the present study were set as 0.1, 0.3 or 1.0 mg/kg/day.

Twelve males per group were dosed for 42 days, beginning 14 days before mating. After the administration period, 5 of 12 males per group were reared for the recovery period of 14 days without administration of PFUA, as satellite groups. The main group females were dosed for 41-46 days, beginning 14 days before mating to day 4 of lactation throughout the mating and gestation period. Females in the satellite group were given PFUA for 42 days, followed by the recovery period of 14 days. The first day of dosing was designated as day 0 of administration and the day after the final dose was designated as day 0 of the recovery period. The volume of each dose was adjusted to 5 ml/kg body weight based on the latest body weight.

## Observations

All rats were observed daily for clinical signs of toxicity. Body weight was recorded twice a week in all males and in the satellite group females, and twice a week during the premating period, on days 0, 4, 7, 11, 14, 17, and 20 of pregnancy and on days 0 and 4 of lactation in main group females. Food consumption was recorded twice a week in all males and in satellite group females, and twice a week during the premating period, on days 1, 4, 7, 11, 14, 17, and 20 of pregnancy and on days 2 and 4 of lactation in main group females. Functional observation battery (FOB) in all animals was recorded once a week during the administration period, as follows: (i) home cage observation; posture, convulsion, and abnormal behavior, (ii) in-the-hand observation; ease of removal from cage and handling, fur and skin condition, eye ball, secretion from nose and/or eye, visible mucous membrane, lacrimation, salivation, piloerection, pupil diameter, and respiration, and (iii) open field observation; arousal, ambulation, posture, shivering, convulsion, rearing frequency, excreta, stereotypical behavior, and abnormal behavior.

Five animals in each group were subjected to the following observations and examinations unless noted otherwise. Sensory reactions for pupillary reflex, approximation reflex, tactile reflex, auditory reflex, pain reflex, righting reflex and width of the landing legs, grip strength of fore and hind limbs, and spontaneous motor activity were tested in main group males on day 37 of administration, in main group females on day 4 of lactation, and in satellite group males and females on day 37 of administration and on day 8 of the recovery period. Fresh urine was sampled from animals using a urine-collecting cage during the last weeks of the dosing and recovery periods. The 4-hr urine samples were collected soon after dosing under fasting (water was allowed *ad libitum*), and the 20-hr urine samples were collected, food and water being allowed *ad libitum*.

After 16-20 hr (overnight) of fasting, the main group of rats was euthanized by exsanguination under anesthesia on the day after the final administration in males and on day 4 of lactation in females, and satellite group rats were euthanized on the day of the completion of the recovery period. The external surfaces of the rats were examined. The abdomen and thoracic cavity were opened, and gross internal examination was performed. Blood samples were drawn from the abdominal aorta. Major organs were removed from all animals, and the brain, thyroid, thymus, heart, liver, spleen, kidney, adrenal glands, testis, epididymis were weighed. The numbers of corpora lutea and implantation sites were counted in all main group females. The testes and epididymides were fixed with Bouin's solution and in 10% phosphate-buffered formalin. Other organs were stored in 10% phosphate-buffered formalin. The cerebrum and cerebellum, pituitary gland, spinal cord, sciatic nerve, thyroid, parathyroid, adrenal glands, thymus, spleen, mandibular lymph nodes, mesenteric lymph node, heart, lung, trachea, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, liver, kidney, bladder, testis, epididymis, uterus, seminal vesicle, sternum, and femur were histopathologically evaluated for five males and females in the control and the highest groups, and organs with macroscopically abnormal findings were also examined histopathologically. The organs for histopathological evaluations were processed routinely for embedding in paraffin, and sections were prepared for staining with hematoxylin–eosin. Test substance-related histopathological changes were found in the liver in males and females, and in the stomach in males; therefore, the liver in all animals and the stomach in all males were also examined histopathologically.

The 4-hr urine samples were tested for color, pH, protein, glucose, ketone body, bilirubin, occult blood, urobilinogen, and urinary sediment. Urinary sediment was stained and examined microscopically. The 20-hr urine samples were tested for osmotic pressure. Urine volume for 4-hr and 20-hr was measured. In the collected blood samples the red blood cell (RBC) count, hemoglobin, platelet count, and white blood cell count were measured. In addition, mean corpuscular volume (MCV), hematocrit, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), reticulocyte rate, and differential leukocyte rates were calculated. Prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrinogen were determined. Blood chemistry was tested for alkaline phosphatase (ALP), total protein, albumin, albumin/globulin (A/G) ratio, total bilirubin, blood

urea nitrogen (BUN), creatinine, glucose, total cholesterol, triglycerides, phospholipid, Na, K, Cl, Ca, inorganic phosphate, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactase dehydrogenase (LDH), and gamma-glutamyltransferase (γ-GTP).

In the main group, daily vaginal lavage samples of each female were evaluated for estrous cyclicity throughout the premating period. Each female rat was mated overnight with a single male rat of the same dosage group until copulation occurred or the 2-week mating period had elapsed. During the mating period, daily vaginal smears were examined for the presence of sperm. The presence of sperm in the vaginal smear and/or a vaginal plug was considered as evidence of successful mating. Once insemination was confirmed, the females were checked twice a day for signs of parturition from day 21 to day 24 of pregnancy. One female in the 0.1 mg/kg/day treatment group did not deliver and did not have implantation. Because of infertility, data for that female for the period corresponding to gestation were excluded from statistical analysis. Other females were allowed to deliver spontaneously and nurse their pups until postnatal day (PND) 4. The day on which parturition was completed by 17:00 was designated as PND 0. Litter size and numbers of live and dead pups were recorded, and live pups were sexed and individually weighed on PNDs 0 and 4. Pups were inspected for external malformations on PND 0. On PND 4, the pups were euthanized by exsanguination under anesthesia, and gross internal examinations were performed.

