TABLE V. *Continued*

		Main Group				Recovery Group	
Dose (mg/kg/day)	Grade	0 (control)	0.1	0.5	2.5	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 $p \leq 0.05$.
**Significantly different from the control, at $p \leq 0.01$.

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, perfluorononanonic 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.

REFERENCES

Ahrens L, Siebert U, Ebinghaus R. 2009. Temporal trends of polyfluoroalkyl compounds in harbor seals (Phoca vitulina) from the German Bight, 1999–2008. Chemosphere 76:151–158.

ATSDR. 2009. Toxicological Profile for Perfluoroalkyls (Draft for Public Comment), US Department of health and human services, Public health service, Agency for Toxic Substances and Disease Registry (ATSDR). Accessed on February 28, 2014.

Bartholomeusz RK, Bruce NW, Lynch AM. 1999. Embryo survival, and fetal and placental growth following elevation of maternal estradiol blood concentrations in the rat. Biol Reprod 61:46–50.

Biegel LB, Hurtt ME, Frame SR, O'Connor JC, Cook JC. 2001. Mechanisms of extrahepatic tumor induction by peroxisome proliferators in male CD rats. Toxicol Sci 60:44–55.

Biegel LB, Liu RC, Hurtt ME, Cook JC. 1995. Effects of ammonium perfluorooctanoate on Leydig cell function: in vitro, in vivo, and ex vivo studies. Toxicol Appl Pharmacol 134:18–25.

Bookstaff RC, Moore RW, Ingall GB, Peterson RE. 1990. Androgenic deficiency in male rats treated with perfluorodecanoic acid. Toxicol Appl Pharmacol 104:322–333.

Chang SC, Das K, Ehresman DJ, Ellefson ME, Gorman GS, Hart JA, Noker PE, Tan YM, Lieder PH, Lau C, Olsen GW, Butenhoff JL. 2008. Comparative pharmacokinetics of perfluorobutyrate in rats, mice, monkeys, and humans and relevance to human exposure via drinking water. Toxicol Sci 104:40–53.

Clara M, Scheffknecht C, Scharf S, Weiss S, Gans O. 2008. Emissions of perfluorinated alkylated substances (PFAS) from point sources—Identification of relevant branches. Water Sci Technol 58:59–66.

Corton JC, Lapinskas PJ, Gonzalez FJ. 2000. Central role of PPARalpha in the mechanism of action of hepatocarcinogenic peroxisome proliferators. Mutat Res 448:139–151.

Ding L, Hao F, Shi Z, Wang Y, Zhang H, Tang H, Dai J. 2009. Systems biological responses to chronic perfluorododecanoic acid exposure by integrated metabonomic and transcriptomic studies. J Proteome Res 8:2882–2891.

Falandysz J, Taniyasu S, Gulkowska A, Yamashita N, Schulte-Oehlmann U. 2006. Is fish a major source of fluorinated surfactants and repellents in humans living on the Baltic Coast? Environ Sci Technol 40:748–751.

Feng Y, Fang X, Shi Z, Xu M, Dai J. 2010. Effects of PFNA exposure on expression of junction-associated molecules and secretory function in rat Sertoli cells. Reprod Toxicol 30:429–437.

Feng Y, Shi Z, Fang X, Xu M, Dai J. 2009. Perfluorononanoic acid induces apoptosis involving the Fas death receptor signaling pathway in rat testis. Toxicol Lett 190:224–230.

Fujii Y, Yan J, Harada KH, Hitomi T, Yang H, Wang P, Koizumi A. 2012. Levels and profiles of long-chain perfluorinated carboxylic acids in human breast milk and infant formulas in East Asia. Chemosphere 86:315–321.

Green S. 1995. PPAR: a mediator of peroxisome proliferator action. Mutat Res 333:101–109.

Griffith FD, Long JE. 1980. Animal toxicity studies with ammonium perfluorooctanoate. Am Ind Hyg Assoc J 41:576–583.

Guruge KS, Taniyasu S, Yamashita N, Wijeratna S, Mohotti KM, Seneviratne HR, Kannan K, Yamanaka N, Miyazaki S. 2005. Perfluorinated organic compounds in human blood serum and seminal plasma: A study of urban and rural tea worker populations in Sri Lanka. J Environ Monit 7:371–377.

Hamid J, Sayeed A, McFarlane H. 1974. The effect of 1-(o-chlorophenyl)−1-(p-chlorophenyl)−2,2-dichloroethane(o,p'-DDD) on the immune response in malnutrition. Br J Exp Pathol 55:94–100.

Harada KH, Hitomi T, Niisoe T, Takanaka K, Kamiyama S, Watanabe T, Moon CS, Yang HR, Hung NN, Koizumi A. 2011. Odd-numbered perfluorocarboxylates predominate over perfluorooctanoic acid in serum samples from Japan, Korea and Vietnam. Environ Int 37:1183–1189.

Haug LS, Huber S, Schlabach M, Becher G, Thomsen C. 2011. Investigation on per- and polyfluorinated compounds in paired samples of house dust and indoor air from Norwegian homes. Environ Sci Technol 45:7991–7998.

