15.5 (15.1–16.2)	13.7 (11.8–14.6)	15.5 (14.9–15.9)	14.9 (14.5–15.3)	13.8 (12.8–14.8)	15.1 (14.3–15.5)	15.0 (14.5–15.4)	14.2 (14.0–14.3)
No. live fetuses	14.1 (12.9–15.0)	14.2 (13.3–15.3)	14.0 (13.5–14.6)	14.9 (14.1–15.6)	14.8 (14.3–15.5)	12.9 (11.6–13.5)	14.7 (14.3–15.2)	13.9 (13.6–14.2)	13.3 (12.4–14.1)	14.2 (12.7–15.0)	14.2 (13.8–14.6)	13.4 (13.2–13.7)
Fetal mortality (%)‡	4.8 (1.9–10.8)	4.2 (2.2–7.3)	3.8 (0.4–5.7)	4.8 (3.6–6.3)	4.7 (3.4–5.8)	5.2 (2.8–7.6)	4.9 (4.0–6.8)	6.4 (5.6–7.1)	4.9 (2.1–7.5)	7.2 (3.1–15.4)	5.6 (4.7–6.8)	5.0 (3.7–6.0)
Body weight (g)												
All fetuses						3.97 (3.84–4.10)				3.85 (3.75–3.98)		3.66 (3.64–3.70)
Male	3.47 (3.34–3.61)	3.88 (3.60–4.01)	3.52 (3.33–3.63)	4.20 (4.07–4.31)	3.62 (3.55–3.70)	4.07 (3.93–4.18)	4.11 (4.00–4.19)	3.62 (3.45–3.77)	5.39 (5.29–5.49)	3.96 (3.83–4.09)	3.81 (3.80–3.83)	3.76 (3.72–3.81)
Female	3.30 (3.13–3.42)	3.67 (3.46–3.77)	3.34 (3.19–3.43)	3.98 (3.89–4.07)	3.44 (3.37–3.50)	3.83 (3.73–3.97)	3.89 (3.82–3.96)	3.43 (3.31–3.58)	5.11 (5.03–5.25)	3.75 (3.67–3.86)	3.62 (3.60–3.65)	3.56 (3.54–3.61)

†V, Vehicle-treated; N, Non-treated.
‡(Number of early resorptions and late fetal deaths/number of implantations) × 100.
Minimum and maximum values from independent experiments are given in parentheses.

Table 4 Mating and cesarean section data from Crl:CD(SD) [former Crj:CD(SD)IGS] rats between 2001 and 2010

Year	2001–2010	2001–2010	2001–2010	2001–2010	2001–2010	2001–2010	2001–2009	2001–2010	2001–2010	2001–2010	2004–2010
Treatment†	V	V	V	V	V	V	V	V	V	V	N/V
Feed	CE-2/CRF-1	NMF	NMF	CRF-1	CRF-1	CRF-1	CRF-1	CRF-1	CR-LPF	CRF-1	CRF-1
No. dams	1064	934	717	565	567	479	346	332	290	279	192
No. experiments	55	47	36	29	28	25	17	16	15	12	10
No. dams/experiment	17–24	17–22	19–24	18–20	19–24	16–20	19–22	18–22	17–20	20–25	17–20
Pregnancy rate (%)	96.0 (85.0–100)	98.1 (85.0–100)	98.5 (95.0–100)	97.4 (90.0–100)	99.2 (95.0–100)	95.8 (80.0–100)	98.8 (95.0–100)	97.9 (90.0–100)	96.7	100	96.0 (85.0–100)
Gestation day (hour) of the cesarean section	20 (9:00–12:00)	20 (13:00–16:00)	20 (13:30–16:00)	20 (8:00–11:00)	20 (9:00–12:00)	20 (9:00–12:00)	20 (9:00–12:00)	20 (13:00–16:00)	20 (9:00–12:00)	20 (9:00–16:00)	20 (9:00–11:00)
No. corpora lutea	15.7 (13.8–17.6)	16.6 (14.6–18.4)	15.6 (14.1–16.3)	15.1 (13.9–16.2)	15.3 (14.4–16.5)	15.3 (14.3–16.1)	15.9 (15.0–16.8)	15.8 (15.1–17.1)	14.9 (14.4–15.9)	15.4 (14.7–16.3)	15.4 (14.9–16.2)
No. implantations	14.8 (13.1–16.4)	14.9 (13.0–16.2)	14.7 (13.1–15.5)	14.2 (12.5–15.2)	14.6 (13.3–14.8)	14.2 (13.3–15.5)	15.0 (13.5–16.2)	14.8 (14.1–15.4)	14.1 (13.5–14.8)	14.8 (13.8–15.8)	14.5 (13.9–15.1)
No. live fetuses	14.1 (12.4–15.4)	14.2 (12.5–15.3)	13.2 (12.3–15.1)	13.5 (11.8–14.6)	13.8 (12.5–14.8)	13.4 (12.4–14.8)	14.2 (12.8–15.5)	14.3 (13.6–15.0)	13.4 (12.9–14.0)	14.0 (13.0–14.9)	13.6 (12.7–14.3)
Fetal mortality (%)‡	4.8 (2.0–9.3)	4.8 (0.8–8.6)	5.1 (2.6–9.0)	4.5 (2.0–8.0)	5.5 (2.3–10.2)	5.5 (2.5–9.1)	6.2 (3.1–9.9)	3.6 (2.1–6.4)	5.3 (1.8–8.0)	5.9 (3.5–8.0)	6.0 (3.3–9.4)
Body weight (g)											
All fetuses						4.07 (3.93–4.20)					
Male	3.81 (3.58–4.01)	4.18 (3.99–4.36)	4.11 (3.95–4.25)	3.94 (3.85–4.09)	3.71 (3.52–3.91)	4.06 (3.85–4.29)	3.53 (3.29–3.86)	4.06 (3.96–4.21)	3.63 (3.51–3.82)	3.73 (3.64–3.81)	3.77 (3.60–4.03)
Female	3.62 (3.38–3.81)	3.96 (3.76–4.16)	3.90 (3.72–4.05)	3.72 (3.63–3.85)	3.52 (3.33–3.64)	3.84 (3.62–4.11)	3.33 (3.13–3.60)	3.85 (3.76–3.97)	3.44 (3.33–3.58)	3.54 (3.42–3.65)	3.56 (3.40–3.81)

†V, Vehicle-treated; N, Non-treated.
‡(Number of early resorptions and late fetal deaths/number of implantations) × 100.
Minimum and maximum values from independent experiments are given in parentheses.