### Data analysis

Statistical analysis of pups was carried out using the litter as the experimental unit. Mean and standard deviation in each dose group were calculated for body weight, food consumption, water consumption, number of feces, rearing frequency, width of the landing legs, grip strength, spontaneous motor activity, urine volume, hematological test results, blood biochemical test results, absolute and relative organ weights, estrous cycle length, length of gestation, numbers of corpora lutea and implantations, implantation index, total number of pups born, number of male and female pups, number of live and dead pups, live birth index, live pups and viability index on day 4 of lactation, and body weight of pups. These were analyzed with Bartlett's test or F-test for homogeneity of variance. If they were homogeneous, the data were analyzed using Dunnett's test or Student's t-test to compare the mean of the control group with that of each dosage group, and if they were not homogeneous, a Dunnett-type rank test or Aspin-Welch t-test was applied. The copulation index, fertility index, gestation index, sex ratio of pups, and data for sensory reactions of reflexes were analyzed with Yates' chi-square test. The 5% levels of probability were used as the criterion for significance. Unless otherwise noted, there are statistically significant differences in the changes described in the following Results section.

## RESULTS

### Parental toxicity

No deaths were observed in any of the groups. A decrease in grip strength of the forefoot was observed in males and females at 1.0 mg/kg/day in the recovery period. No other treatment-related effects on clinical signs of toxicity, FOB, sensory reactivity, or spontaneous motor activity were observed in males and females in the main and satellite groups (data not shown).

Body weight changes in each group are shown in Figs. 1 and 2. In males at 1.0 mg/kg/day, body weight gains decreased during the dosing period and during the recovery period. In females at 1.0 mg/kg/day, body weight gains decreased during the lactation period in the main group and during the dosing period and the recovery period in the satellite group, and lowered body weight was observed on days 38 and 41 of the dosing period and on days 0-13 of the recovery period in the satellite group. No effects on body weight in male and female groups were observed at any other dosing. Food consumption (data not shown) was decreased on day 4 of the delivery period at 1.0 mg/kg/day in females. Urinalysis revealed no significant differences in any parameters between the control and treatment groups in males and females in the main and satellite groups (data not shown).

Table 1 shows hematological findings in male and female rats. At 1.0 mg/kg/day, low values of fibrinogen and APTT were observed in males of the main and satellite groups, and a low value of fibrinogen was observed in females of the main group. The other significant changes in hematological findings were incidental because they were slight without related changes or did not occur in a dose-dependent manner.

Blood biochemical findings are shown in Table 2. At 1.0 mg/kg/day in the main group, increases in BUN and ALP and decreases in total protein and albumin were observed in males, and an increase in BUN and a decrease in total protein were observed in females. At 1.0 mg/kg/day in the satellite group, increases in BUN and ALP in males and females, and a decrease in total protein in females were observed. The other changes with statistical significances in blood biochemical findings were incidental because they were slight without related changes or did not occur in a dose-dependent manner.

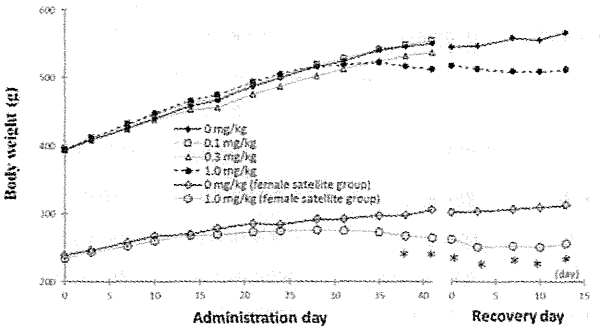


**Fig. 1.** Body weight of males in main groups and satellite groups for recovery period and females in satellite groups.
*: Significantly different from the control, $p \leqq 0.05$.

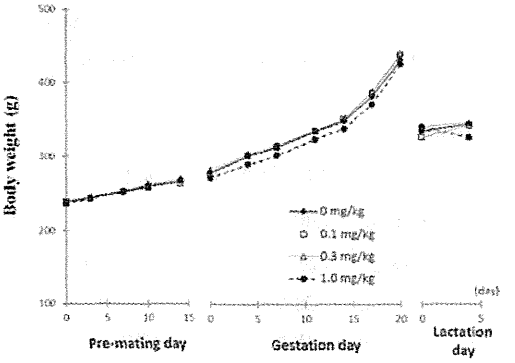


**Fig. 2.** Body weight of females in main groups.

Organ weights in males and females are shown in Table 3. Relative weight of the liver was increased at 0.3 mg/kg/day in main group males, and absolute and relative weights of the liver were increased in males and females at 1.0 mg/kg/day in main and satellite groups. Absolute and relative weights of the spleen were decreased at 1.0 mg/kg/day in main group males. Enlargement of the liver in two males and a dark red focus in the stomach in three males were observed at 1.0 mg/kg/day in the main group. No other treatment-related findings at necropsy were observed in males and females in main and satellite groups. Histopathological findings are shown in Table 4. Possibly treatment-related changes were observed in the liver and stomach: In the main groups, centrilobular hypertrophy of hepatocytes in males and females were observed at 0.3 mg/kg/day and above, diffuse vacuolation of hepatocytes in males, and minimal focal necrosis in males and females were observed at 1.0 mg/kg/day, and in the satellite groups, minimal diffuse vacuolation of hepatocytes in males, centrilobular hypertrophy/degeneration of hepatocytes in males and females, and Glisson's sheath cell infiltration in females were observed at 1.0 mg/kg/day. In the glandular stomach, minimal erosion was observed in 3/7 males at 1.0 mg/kg/day. Although a similar change was observed in 2/6 control females, the possibility that PFUA treatment affected the stomach in males could not be ruled out. The findings in other organs were considered to be incidental in main and satellite groups, because there was no dose-dependent increase in incidence or severity. On reproductive organs, no treatment-related histopathological changes were found in the epididymides, testis, and uterus in PFUA-treated groups.

### Reproductive and developmental findings

There were no significant differences in the mean estrous cycle and in the incidence of females with a normal estrous cycle between the control and PFUA groups either in the main or recovery group (data not shown). The data for reproductive and developmental parameters are shown in Table 5. Reproduction performance of parental rats, delivery and nursing were not significantly different between the control and PFUA-treated groups. Regarding the general appearance of pups, there were no abnormal findings in any groups. The body weights of male and female pups on PNDs 0 and 4 were lowered at 1.0 mg/kg/day. There were no significant differences in the sex ratio of live pups or the viability index on PND 4. At gross pathology in pups on PND 4, thymic remnant in the neck was observed in one male and one female at 0.3 mg/kg/day, and in two females at 1.0 mg/kg/day, and these were considered to be incidental because of the low incidence. There were no other changes in gross internal findings of pups in any PFUA-treated groups.