Haug LS, Thomsen C, Becher G. 2009. Time trends and the influence of age and gender on serum concentrations of perfluorinated compounds in archived human samples. Environ Sci Technol 43:2131–2136.

Hirata-Koizumi M, Fujii S, Furukawa M, Ono A, Hirose A. 2012. Repeated dose and reproductive/developmental toxicity of perfluorooctadecanoic acid in rats. J Toxicol Sci 37:63–79.

Hoff PT, Scheirs J, Van de Vijver K, Van Dongen W, Esmans EL, Blust R, De Coen W. 2004. Biochemical effect evaluation of perfluorooctane sulfonic acid-contaminated wood mice (Apodemus sylvaticus). Environ Health Perspect 112:681–686.

Holden PR, Tugwood JD. 1999. Peroxisome proliferator-activated receptor alpha: Role in rodent liver cancer and species differences. J Mol Endocrinol 22:1–8.

Inoue Y, Hashizume N, Yakata N, Murakami H, Suzuki Y, Kikushima E, Otsuka M. 2012. Unique physicochemical properties of perfluorinated compounds and their bioconcentration in common Carp Cyprinus carpio L. Arch Environ Contam Toxicol 62:672–680.

JALAS. 1987. Guidelines for Animal Experimentation, Japanese Association for Laboratory Animal Science, dated May 22, 1987.

Japanese Animal Welfare Law. 2006. Act on Welfare and Management of Animals. Act No. 105 of October 1, 1973. As amended up to Act No. 50 of June 2, 2006.

Jensen AA, Leffers H. 2008. Emerging endocrine disrupters: Perfluoroalkylated substances. Int J Androl 31:161–169.

Kemper RA. 2003. Perfluorooctanoic acid: Toxicokinetics in the rat. Association of Plastics Manufactures of Europe. Submitted to the US EPA's Admistrative Record. AR226–1499, cited in ASTDR (2009).

Kotsuji F, Goto K, Aso T, Tominaga T. 1986. The influence of weight loss on the reproductive function of the female rat: Changes in the estrous cycle and hypothalamo-pituitary-ovarian function during feed restriction and subsequent refeeding period (in Japanese). Nihon Sanka Fujinka Gakkai Zasshi 38: 1713–1721.

Kudo N, Katakura M, Sato Y, Kawashima Y. 2002. Sex hormone-regulated renal transport of perfluorooctanoic acid. Chem Biol Interact 139:301–316.

Kudo N, Suzuki-Nakajima E, Mitsumoto A, Kawashima Y. 2006. Responses of the liver to perfluorinated fatty acids with different carbon chain length in male and female mice: In relation to induction of hepatomegaly, peroxisomal beta-oxidation and microsomal 1-acylglycerophosphocholine acyltransferase. Biol Pharm Bull 29:1952–1957.

Kunacheva C, Tanaka S, Fujii S, Boontanon SK, Musirat C, Wongwattana T, Shivakoti BR. 2011. Mass flows of perfluorinated compounds (PFCs) in central wastewater treatment plants of industrial zones in Thailand. Chemosphere 83:737–744.

Lau C, Anitole K, Hodes C, Lai D, Pfahles-Hutchens A, Seed J. 2007. Perfluoroalkyl acids: A review of monitoring and toxicological findings. Toxicol Sci 99:366–394.

Li Y, Ramdhan DH, Naito H, Yamagishi N, Ito Y, Hayashi Y, Yanagiba Y, Okamura A, Tamada H, Gonzalez FJ, Nakajima T. 2011. Ammonium perfluorooctanoate may cause testosterone reduction by adversely affecting testis in relation to PPARalpha. Toxicol Lett 205:265–272.

Martin JW, Mabury SA, Solomon KR, Muir DC. 2003. Bioconcentration and tissue distribution of perfluorinated acids in rainbow trout (Oncorhynchus mykiss). Environ Toxicol Chem 22: 196–204.

MOE. 2006. Standards Relating to the Care, Management of Laboratory Animals and Relief of Pain. Announcement No. 88 of Ministry of the Environment (MOE), Japan, dated April 28, 2006.

MOE, METI, MHLW. 2008. Standard concerning testing laboratories implementing tests for new chemical substances etc. Joint notification by director generals of Environmental Policy Bureau, Ministry of the Environment (MOE), Japan (Kanpokihatsu No. 031121004) and Manufacturing Industries Bureau, Ministry of Economy, Trade and Industry (METI), Japan (Seikyokuhatsu No. 3), dated November 17, 2003 and by director general of Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare (MHLW), Japan (Yakusyokuhatsu No. 1121003), dated November 21, 2003. As amended up to July 4, 2008.

Moriyama T, Tsujioka S, Ohira T, Nonaka S, Ikeda H, Sugiura H, Tomohiro M, Samura K, Nishikibe M. 2008. Effects of reduced food intake on toxicity study parameters in rats. J Toxicol Sci 33:537–547.

Murakami M, Shinohara H, Takada H. 2009. Evaluation of wastewater and street runoff as sources of perfluorinated surfactants (PFSs). Chemosphere 74:487–493.

Narita K, Nagao K, Bannai M, Ichimaru T, Nakano S, Murata T, Higuchi T, Takahashi M. 2011. Dietary deficiency of essential amino acids rapidly induces cessation of the rat estrous cycle. PLoS One 6:e28136.

Nishikoori H, Murakami M, Sakai H, Oguma K, Takada H, Takizawa S. 2011. Estimation of contribution from non-point sources to perfluorinated surfactants in a river by using boron as a wastewater tracer. Chemosphere 84:1125–1132.

O'Connor JC, Frame SR, Ladics GS. 2002. Evaluation of a 15-day screening assay using intact male rats for identifying steroid biosynthesis inhibitors and thyroid modulators. Toxicol Sci 69:79–91.

OECD. 1996. Organisation for Economic Co-operation and Development (OECD) Guidelines for the Testing of Chemicals, Section 4: Health Effects, Test No. 422: Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test. Adopted on 22 March, 1996.

OECD. 2009. Guidance Document for Histologic Evaluation of Endocrine and Reproductive Tests in Rodents Series on testing and assessment, number 106, OECD, Environmenta directorate, Joint meeting of the chemicals committee and the working party on chemicals, pesticides and iotechnology, Available at: http://www.oecd.org/officialdocuments/displaydocumentpdf/?cote=env/jm/mono(2009)11&doclanguage=en, accessed on July 28, 2011.

Ogle TF, Mills TM, Costoff A. 1990. Progesterone maintenance of the placental progesterone receptor and placental growth in ovariectomized rats. Biol Reprod 43:276–284.

Ohmori K, Kudo N, Katayama K, Kawashima Y. 2003. Comparison of the toxicokinetics between perfluorocarboxylic acids with different carbon chain length. Toxicology 184:135–140.

Olsen GW, Lange CC, Ellefson ME, Mair DC, Church TR, Goldberg CL, Herron RM, Medhdizadehkashi Z, Nobiletti JB, Rios JA, Reagen WK, Zobel LR. 2012. Temporal trends of perfluoroalkyl concentrations in American Red Cross Adult Blood Donors, 2000–2010. Environ Sci Technol 46:6330–6338.

Parker RM. 2006. Testing for reproductive toxicity. In: Hood RD, editor. Developmental and Reproductive Toxicology—A Practical Approach. Florida: CRC Press, Taylor & Fransis Group. pp 425–487.

Perkins RG, Butenhoff JL, Kennedy GL Jr, Palazzolo MJ. 2004. 13-week dietary toxicity study of ammonium perfluorooctanoate (APFO) in male rats. Drug Chem Toxicol 27: 361–378.

Permadi H, Lundgren B, Andersson K, Sundberg C, DePierre JW. 1993. Effects of perfluoro fatty acids on peroxisome proliferation and mitochondrial size in mouse liver: dose and time factors and effect of chain length. Xenobiotica 23:761–770.

Prevedouros K, Cousins IT, Buck RC, Korzeniowski SH. 2006. Sources, fate and transport of perfluorocarboxylates. Environ Sci Technol 40:32–44.

Rosol TJ, Yarrington JT, Latendresse J, Capen CC. 2001. Adrenal gland: structure, function, and mechanisms of toxicity. Toxicol Pathol 29:41–48.

Shallie PD, Fakoya FA, Fakunle PB, Haruna MT, Shotunde DF. 2012. Dietary stress and energy metabolism: Evaluation of the adrenal cortex. Webmed Central BIOCHEMISTRY 3: WMC003472.

Shi Z, Ding L, Zhang H, Feng Y, Xu M, Dai J. 2009a. Chronic exposure to perfluorododecanoic acid disrupts testicular steroidogenesis and the expression of related genes in male rats. Toxicol Lett 188:192–200.

Shi Z, Feng Y, Wang J, Zhang H, Ding L, Dai J. 2010a. Perfluorododecanoic acid-induced steroidogenic inhibition is associated with steroidogenic acute regulatory protein and reactive oxygen species in cAMP-stimulated Leydig cells. Toxicol Sci 114:285–294.

Shi Z, Zhang H, Ding L, Feng Y, Wang J, Dai J. 2010b. Proteomic analysis for testis of rats chronically exposed to perfluorododecanoic acid. Toxicol Lett 192:179–188.

Shi Z, Zhang H, Ding L, Feng Y, Xu M, Dai J. 2009b. The effect of perfluorododecanonic acid on endocrine status, sex hormones and expression of steroidogenic genes in pubertal female rats. Reprod Toxicol 27:352–359.

Shi Z, Zhang H, Liu Y, Xu M, Dai J. 2007. Alterations in gene expression and testosterone synthesis in the testes of male rats exposed to perfluorododecanoic acid. Toxicol Sci 98:206–215.

Tao L, Kannan K, Kajiwara N, Costa MM, Fillmann G, Takahashi S, Tanabe S. 2006. Perfluorooctanesulfonate and related fluorochemicals in albatrosses, elephant seals, penguins, and polar skuas from the Southern Ocean. Environ Sci Technol 40:7642–7648.

Thompson J, Roach A, Eaglesham G, Bartkow ME, Edge K, Mueller JF. 2011. Perfluorinated alkyl acids in water, sediment and wildlife from Sydney Harbour and surroundings. Mar Pollut Bull 62:2869–2875.