## DISCUSSION

The present study of rats was conducted to examine the possible effects of PFUA on reproduction and development as well as the possible general toxic effects. The dosage of PFUA used in this study was sufficiently high to be expected to induce general toxic effects in parental animals. The following results suggest that the liver is a sensitive target organ. The weight of the liver was increased in males at 0.3 mg/kg/day and above, and in females at 1.0 mg/kg/day, and centrilobular hypertrophy of hepatocytes was observed in both sexes at 0.3 mg/kg/day and above, focal necrosis and/or diffuse vacuolation of hepatocytes were also found in the 1.0

**Table 1.** Hematological findings

| | | Main group | | | | Satellite group | |
|---|---|---|---|---|---|---|---|
| | Group | 0 mg/kg/day | 0.1 mg/kg/day | 0.3 mg/kg/day | 1.0 mg/kg/day | 0 mg/kg/day | 1.0 mg/kg/day |
| Males | | | | | | | |
| Number of animals | | 5 | 5 | 5 | 5 | 5 | 5 |
| WBC | ($10^2$/μl) | 121.2 ± 31.4 | 94.8 ± 21.1 | 127.6 ± 35.4 | 129.8 ± 23.5 | 73.4 ± 26.8 | 111.6 ± 19.5* |
| RBC | ($10^4$/μl) | 830 ± 40 | 846 ± 25 | 852 ± 20 | 869 ± 23 | 894 ± 34 | 886 ± 47 |
| HGB | (g/dl) | 15.6 ± 0.4 | 15.7 ± 0.6 | 15.4 ± 0.4 | 15.6 ± 0.7 | 16.0 ± 0.4 | 15.3 ± 0.9 |
| MCV | fl | 52.5 ± 1.8 | 51.4 ± 1.7 | 50.6 ± 0.7 | 50.1 ± 1.4* | 50.9 ± 1.5 | 49.4 ± 1.9 |
| MCH | pg | 18.8 ± 0.5 | 18.6 ± 0.8 | 18.1 ± 0.4 | 17.9 ± 0.4* | 17.9 ± 0.3 | 17.3 ± 0.7 |
| Platelet | ($10^4$/μl) | 98.7 ± 3.7 | 121.4 ± 5.2** | 109.2 ± 8.8 | 111.2 ± 8.8* | 107.8 ± 12.4 | 122.7 ± 18.6 |
| APTT | (sec) | 22 ± 4.1 | 19.2 ± 1.9 | 20.8 ± 4.2 | 16.6 ± 0.7* | 20.4 ± 1.7 | 17.2 ± 2.6* |
| Fibrinogen | mg/dl | 294 ± 20 | 273 ± 35 | 283 ± 31 | 200 ± 23** | 304 ± 35 | 245 ± 22* |
| Females | | | | | | | |
| Number of animals | | 5 | 5 | 5 | 5 | 5 | 5 |
| WBC | ($10^2$/μl) | 143.4 ± 43.8 | 128.7 ± 25.4 | 151.8 ± 33.5 | 159.2 ± 45.1 | 58.6 ± 14.9 | 65.1 ± 13.6 |
| RBC | ($10^4$/μl) | 702 ± 46 | 680 ± 67 | 692 ± 50 | 645 ± 51 | 830 ± 30 | 846 ± 56 |
| HGB | (g/dl) | 13.1 ± 1.0 | 13.5 ± 1.0 | 13.5 ± 1.1 | 13.2 ± 0.8 | 15.4 ± 0.4 | 15.4 ± 1.0 |
| MCV | fl | 52.7 ± 1.3 | 56.7 ± 4.5 | 55.0 ± 1.2 | 58.0 ± 3.1* | 51.4 ± 1.4 | 50.1 ± 1.2 |
| MCH | pg | 18.6 ± 0.5 | 20.0 ± 1.6 | 19.5 ± 0.6 | 20.5 ± 1.1* | 18.6 ± 0.6 | 18.2 ± 0.6 |
| Platelet | ($10^4$/μl) | 159.4 ± 27.4 | 141.0 ± 22.7 | 164.8 ± 19.6 | 161.8 ± 30.9 | 130.6 ± 13.7 | 125.7 ± 18.1 |
| APTT | (sec) | 17.6 ± 1.8 | 17.5 ± 2.4 | 17.9 ± 2.3 | 15.2 ± 3.3 | 17.9 ± 2.3 | 17 ± 2.9 |
| Fibrinogen | mg/dl | 335 ± 53 | 319 ± 95 | 282 ± 49 | 228 ± 42* | 207 ± 10 | 176 ± 31 |

Values are given as the mean ± S.D.
*: Significantly different from the control, $p \leqq 0.05$. **: Significantly different from the control, $p \leqq 0.01$.

mg/kg/day group. In rodents, it is clear that the hepatic response to exposure to many perfluoroalkyl compounds is initiated by the activation of the nuclear hormone receptor, PPARα (ATSDR, 2009), and PFUA activates mouse PPARα *in vitro* (Wolf *et al.*, 2012). The hepatic proliferative responses, including an increase in the liver weight and centrilobular hypertrophy of hepatocytes, observed in the present study might have been initiated by the activation of PPARα, although there is a scientific consensus that compounds which are peroxisome proliferators in rodents have little or no effect on human liver (IARC, 1995). Regarding the toxicity of PFAAs, the involvement of mechanisms other than PPARα has been suggested (Peters and Gonzalez, 2011), so further research on the toxicity mechanism of PFUA is desired.

Effects on the body weight of adult males/females and pups were observed only at 1.0 mg/kg/day. In adult animals, suppression of body weight gain was observed in males/females in the administration and/or recovery periods, although not in females in the premating and gestation periods. It is considered that these body weight changes were a direct effect of PFUA because they were not related to food consumption. There is a possibility of maternal-fetal/infant transfer of PFUA, because maternal-fetal transfer and maternal-infant transfer of PFOA through breast milk have been observed in rats (Hinderliter *et al.*, 2005). Because there was no difference in the length of the gestation period in dams dosed at 1.0 mg/kg/day compared to the controls, and because sup-