UK COT. 2006. COT statement on the tolerable risk daily intake for perfluorooctanoic acid, Committee on toxicity of chemicals on food, consumer products and the environment, October 2006.

US EPA. 2013. Perfluorooctanoic acid (PFOA) and fluorinated telomers, last updated on November 4, 2013. United States Environmental Protection Agency (US EPA). Available at: http://www.epa.gov/oppt/pfoa/. Accessed on February 28, 2014.

Van de Vijver KI, Holsbeek L, Das K, Blust R, Joiris C, De Coen W. 2007. Occurrence of perfluorooctane sulfonate and other perfluorinated alkylated substances in harbor porpoises from the Black Sea. Environ Sci Technol 41: 315–320.

Wang T, Lu Y, Chen C, Naile JE, Khim JS, Giesy JP. 2012. Perfluorinated compounds in a coastal industrial area of Tianjin, China. Environ Geochem Health 34:301–311.

Wolf CJ, Schmid JE, Lau C, Abbott BD. 2012. Activation of mouse and human peroxisome proliferator-activated receptor-alpha (PPARalpha) by perfluoroalkyl acids (PFAAs): Further investigation of C4-C12 compounds. Reprod Toxicol 33:546–551.

Yang Q, Xie Y, Alexson SE, Nelson BD, DePierre JW. 2002. Involvement of the peroxisome proliferator-activated receptor alpha in the immunomodulation caused by peroxisome proliferators in mice. Biochem Pharmacol 63:1893–1900.

Zhang H, Shi Z, Liu Y, Wei Y, Dai J. 2008. Lipid homeostasis and oxidative stress in the liver of male rats exposed to perfluorododecanoic acid. Toxicol Appl Pharmacol 227: 16–25.

Congenital Anomalies
THE JAPANESE TERATOLOGY SOCIETY
150 *Congenital Anomalies* 2014; **54**, 150–161 doi:10.1111/cga.12050

ORIGINAL ARTICLE

Historical control data on developmental toxicity studies in rodents

Makoto Ema, Katsumi Endoh, Ryou Fukushima, Sakiko Fujii, Hiroaki Hara, Mutsuko Hirata-Koizumi, Akihiko Hirose, Hitoshi Hojo, Masao Horimoto, Nobuhito Hoshino, Yoshinori Hosokawa, Yukari Imai, Hiroshi Inada, Kunifumi Inawaka, Keiichi Itoh, Yoshihiro Katsumata, Hiroyuki Izumi, Hirohito Kato, Maki Maeda, Kiyoshi Matsumoto, Seiki Matsuo, Toshiki Matsuoka, Ikuo Matsuura, Hiroshi Mineshima, Yoji Miwa, Nao Nakano, Masato Naya, Hiroko Noyori, Takafumi Ohta, Harutaka Oku, Atsushi Ono, Tatsuya Shimizu, Kazuhiro Shimomura, Ikuro Takakura, Ryota Tanaka, Taishi Tateishi, Yuko Tominaga, Tohru Uesugi, Chizuru Urakawa, Kaoru Yabe, Akihito Yamashita, Toshiaki Yamauchi, and Ryohei Yokoi
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

Correspondence: Makoto Ema, DVM, PhD, Research Institute of Science for Safety and Sustainability, National Institute of Advanced Industrial Science and Technology (AIST), 16-1 Onogawa, Tsukuba, Ibaraki 305-8569, Japan. Email: ema-makoto@aist.go.jp
Received October 2, 2013; revised and accepted December 25, 2013.

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.

Table 5 Summary of historical control data on developmental toxicity studies in rodents

Animals	Crlj:CD(SD) rats	Crl:CD(SD) rats		Jcl:SD rats		Slc:SD rats	Crlj:WI rats		Jcl:Wistar rats	Wistar Hannover rats	Crlj:CD1(ICR) mice	Slc:Syrian hamsters
Year	1994–2000	1994–2000	2001–2010	1994–2000	2001–2005	1995–1997	1997–1999	2002–2009	2001	2001–2010	2000–2009	1999
Pregnancy rate (%)	91.3–98.5	93.0–100	95.8–100	90.6–100	95.8–97.5	95.0	95.0–95.5	95.0–98.3	91.7	87.2–100	72.7–100	88.9
No. corpora lutea	16.4–18.7	14.6–17.4	14.9–16.6	16.7–18.4	17.1–19.9	15.7	16.4–17.9	17.3–17.6	15.9	11.5–14.1	13.1–15.6	15.6
No. implantations	14.7–16.8	13.7–15.6	14.1–15.0	16.1–16.8	16.2–17.0	14.7	15.7–16.4	16.1–16.4	14.9	9.6–12.7	11.6–14.6	14.7
No. live fetuses	13.9–15.8	12.9–14.9	13.2–14.3	14.9–15.6	15.3–15.7	13.3	15.2–15.5	15.2–15.3	13.5	9.0–12.2	11.2–14.1	12.1
Fetal mortality (%)	4.3–8.1	3.8–7.2	3.6–6.2	4.9–7.7	5.8–7.1	5.6	3.5–5.4	5.7–7.2	9.4	4.2–7.9	3.2–9.3	17.6
Incidence of fetuses with external malformations (%)†	0.04–0.53	0–0.36	0.05–0.18	0–0.27	0–0.16	0.13	0–0.34	0–0.11	0	0–0.59	0–0.36	1.44
Incidence of fetuses with visceral malformations (%)†	0.45–16.57	0–11.09	0.32–8.27	0–11.93	0.58–5.05	20.16	1.45–15.09	0.71–8.88	0	0–19.28	0–15.17	2.27
Incidence of fetuses with skeletal malformations (%)†	0–3.97	0–8.02	0.10–0.56	0–1.07	0–1.12	0.49	0–4.00	0	1.29	0–24.49	0–2.02	4.79
Incidence of fetuses with skeletal variations ('%)†	3.60–8.36	6.98–22.98	9.42–17.63	30.60–62.37	38.55–43.45	6.85	18.00–43.11	13.91–36.99	11.61	31.56–67.35	33.16–64.71	78.77

Data are expressed as minimum and maximum values.

†The incidence of fetuses with malformations is expressed as a proportion of the total number of fetuses with malformations to the total number of fetuses examined.

Table 6 Mating and cesarean section data from Jcl:SD, Slc:SD, and Crlj:WI rats between 1994 and 2000

Strain	Jcl:SD	Jcl:SD	Jcl:SD	Jcl:SD	Jcl:SD	Slc:SD	Crlj:WI	Crlj:WI
Year	1994–2000	1994–1997	1997–2000	1994	1998	1995–1997	1999	1998
Treatment†	V	V	V	V	V	V	V	V
Feed	CA-1	CE-2	MF	NMF	CRF-1	NMF	NMF	CRF-1
No. dams	216	76	48	24	19	57	21	19
No. experiments	11	4	2	1	1	3	1	1
No. dams/experiment	18–23	16–21	24	24	19	19–19	21	19
Pregnancy rate (%)	96.8 (90.0–100)	90.6 (80.0–100)	100	100	95.0	95.0	95.5	95.0
Gestation day (hour) of the cesarean section	21 (9:00–12:00)	20 (9:00–12:00)	20 (9:00–12:00)	20 (13:30–16:00)	21 (8:00–11:00)	20 (13:30:16:00)	20 (13:00–16:00)	20 (8:00–11:00)
No. corpora lutea	18.4 (17.2–19.5)	17.9 (16.5–18.8)	16.7 (16.6–16.8)	17.3	18.3	15.7 (15.1–16.7)	17.9	16.4
No. implantations	16.8 (15.8–17.9)	16.5 (15.3–17.5)	16.2 (15.9–16.4)	16.8	16.1	14.7 (14.6–14.9)	16.4	15.7
No. live fetuses	15.5 (14.4–16.4)	15.3 (14.4–16.0)	14.9 (14.5–15.3)	15.6	15.2	13.3 (13.3–14.5)	15.5	15.2
Fetal mortality (%)‡	7.7 (4.0–11.0)	7.0 (5.3–8.5)	7.6 (6.7–8.5)	6.7	4.9	5.6 (2.9–8.9)	5.4	3.5
Body weight (g)								
All fetuses	5.17 (5.06–5.33)	4.11 (4.04–4.19)	3.94 (3.79–4.07)					
Male	5.30 (5.17–5.51)	4.20 (4.13–4.24)	4.07 (4.07–4.07)	4.13	5.77	3.98 (3.92–4.03)	4.20	3.95
Female	5.03 (4.90–5.16)	4.00 (3.93–4.08)	3.80 (3.79–3.81)	3.91	5.40	3.79 (3.73–3.85)	4.00	3.77

†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 7 Mating and cesarean section data from Jcl:SD, Crlj:WI, Jcl:Wistar, and Wistar Hannover rats between 2001 and 2010