**Table 2.** Blood biochemical findings

| | | Main group | | | | Satellite group | |
|---|---|---|---|---|---|---|---|
| | Group | 0 mg/kg /day | 0.1 mg/kg/day | 0.3 mg/kg/day | 1.0 mg/kg/day | 0 mg/kg/day | 1.0 mg/kg/day |
| Males | | | | | | | |
| Number of animals | | 5 | 5 | 5 | 5 | 5 | 5 |
| AST | (IU/l) | 67 ± 9 | 70 ± 4 | 73 ± 17 | 77 ± 6 | 62 ± 9 | 73 ± 12 |
| ALT | (IU/l) | 31 ± 3 | 32 ± 3 | 34 ± 3 | 39 ± 7* | 31 ± 5 | 37 ± 5 |
| ALP | (IU/l) | 427 ± 12.6 | 461 ± 85 | 514 ± 96 | 1021 ± 179** | 379 ± 95 | 707 ± 152** |
| Total cholesterol | (mg/dl) | 56 ± 14 | 47 ± 8 | 34 ± 6** | 46 ± 11 | 55 ± 18 | 53 ± 13 |
| Triglyceride | (mg/dl) | 48 ± 10 | 70 ± 42 | 41 ± 9 | 46 ± 16 | 52 ± 17 | 45 ± 27 |
| Phospholipid | (mg/dl) | 90 ± 13 | 82 ± 14 | 65 ± 9* | 87 ± 11 | 87 ± 19 | 92 ± 21 |
| BUN | (mg/dl) | 13 ± 2 | 14 ± 3 | 15 ± 1 | 21 ± 4** | 17 ± 2 | 23 ± 5* |
| Na | (mmol/l) | 147 ± 2 | 146 ± 2 | 147 ± 1 | 145 ± 1 | 145 ± 1 | 143 ± 1** |
| Cl | (mmol/l) | 108 ± 2 | 108 ± 1 | 109 ± 1 | 109 ± 3 | 107 ± 1 | 108 ± 1 |
| Ca | (mg/dl) | 10.1 ± 0.2 | 10.0 ± 0.3 | 10.0 ± 0.3 | 9.7 ± 0.2* | 9.9 ± 0.3 | 9.5 ± 0.3 |
| Total protein | (g/dl) | 6.2 ± 0.2 | 6.0 ± 0.3 | 6.1 ± 0.1 | 5.5 ± 0.3** | 6.3 ± 0.1 | 5.8 ± 0.5 |
| Albumin | (g/dl) | 2.8 ± 0.1 | 2.8 ± 0.1 | 2.9 ± 0.0 | 2.6 ± 0.1* | 2.7 ± 0.1 | 2.8 ± 0.2 |
| A/G | | 0.80 ± 0.07 | 0.86 ± 0.03 | 0.93 ± 0.05** | 0.88 ± 0.06 | 0.77 ± 0.04 | 0.93 ± 0.09** |
| Females | | | | | | | |
| Number of animals | | 5 | 5 | 5 | 5 | 5 | 5 |
| AST | (IU/l) | 84 ± 21 | 92 ± 12 | 86 ± 15 | 81 ± 12 | 59 ± 4 | 68 ± 11 |
| ALT | (IU/l) | 53 ± 9 | 55 ± 12 | 50 ± 18 | 49 ± 1 | 26 ± 4 | 28 ± 4 |
| ALP | (IU/l) | 219 ± 72 | 242 ± 42 | 286 ± 176 | 263 ± 18 | 158 ± 28 | 289 ± 54** |
| Total cholesterol | (mg/dl) | 60 ± 11 | 52 ± 13 | 41 ± 13* | 49 ± 8 | 78 ± 16 | 64 ± 14 |
| Triglyceride | (mg/dl) | 54 ± 11 | 38 ± 12 | 41 ± 18 | 60 ± 25 | 28 ± 11 | 20 ± 3 |
| Phospholipid | (mg/dl) | 112 ± 13 | 94 ± 18 | 80 ± 20* | 98 ± 11 | 141 ± 20 | 108 ± 15* |
| BUN | (mg/dl) | 13 ± 2 | 13 ± 4 | 16 ± 3 | 19 ± 2** | 20 ± 3 | 29 ± 7* |
| Na | (mmol/l) | 141 ± 1 | 141 ± 2 | 143 ± 1 | 142 ± 1 | 143 ± 1 | 143 ± 1 |
| Cl | (mmol/l) | 106 ± 1 | 107 ± 2 | 108 ± 2 | 108 ± 2* | 109 ± 1 | 112 ± 2 |
| Ca | (mg/dl) | 10.3 ± 0.2 | 10.2 ± 0.4 | 10.3 ± 0.1 | 10.0 ± 0.3 | 10.2 ± 0.3 | 9.9 ± 0.2 |
| Total protein | (g/dl) | 6.2 ± 0.2 | 5.8 ± 0.3* | 6.0 ± 0.1 | 5.6 ± 0.2** | 6.7 ± 0.2 | 5.8 ± 0.3** |
| Albumin | (g/dl) | 2.8 ± 0.2 | 2.8 ± 0.2 | 2.8 ± 0.1 | 2.7 ± 0.2 | 3.1 ± 0.2 | 2.9 ± 0.3 |
| A/G | | 0.85 ± 0.05 | 0.92 ± 0.05 | 0.89 ± 0.07 | 0.91 ± 0.11 | 0.87 ± 0.04 | 1.01 ± 0.09* |

Values are given as the mean ± S.D.
*: Significantly different from the control, $p \leq 0.05$. **: Significantly different from the control, $p \leq 0.01$.

pression of body weight gain in females during pregnancy was not observed, the lowered body weight on PND 0 was considered a direct effect of PFUA due to intrauterine exposure. Also in other PFCAs, low values of body weight of pups at birth without effects on the body weight of dams in the gestation period were observed (Butenhoff *et al.*, 2004; Loveless *et al.*, 2009). The lowered body weight on PND 4 was considered to be a direct effect of PFUA by ingestion of breast milk, as well as a secondary effect of PFUA caused by the lowered body weight in