Strain	Jcl:SD	Jcl:SD	Crlj:WI	Crlj :WI	Jcl:Wistar	BrHan: WIST@Jcl (GALAS)	BrlHan: Wist@Jcl (GALAS)	BrlHan: WIST@Jcl (GALAS)	BrlHan: WIST@Jcl (GALAS)	Crl:WI(Han)	Crl:WI(Han)	RccHan: WIST
Year	2001–2003	2005	2002–2009	2007	2001	2002–2010	2001–2010	2004	2009	2010	2001	2010
Treatment†	V	V	V	V	V	V	V	V	V	V	V	N
Feed	NMF	MF	NMF	CE-2	MF	MF	CRF-1	CE-2	CE-2	CRF-1	CE-2	NMF
No. dams	39	24	59	19	24	191	134	20	19	41	19	79
No. experiments	2	1	3	1	1	8	6	1	1	1	1	1
No. dams/experiment	19–20	24	19–20	19	24	23–24	21–23	20	19	41	19	79
Pregnancy rate (%)	97.5 (95.0–100)	95.8	98.3 (95.0–100)	95.0	91.7	97.4 (95.8–100)	95.8 (91.7–100)	100	95.0	87.2	95.0	98.8
Gestation day (hour) of the cesarean section	20 (13:00–16:00)	20 (9:00–12:00)	20 (13:00–16:00)	20 (9:00–12:00)	20 (9:00–12:00)	20 (9:00–12:00)	20 (9:00–16:00)	20 (9:00–12:00)	20 (9:00–12:00)	20 (9:00–11:30)	20 (9:00–12:00)	20 (13:00–16:00)
No. corpora lutea	19.9 (18.9–20.8)	17.1	17.3 (16.4–18.1)	17.6	15.9	13.8 (13.3–14.4)	13.2 (12.6–14.1)	13.6	14.1	11.5	13.4	13.8
No. implantations	17.0 (16.4–17.5)	16.2	16.1 (15.9–16.5)	16.4	14.9	12.7 (12.0–13.3)	12.3 (11.7–13.0)	12.2	12.7	9.6	12.2	12.1
No. live fetuses	15.7 (15.0–16.4)	15.3	15.2 (14.9–15.4)	15.3	13.5	11.9 (11.4–12.2)	11.3 (10.9–12.1)	11.7	12.2	9.0	11.7	11.3
Fetal mortality (%)‡	7.1 (6.0–8.2)	5.8	5.7 (4.6–6.3)	7.2	9.4	5.8 (3.7–8.9)	7.9 (4.9–14.0)	4.5	4.7	6.3	4.2	6.3
Body weight (g)												
All fetuses		3.99			3.15	3.48 (3.35–3.62)		3.37		3.51		
Male	4.43 (4.40–4.45)	4.06	4.29 (4.21–4.35)	3.83	3.25	3.57 (3.52–3.62)	3.40 (3.31–3.52)	3.45	3.49		3.79	3.89
Female	4.15 (4.07–4.23)	3.91	4.02 (3.91–4.09)	3.59	3.05	3.39 (3.35–3.47)	3.22 (3.12–3.35)	3.23	3.34		3.61	3.71

†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 8 Mating and cesarean section data from mice and hamsters

Species	Mice	Mice	Mice	Mice	Mice	Hamsters
Strain	Crlj:CD1(ICR)	Crlj:CD1(ICR)	Crlj:CD1(ICR)	Crlj:CD1(ICR)	Crlj:CD1(ICR)	Slc:Syrian
Year	2000	2001–2009	2002–2009	2004	2002	1999
Treatment†	V	V	V	V	V	V
Feed	CE-2	CRF-1	CE-2	NMF	CRF-1	CRF-1
No. dams	16	254	98	21	20	23
No. experiments	1	13	5	1	1	1
No. dams/experiment	16	16–23	16–23	21	20	23
Pregnancy rate (%)	72.7	82.7 (72.0–92.0)	78.9 (68.0–92.0)	95.5	100	88.9
Gestation day (hour) of the cesarean section	18 (9:00–12:00)	18 (7:00–10:00)	18 (9:00–12:00)	17 (13:00–16:00)	17 (9:00–12:00)	14 (9:00–11:00)
No. corpora lutea	13.1	14.0 (11.7–16.2)	14.0 (13.3–14.8)	15.6	14.7	15.6
No. implantations	11.6	12.4 (9.9–14.3)	12.6 (12.1–13.5)	14.4	14.6	14.7
No. live fetuses	11.2	11.5 (9.0–13.1)	11.7 (11.3–12.3)	13.4	14.1	12.1
Fetal mortality (%)‡	3.2	7.7 (4.6–9.4)	9.3 (6.2–14.0)	7.7	3.0	17.6
Body weight (g)						
All fetuses					1.02	
Male	1.51	1.45 (1.39–1.50)	1.45 (1.39–1.51)	1.19	1.05	1.60
Female	1.44	1.39 (1.35–1.43)	1.39 (1.32–1.41)	1.13	0.99	1.50

†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.

Skeletal anomalies

Table S13 shows data on skeletal anomalies in Crlj:CD(SD) rats between 1994 and 2000. The incidence of fetuses with skeletal malformations ranged from 0 to 3.97% and was slightly over the range reported previously in this rat strain (0–0.85% in Morita et al. 1987; 0–2.74% in Nakatsuka et al. 1997). This appears to be due to the higher incidence of cleft sternebrae detected in one laboratory. This anomaly was also observed in a previous survey (Nakatsuka et al. 1997).

Data for Crl:CD(SD) rats between 1994 and 2000 and between 2001 and 2010 are presented in Tables S14 and S16, respectively. The incidence of fetuses with skeletal malformations ranged from 0 to 8.02% between 1994 and 2000 and 0.10 to 0.56% between 2001 and 2010. Although this incidence between 2001 and 2010 was within the ranges of that previously reported in this rat strain (0–5.2%) (CD(SD)IGS Study Group 1998, 1999, 2000, 2001, 2003), the incidence between 1994 and 2000 was slightly over the ranges previously reported. This appears to be due to the higher incidence of split costal cartilage and cleft sternebrae (5.28%) found in one laboratory. These anomalies were also observed in previous surveys (Morita et al. 1987; Nakatsuka et al. 1997). No clear difference was observed in the types of anomalies between the previous and present surveys.

Skeletal variations

Data on skeletal variations in Crlj:CD(SD) rats between 1994 and 2000 are presented in Table S19. The incidence of fetuses with skeletal variations ranged from 3.60 to 8.36%. This incidence was within the range of a previous survey of this rat strain (1.82–28.13%) (Nakatsuka et al. 1997). No noticeable difference was found in the 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 S20 and S22, respectively. The incidence of fetuses with skeletal variations ranged from 6.98 to 22.98% between 1994 and 2000 and 9.42 to 17.63% between 2001 and 2010. These incidences were within the ranges of those in previous surveys of this rat strain (6.8–35.7%) (CD(SD)IGS Study Group 1998, 1999, 2000, 2001, 2003). No clear difference was observed in the types of variations between the previous and present surveys. No noticeable difference was found in the types of skeletal variations between the two intervals evaluated (1994–2000 and 2001–2010).

CONCLUSION

Historical control data on rodent developmental toxicity studies, which were performed between 1994 and 2010, were obtained from 19 laboratories in Japan. Summary of historical control data on developmental toxicity studies in rodents was shown in Table 5. Inter-laboratory variations in the incidences of fetuses with alterations appear to be due to differences in the selection of observation parameters, observation criteria, classification, and terminology of fetal alterations. This survey provides information on historical control data of Crlj:CD(SD), which was completely withdrawn from the Japanese market in 2007, and Crl:CD(SD) rats, which have been developed and completely replaced Crlj:CD(SD) in 2007. Initial information on Wistar Hannover rats, which have been recently introduced into Japan, mice, and hamsters has also been provided in this survey. These historical control data may be helpful in interpreting the effect of chemicals in reproductive and developmental toxicity studies. However, the continuous accumulation of historical control data is needed for an adequate evaluation of reproductive and developmental toxicity data. To further interpret this data and its assessment for human health, it is necessary to harmonize the classification and terminology of fetal alterations.

ACKNOWLEDGMENTS

This work was partially supported by Health and Labour Sciences Research Grants (Research on Regulatory Science of Pharmaceuticals and Medical Devices) and by the Ministry of Health, Labour and Welfare of Japan.

REFERENCES

Aoyama H, Kikuta M, Shirasaka N et al. 2002. Historical control data on reproductive abnormalities and incidences of spontaneous fetal malformations in Wistar Hannover GALAS rats. Congenit Anom 42:194–201.

Barrow PC. 2009. Reproductive toxicity testing for pharmaceuticals under ICH. Reprod Toxicol 28:172–179.

CD(SD)IGS Study Group. 1998. Maeda Y and Shibuya K, eds. Biological Reference Data on CD(SD)IGS Rats-1998. https://www.google.co.jp/#q=Biological+Reference+Data+on+CD(SD)IGS+Rats-1998, accessed January 24, 2014.

CD(SD)IGS Study Group. 1999. Maeda Y and Shibuya K, eds. Biological Reference Data on CD(SD)IGS Rats-1999. https://www.google.co.jp/#q=Biological+Reference+Data+on+CD(SD)IGS+Rats-1998, accessed January 24, 2014.

CD(SD)IGS Study Group. 2000. Matsuzawa T and Inoue H, eds. Biological Reference Data on CD(SD)IGS Rats-2000. https://www.google.co.jp/#q=Biological+Reference+Data+on+CD(SD)IGS+Rats-2000, accessed January 24, 2014.

CD(SD)IGS Study Group. 2001. Maeda Y and Inoue H, eds. Biological Reference Data on CD(SD)IGS Rats-2002/2003. https://www.google.co.jp/#q=Biological+Reference+Data+on+CD(SD)IGS+Rats-2001, accessed January 24, 2014.

CD(SD)IGS Study Group. 2003. Maeda Y and Shibuya K, eds. Biological Reference Data on CD(SD)IGS Rats-2002/2003. https://www.google.co.jp/#q=Biological+Reference+Data+on+CD(SD)IGS+Rats-2002%2F2003, accessed January 24, 2014.

CLEA Japan, Inc. 2007. Global alliance for laboratory animal standardization (GALAS) –Wistar Hannover GALAS rats. http://www.clea-japan.com/REPORT/pdf/galas.pdf, accessed January 24, 2014.

Ema M, Aoyama H, Arima A et al. 2012. Historical control data on prenatal developmental toxicity studies in rabbits. Congenit Anom 52:155–161.

Giknis MLA, Clifford CB. 2007. Reproductive and behavioral evaluations in Crl:CD-1®(ICR) mice. http://www.google.co.jp/url?sa=t&rct=j&q=&esrc=s&frm=1&source=web&cd=1&ved=0CCwQFjAA&url=http%3A%2F%2Fwww.crj.co.jp%2Fcms%2Fpdf%2Finfo_common%2F49%2F1496903%2Frm_rm_r_reproductive_behavioral_evaluations_CD1_mice.pdf&ei=1PzhUpuRAoOklQXQ8oDICA&usg=AFQjCNFKFWanP9FKm2fSR1LONmxtI-129w&sig2=cJ4jTcvyc0DSrqhQkhuT9g, accessed January 24, 2014.