**Table 3.** Organ weights

| | | Main Group | | | | Satellite Group | |
|---|---|---|---|---|---|---|---|
| Dose (mg/kg/day) | | 0 (control) | 0.1 | 0.3 | 1.0 | 0 (control) | 1.0 |
| Males | | | | | | | |
| No. of animals examined | | 5 | 5 | 5 | 5 | 5 | 5 |
| Brain | (g) | 2.18 ± 0.08 | 2.18 ± 0.09 | 2.15 ± 0.08 | 2.17 ± 0.08 | 2.09 ± 0.04 | 2.14 ± 0.13 |
| | (%)[a] | 0.42 ± 0.03 | 0.4 ± 0.03 | 0.41 ± 0.01 | 0.44 ± 0.05 | 0.39 ± 0.03 | 0.44 ± 0.03** |
| Throid[b] | (mg) | 22.4 ± 1.8 | 25.7 ± 2.5 | 21.1 ± 2.9 | 22.3 ± 3.5 | 23.5 ± 3.8 | 18.7 ± 2.9 |
| | (%)[a] | 4.3 ± 0.4 | 4.7 ± 0.4 | 4.1 ± 0.6 | 4.5 ± 0.7 | 4.4 ± 0.5 | 3.8 ± 0.4 |
| Thymus | (mg) | 297 ± 90 | 432 ± 173 | 342 ± 106 | 260 ± 61 | 250 ± 80 | 251 ± 67 |
| | (%)[a] | 57 ± 19 | 79 ± 27 | 66 ± 21 | 53 ± 16 | 47 ± 17 | 51 ± 11 |
| Heart | (g) | 1.52 ± 0.1 | 1.5 ± 0.2 | 1.51 ± 0.03 | 1.38 ± 0.17 | 1.46 ± 0.17 | 1.29 ± 0.19 |
| | (%)[a] | 0.29 ± 0.02 | 0.28 ± 0.04 | 0.29 ± 0 | 0.28 ± 0.02 | 0.28 ± 0.02 | 0.27 ± 0.02 |
| Liver | (g) | 15.12 ± 2.14 | 16.45 ± 2.06 | 17.54 ± 0.73 | 20.95 ± 2.56** | 14.19 ± 1.56 | 19.85 ± 3.03** |
| | (%)[a] | 2.88 ± 0.27 | 3.02 ± 0.19 | 3.39 ± 0.16** | 4.18 ± 0.19** | 2.67 ± 0.22 | 4.07 ± 0.36** |
| Spleen | (g) | 0.84 ± 0.16 | 0.76 ± 0.09 | 0.79 ± 0.05 | 0.65 ± 0.09* | 0.72 ± 0.11 | 0.72 ± 0.04 |
| | (%)[a] | 0.16 ± 0.03 | 0.14 ± 0.01 | 0.15 ± 0.01 | 0.13 ± 0.01* | 0.14 ± 0.02 | 0.15 ± 0.01 |
| Kidney[b] | (g) | 3.43 ± 0.31 | 3.44 ± 0.38 | 3.51 ± 0.08 | 3.4 ± 0.17 | 3.51 ± 0.31 | 3.32 ± 0.43 |
| | (%)[a] | 0.65 ± 0.06 | 0.63 ± 0.04 | 0.68 ± 0.03 | 0.68 ± 0.06 | 0.66 ± 0.03 | 0.68 ± 0.04 |
| Adrenal[b] | (mg) | 64 ± 13 | 70 ± 8 | 68 ± 3 | 58 ± 9 | 61 ± 9 | 46 ± 8* |
| | (%)[a] | 12 ± 2 | 13 ± 1 | 13 ± 1 | 12 ± 1 | 12 ± 2 | 9 ± 1 |
| Testis[b,c] | (g) | 3.34 ± 0.21 | 3.57 ± 0.26 | 3.48 ± 0.28 | 2.98 ± 0.86 | 3.49 ± 0.26 | 3.57 ± 0.35 |
| | (%)[a] | 0.63 ± 0.07 | 0.67 ± 0.05 | 0.68 ± 0.06 | 0.62 ± 0.17 | 0.66 ± 0.03 | 0.74 ± 0.07* |
| Epididymis[b,c] | (mg) | 1339 ± 84 | 1420 ± 112 | 1368 ± 199 | 1578 ± 950 | 1337 ± 51 | 1388 ± 87 |
| | (%)[a] | 252 ± 21 | 265 ± 25 | 268 ± 36 | 335 ± 220 | 252 ± 11 | 288 ± 34 |
| Females | | | | | | | |
| No. of animals examined | | 5 | 5 | 5 | 5 | 5 | 5 |
| Brain | (g) | 1.99 ± 0.05 | 1.97 ± 0.08 | 1.98 ± 0.09 | 2 ± 0.04 | 1.96 ± 0.09 | 1.86 ± 0.06 |
| | (%)[a] | 0.64 ± 0.04 | 0.66 ± 0.03 | 0.65 ± 0.05 | 0.67 ± 0.06 | 0.68 ± 0.08 | 0.78 ± 0.02** |
| Throid[b] | (mg) | 17.2 ± 1.8 | 19.2 ± 3.2 | 17.5 ± 3 | 16.9 ± 0.7 | 17.2 ± 2.7 | 14.7 ± 1 |
| | (%)[a] | 5.5 ± 0.8 | 6.5 ± 1.1 | 5.8 ± 1.1 | 5.6 ± 0.6 | 6 ± 1 | 6.2 ± 0.3 |
| Thymus | (mg) | 192 ± 16 | 170 ± 102 | 243 ± 82 | 249 ± 58 | 245 ± 98 | 147 ± 59 |
| | (%)[a] | 61 ± 4 | 56 ± 32 | 79 ± 24 | 82 ± 14 | 85 ± 39 | 62 ± 23 |
| Heart | (g) | 1.02 ± 0.08 | 0.96 ± 0.06 | 0.92 ± 0.04 | 0.94 ± 0.11 | 0.86 ± 0.05 | 0.73 ± 0.03** |
| | (%)[a] | 0.33 ± 0.03 | 0.32 ± 0.01 | 0.3 ± 0.01 | 0.31 ± 0.02 | 0.29 ± 0.02 | 0.31 ± 0.01 |
| Liver | (g) | 10.56 ± 0.68 | 10.61 ± 0.48 | 10.55 ± 1.48 | 12.76 ± 1.00** | 7.22 ± 0.38 | 8.63 ± 1.04* |
| | (%)[a] | 3.37 ± 0.12 | 3.57 ± 0.13 | 3.46 ± 0.36 | 4.21 ± 0.15** | 2.48 ± 0.14 | 3.64 ± 0.47** |
| Spleen | (g) | 0.62 ± 0.06 | 0.65 ± 0.16 | 0.65 ± 0.1 | 0.66 ± 0.15 | 0.49 ± 0.05 | 0.43 ± 0.05 |
| | (%)[a] | 0.2 ± 0.02 | 0.22 ± 0.05 | 0.22 ± 0.02 | 0.21 ± 0.03 | 0.17 ± 0.02 | 0.18 ± 0.01 |
| Kidney[b] | (g) | 2.24 ± 0.42 | 1.96 ± 0.18 | 2.06 ± 0.19 | 2.05 ± 0.09 | 1.89 ± 0.14 | 1.93 ± 0.17 |
| | (%)[a] | 0.72 ± 0.14 | 0.66 ± 0.06 | 0.68 ± 0.07 | 0.68 ± 0.04 | 0.64 ± 0.02 | 0.81 ± 0.07** |
| Adrenal[b] | (mg) | 82 ± 4 | 84 ± 10 | 89 ± 14 | 80 ± 13 | 70 ± 8 | 49 ± 5** |
| | (%)[a] | 26 ± 2 | 28 ± 5 | 30 ± 5 | 26 ± 3 | 25 ± 5 | 21 ± 1 |

Values are given as the mean ± S.D.

[a]: Ratio of organ weight to body weight (relative organ weight). [b]: Values are represented as the total weights of the organs on both sides. [c]: Organ weight was measured for all animals (number of examined animals: 7 at 0 and 1.0 mg/kg/day and 12 at 0.1 and 0.3 mg/kg/day in the main group, and 5 at 0 and 1.0 mg/kg/day in the recovery group.)

*: Significantly different from the control, $p \leqq 0.05$. **: Significantly different from the control, $p \leqq 0.01$.

**Table 4.** Histopathological findings

| | Males | | | | | | Females | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Main | | | | Satellite | | Main | | | | Satellite | |
| Dose (mg/kg/day) | 0 | 0.1 | 0.3 | 1.0 | 0 | 1.0 | 0 | 0.1 | 0.3 | 1.0 | 0 | 1.0 |
| Heart | | | | | | | | | | | | |
| Number examined | 5 | 0 | 0 | 5 | | | 5 | 0 | 0 | 5 | | |
| Cardiomyopathy (minimal) | 1 | | | 1 | | | 0 | | | 0 | | |
| Kidney | | | | | | | | | | | | |
| Number examined | 5 | 0 | 1 | 5 | | | 5 | 0 | 0 | 5 | | 1 |
| Dilatation, pelvic | 0 | | 1 | 0 | | | 1 | | | 0 | | 1 |
| (minimal) | | | 1 | | | | | | | | | 1 |
| (moderate) | | | | | | | 1 | | | | | |
| Regeneration, tubular | 4 | | 1 | 1 | | | 1 | | | 1 | | 0 |
| (minimal) | 3 | | 1 | 1 | | | 1 | | | 1 | | |
| (mild) | 1 | | | | | | | | | | | |
| Liver | | | | | | | | | | | | |
| Number examined | 7 | 12 | 12 | 7 | 5 | 5 | 12 | 12 | 12 | 12 | 5 | 5 |
| Vacuolation, hepatocytes, diffuse | 0 | 0 | 0 | 3 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| (minimal) | | | | 2 | | 1 | | | | | | |
| (mild) | | | | 1 | | | | | | | | |
| Necrosis, focal (minimal) | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 |
| Cell infiltration, Glisson's sheath (mild) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| Microgranuloma | 4 | 3 | 1 | 2 | 3 | 3 | 1 | 1 | 0 | 2 | 4 | 4 |
| (minimal) | 4 | 3 | 1 | 2 | 3 | 3 | 1 | 1 | | 2 | 4 | 2 |
| (mild) | | | | | | | | | | | | 2 |
| Degeneration, hepatocytes, centrilobular (minimal) | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 3 |
| Hypertrophy, hepatocytes, centrilobular | 0 | 0 | 3 | 7 | 0 | 5 | 0 | 0 | 1 | 11 | 0 | 5 |
| (minimal) | | | 2 | | | | | | 1 | 8 | | |
| (mild) | | | 1 | 2 | | 3 | | | | 3 | | 2 |
| (moderate) | | | | 5 | | 2 | | | | | | 3 |
| Spleen | | | | | | | | | | | | |
| Number examined | 5 | 0 | 0 | 5 | | | 5 | 0 | 0 | 5 | | |
| Hematopoiesis, extramedullary (minimal) | 4 | | | 1 | | | 5 | | | 4 | | |
| Stomach | | | | | | | | | | | | |
| Number examined | 7 | 12 | 12 | 7 | 5 | 5 | 6 | 0 | 0 | 5 | | |
| Erosion, glandular stomach (minimal) | 0 | 0 | 0 | 3 | 0 | 0 | 2 | | | 0 | | |
| Thymus | | | | | | | | | | | | |
| Number examined | 5 | 0 | 0 | 5 | | | 5 | 1 | 0 | 5 | | |
| Atrophy, lymphoid (mild) | 0 | | | 0 | | | 0 | 1 | | 0 | | |
| Thyroid | | | | | | | | | | | | |
| Number examined | 5 | 0 | 0 | 5 | | | 5 | 0 | 0 | 5 | | |
| Ectopic thymus (minimal) | 0 | | | 0 | | | 0 | | | 1 | | |
| Cyst, ultimobranchial (minimal) | 1 | | | 2 | | | 2 | | | 0 | | |
| Testis | | | | | | | | | | | | |
| Number examined | 5 | 0 | 0 | 5 | | | | | | | | |
| Not remarkable | 5 | | | 5 | | | | | | | | |
| Epididymis | | | | | | | | | | | | |
| Number examined | 5 | 1 | 0 | 5 | | | | | | | | |
| Granuloma, spermatic | 1 | 1 | | 1 | | | | | | | | |
| (minimal) | | 1 | | 1 | | | | | | | | |
| (mild) | 1 | | | | | | | | | | | |
| Uterus | | | | | | | | | | | | |
| Number examined | | | | | | | 5 | 1 | 0 | 5 | | |
| Dilatation, lumina (minimal) | | | | | | | 0 | 1 | | 0 | | |