Horimoto M, Ariyuki F, Daidohji S et al. 1998. Terminology of developmental abnormalities in common laboratory mammals. Congenit Anom 38:153–237.

Kameyama Y, Tanimura T, Yasuda M. 1980. Spontaneous malformations in laboratory animals – photographic atlas and reference data. Congenit Anom 20:25–106.

Kimmel CA, Price CJ. 1990. Developmental toxicity studies. In: Arnold DL, Grice HC, Krewski DR, editors. Handbook of in vivo toxicity testing. San Diego: Academic Press. p 271–301.

Liberati TA, Roe BJ, Feuston MH. 2002. An oral (gavage) control embryo-fetal development study in the Wistar Hannover rats. Drug Chem Toxicol 25:109–130.

Makris S, Solomon HM, Clark R et al. 2009. Terminology of developmental abnormalities in common laboratory mammals (version 2). Congenit Anom 49:123–246.

Marty MS, Allen B, Chapin RE et al. 2009. Inter-laboratory control data for reproductive endopoints required in the OPPTS 870.3800/OECD 416 reproduction and fertility test. Birth Defects Res B Dev Reprod Toxicol 86:470–489.

Morita H, Ariyuki F, Inomata N et al. 1987. Spontaneous malformations in laboratory animals: frequency of external, internal and skeletal malformations in rats, rabbits and mice. Congenit Anom 27:147–206.

Nakatsuka T, Hoimoto M, Ito M, Matsubara Y, Akaike M, Ariyuki F. 1997. Japan Pharmaceutical Manufacturers Association (JPMA) survey on background control data of developmental and reproductive toxicity studies in rats, rabbits and mice. Congenit Anom 37:47–138.

Schardein JL. 2000. Chemically induced birth defects. New York: Marcel Decker.

Takeuchi T, Okuda H, Kasahara Y, Ushigome S, Aihara I, Fukushima S. 2011. Differences in spontaneous abnormalities among three outbred stocks of Wistar Hannover rats in Japan. Congenit Anom 51:149–152.

Tyl RW, Marr MC. 2006. Developmental toxicity testing – methodology. In: Hood RD, editor. Developmental and reproductive toxicology – a practical approach, 2nd edn. Boca Raton: Taylor & Francis. p 201–261.

Wilson JG. 1973. Environment and Birth Defects. New York: Academic Press.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. External anomalies in Crlj:CD(SD) [former Crj:CD(SD)] rats between 1994 and 2000.
Table S2. External anomalies in Crl:CD(SD) [former Crj:CD(SD)IGS] between 1994 and 2000.
Table S3. External anomalies in Jcl:SD, Slc:SD, and Crlj:WI rats between 1994 and 2000.
Table S4. External anomalies in Crl:CD(SD) [former Crj:CD(SD)IGS] rats between 2001 and 2010.
Table S5. External anomalies in Jcl:SD, Crlj:WI, Jcl:Wistar, and Wistar Hannover rats between 2001 and 2010.
Table S6. External anomalies in mice and hamsters.
Table S7. Visceral anomalies in Crlj:CD(SD) [former Crj:CD(SD)] rats between 1994 and 2000.
Table S8. Visceral anomalies in Crl:CD(SD) [former Crj:CD(SD)IGS] between 1994and 2000.
Table S9. Visceral anomalies in Jcl:SD, Slc:SD, and Crlj:WI rats between 1994 and 2000.
Table S10. Visceral anomalies in Crl:CD(SD) [former Crj:CD(SD)IGS] rats between 2001 and 2010.
Table S11. Visceral anomalies in Jcl:SD, Crlj:WI, Jcl:Wistar, and Wistar Hannover rats between 2001 and 2010.
Table S12. Visceral anomalies in mice and hamsters.
Table S13. Skeletal anomalies in Crlj:CD(SD) [former Crj:CD(SD)] rats between 1994 and 2000.
Table S14. Skeletal anomalies in Crl:CD(SD) [former Crj:CD(SD)IGS] between 1994 and 2000.
Table S15. Skeletal anomalies in Jcl:SD, Slc:SD, and Crlj:WI rats between 1994 and 2000.
Table S16. Skeletal anomalies in Crl:CD(SD) [former Crj:CD(SD)IGS] rats between 2001 and 2010.
Table S17. Skeletal anomalies in Jcl:SD, Crlj:WI, Jcl:Wistar, and Wistar Hannover rats between 2001 and 2010.
Table S18. Skeletal anomalies in mice and hamsters.
Table S19. Skeletal Variations in Crlj:CD(SD) [former Crj:CD(SD)] rats between 1994 and 2000.
Table S20. Skeletal variations in Crl:CD(SD) [former Crj:CD(SD)IGS] between 1994 and 2000.
Table S21. Skeletal variations in Jcl:SD, Slc:SD, and Crlj:WI rats between 1994 and 2000.
Table S22. Skeletal variations in Crl:CD(SD) [former Crj:CD(SD)IGS] rats between 2001 and 2010
Table S23. Skeletal variations in Jcl:SD, Crlj:WI, Jcl:Wistar, and Wistar Hannover rats between 2001 and 2010.
Table S24. Skeletal variations in mice and hamsters.