**Table 5.** Reproductive and developmental parameters

| | 0 mg/kg/day | 0.1 mg/kg/day | 0.3 mg/kg/day | 1.0 mg/kg/day |
|---|---|---|---|---|
| Number of animals (males/females) | 12/12 | 12/12 | 12/12 | 12/12 |
| Copulation index (males/females) (%) | 100/100 | 100/100 | 100/100 | 100/100 |
| Fertility index (%) | 100 | 91.7 | 100 | 100 |
| Gestation index (%) | 100 | 100 | 100 | 100 |
| Number of pregnant animals | 12 | 11 | 12 | 12 |
| Gestation length (days) | 22.0 ± 0.3 | 22.1 ± 0.5 | 22.1 ± 0.5 | 21.7 ± 0.2 |
| Number of corpora lutea | 15.8 ± 1.9 | 16.8 ± 1.8 | 16.2 ± 1.9 | 16.2 ± 1.5 |
| Number of implantation sites | 14.6 ± 2.0 | 15.5 ± 3.3 | 15.0 ± 1.9 | 15.3 ± 1.6 |
| Implantation index (%) | 92.0 ± 5.5 | 91.0 ± 15.4 | 92.8 ± 6.0 | 94.8 ± 4.4 |
| Number of litters | 12 | 11 | 12 | 12 |
| Number of live pups on PND 0 | 13.9 ± 2.2 | 14.5 ± 3.4 | 13.1 ± 3.1 | 13.5 ± 2.2 |
| Live birth index (%) | 98.9 ± 2.6 | 97.3 ± 5.3 | 93.2 ± 18.2 | 97.9 ± 4.1 |
| Sex ratio | 0.51 | 0.47 | 0.55 | 0.52 |
| Number of live pups on PND 4 | 13.7 ± 1.9 | 14.0 ± 3.2 | 12.8 ± 3.1 | 13.4 ± 2.2 |
| Viability index (%) | 98.5 ± 2.8 | 97.1 ± 3.3 | 97.7 ± 5.9 | 99.4 ± 2.2 |
| Body weight of male pups (g) | | | | |
| on PND 0 | 6.7 ± 0.3 | 6.7 ± 0.6 | 6.4 ± 0.5 | 5.8 ± 0.3** |
| on PND 4 | 10.5 ± 0.5 | 10.1 ± 1.8 | 10.2 ± 1.2 | 8.5 ± 0.7** |
| Body weight of female pups (g) | | | | |
| on PND 0 | 6.4 ± 0.4 | 6.3 ± 0.6 | 6.1 ± 0.6 | 5.6 ± 0.2** |
| on PND 4 | 9.9 ± 0.6 | 9.7 ± 1.7 | 9.5 ± 0.8 | 8.3 ± 0.7** |

Values are given as the mean ± S.D.
**: Significantly different from the control, $p \leqq 0.01$.

dams. In the PFOA oral dose study (Abbott *et al.*, 2007), the reduction of postnatal weight gain appeared to depend on PPARα expression.

The elimination rate of PFOA in female rats is approximately 40 times faster than in male rats (ATSDR, 2009). Organic anion transport proteins play a key role in PFCAs (C4 to C10) renal tubular reabsorption (Han *et al.*, 2012), and the slower elimination of PFOA in male rats compared to female rats has been attributed to sex hormone modulation of organic anion transporters in the kidney (ATSDR, 2009). In the present study, there were slight gender differences in the hepatotoxicity of PFUA: liver weight increased in males at 0.3 mg/kg/day and above and in females at 1.0 mg/kg/day, and histopathological findings observed in the 1.0 mg/kg/day groups were more numerous and severer in males than in females. The gender differences in hepatotoxicity observed in the present study are considered to be attributable to faster elimination in female rats, as with other PFCAs.

Increased liver weight and hepatocellular hypertrophy, induced by activation of PPARα, were generally observed in previous studies on PFAAs. Significant peroxisome proliferative activity seems to require a carbon length more than 7 (ATSDR, 2009). In gavage studies of PFAAs in male rats, which are more sensitive than females, the following results were observed; for PFOA (C8), increased liver weight and hepatocellular hypertrophy at 5 mg/kg/day for 28 days (Cui *et al.*, 2009); for perfluorononanoic acid (C9), increased liver weight at 1 mg/kg/day for 14 days (Fang *et al.*, 2012); for perfluorododecanoic acid (C12), increased liver weight at 0.02 mg/kg/day for 110 days (Ding *et al.*, 2009). In the current study of PFUA (C11), increased liver weight and centrilobular hypertrophy of hepatocytes were observed from 0.3 mg/kg/day for 42 days. In consideration of differences in the administration period or doses in these studies, the intensity of the liver toxicity of PFUA (C11) was estimated to be between C9 and C12, suggesting that the toxic potency of PFAAs (C8-C12) increases by lengthening their carbon chain. This is because hydrophobicity, which increases as carbon length increases, seems to favor biliary enterohepatic recirculation, resulting in more protracted toxicity (ATSDR, 2009). In contrast, 42-day administration of PFOdA (C18) increased liver

weight at 200 mg/kg/day but not at 40 mg/kg/day in male rats (Hirata-Koizumi *et al.*, 2012). In comparison with other PFAAs (C8-C12), including PFUA (C11), PFOdA induced liver toxicity at higher doses, and this may be due to the low absorption of PFOdA into the body.

At 1.0 mg/kg/day in the main group, the following effects on hematological and blood biochemical parameters were observed; a decrease in fibrinogen was observed in males and females, but increases in APTT and PT were not observed, suggesting that there would be no toxicologically significant effects on the blood coagulation system; decreases in fibrinogen, total protein and albumin observed in males and/or females may be due to reduced synthesis in the damaged liver; the increase in BUN observed in males and females could be due to increased hepatic protein catabolism, because urinalysis parameters and the gross and microscopic appearance of the kidneys were not changed; and the increase of ALP in males was related to the histopathological findings in the liver. These effects except for the decrease in fibrinogen in females were observed also at the end of the recovery period, and the increase of ALP was observed in females only after the recovery period. Moreover, in histopathological findings, centrilobular degeneration of hepatocytes in both sexes and Glisson's sheath cell infiltration in females were observed only at the end of the recovery period, and in females, centrilobular hypertrophy of hepatocytes was more serious at the end of the recovery period. These results suggest that the whole body elimination of PFUA in rats, as well as other PFCAs, is slow. There are some reports indicating that PFCAs are secreted in bile and undergo extensive reabsorption from the gastrointestinal tract (Kudo *et al.*, 2001; Vanden Heuvel *et al.*, 1991a, 1991b; reviewed in ATSDR, 2009). In general, PFCAs with longer carbon chains (C4-C10) have a longer half-life (Hirata-Koizumi *et al.*, 2012). Although the elimination half-life of PFUA is unknown, the half-life after intravenous injection of perfluorodecanoic acid (PFDeA, C10) in rats was about 40 to 60 days (Ohmori *et al.*, 2003), It is estimated that the half-life of PFUA is longer than the recovery period, 14 days, and it is reasonable that some effects of PFUA appear after the recovery period. The above findings may be effects of PFUA caused by enterohepatic recirculation, which lasted through the dosing and recovery periods. The decrease in grip strength of the forefoot observed in males and females at 1.0 mg/kg/day in the satellite group was considered a secondary effect related to suppression of body weight gain.

In conclusion, the NOAEL for repeated dose toxicity is considered to be 0.1 mg/kg/day based on the observed centrilobular hypertrophy of hepatocytes in both sexes at 0.3 mg/kg/day, and the NOAEL for reproductive/developmental toxicity is considered to be 0.3 mg/kg/day based on the lowered body weight of pups at birth and body weight gain at 4 days after birth inhibited at 1.0 mg/kg/day.

## ACKNOWLEDGMENTS

This study was undertaken under the Japanese safety programme for existing chemicals funded by the Ministry of Health, Labour and Welfare, Japan, and was supported by a Health and Labour Sciences Research Grant (H22-Kenki-Ippan-006, H25-Kenki-Ippan-007) from the Ministry of Health, Labour and Welfare, Japan.

## REFERENCES

Abbott, B.D., Wolf, C.J., Schmid, J.E., Das, K.P., Zehr, R.D., Helfant, L., Nakayama, S., Lindstrom, A.B., Strynar, M.J. and Lau, C. (2007): Perfluorooctanoic acid-induced developmental toxicity in the mouse is dependent on expression of peroxisome proliferator-activated receptor-alpha. Toxicol. Sci., **98**, 571-581.

ATSDR (2009): Toxicological profile for Perfluoroalkyls (Draft for Public Comment), U.S. Department of health and human services, Public health service, Agency for Toxic Substances and Disease Registry (ATSDR). May 2009.

Butenhoff, J.L., Kennedy, G.L.Jr., Frame, S.R., O'Connor, J.C. and York, R.G. (2004): The reproductive toxicology of ammonium perfluorooctanoate (APFO) in the rat. Toxicology, **196**, 95-116.

Calafat, A.M., Needham, L.L., Kuklenyik, Z., Reidy, J.A., Tully, J.S., Aguilar-Villalobos, M. and Naeher, L.P. (2006): Perfluorinated chemicals in selected residents of the American continent. Chemosphere, **63**, 490-496.

Calafat, A.M., Kuklenyik, Z., Reidy, J.A., Caudill, S.P., Tully, J.S. and Needham, L.L. (2007a): Serum concentrations of 11 polyfluoroalkyl compounds in the U.S. population: Data from the National Health and Nutrition Examination Survey (NHANES) 1999-2000. Environ. Sci. Technol., **41**, 2237-2242.

Calafat, A.M., Wong, L.Y., Kuklenyik, Z., Reidy, J.A. and Needham, L.L. (2007b): Polyfluoroalkyl chemicals in the U.S. population: Data from the National Health and Nutrition Examination Survey (NHANES) 2003-2004 and comparisons with NHANES 1999-2000. Environ. Health Perspect., **115**, 1596-1602.

CHRIP (2013): Chemical Risk Information Platform. Available at: http://www.safe.nite.go.jp/japan/db.html, accessed in March and October 2013, or at: http://www.meti.go.jp/policy/chemical_management/kasinhou/information/volume_index.html.

Cui, L., Zhou, Q.F., Liao, C.Y., Fu, J.J. and Jiang, G.B. (2009): Studies on the toxicological effects of PFOA and PFOS on rats using histological observation and chemical analysis. Arch. Environ. Contam. Toxicol., **56**, 338-349.

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

Domingo, J.L., Ericson-Jogsten, I., Eriksson, U., Martorell, I., Perelló, G., Nadal, M. and van Bavel, B. (2012): Human dietary exposure to perfluoroalkyl substances in Catalonia, Spain. Tem-

poral trend. Food Chem., **135**, 1575-1582.

ECHA (2012): European Chemicals Agency. Member state committee, support document for identification of Henicosafluoroundecanoic Acid as a substance of very high concern because of its vPvB properties. Adopted on 13 December 2012.

Environment Canada (2010): Draft ecological screening assessment report. Long-chain (C9-C20) Perfluorocarboxylic acids, their salts and their precursors. October 2010.

EPA (2013a): U.S. Environmental Protection Agency. Perfluorooctanoic Acid (PFOA) and Fluorinated Telomers. Available at: http://www.epa.gov/opptintr/pfoa/index.html, accessed in Mar 2013.

EPA (2013b): U.S. Environmental Protection Agency. PFOA Stewardship Program Reporting Guidance. Available at: http://www.epa.gov/opptintr/pfoa/pubs/stewardship/pfoaguidance.html#minimize, accessed in Mar 2013.

Fang, X., Zou, S., Zhao, Y., Cui, R., Zhang, W., Hu, J. and Dai, J. (2012): Kupffer cells suppress perfluorononanoic acid-induced hepatic peroxisome proliferator-activated receptor α expression by releasing cytokines. Arch. Toxicol., **86**, 1515-1525.

Han, X., Nabb, D.L., Russell, M.H., Kennedy, G.L. and Rickard, R.W. (2012): Renal elimination of perfluorocarboxylates (PFCAs). Chem. Res. Toxicol., **25**, 35-46.

Hinderliter, P.M., Mylchreest, E., Gannon, S.A., Butenhoff, J.L. and Kennedy, G.L.Jr. (2005): Perfluorooctanoate: Placental and lactational transport pharmacokinetics in rats. Toxicology, **211**,139-148.

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

IARC (1995). Peroxisome Proliferation and its Role in Carcinogenesis. International Agency for Research on Cancer (IARC), Working Group of 7-11 December 1994, Report N° 24, Lyon, France, 11.

Japanese law (2005): Act on Welfare and Management of Animals. Act No.105 of October 1, 1973. As amended up to Act No.68 of June 22, 2005.

Japanese law (2009): Act on the Evaluation of Chemical Substances and Regulation of Their Manufacture, etc. Law number: Act No.117 of 1973. Amendment: Act No.39 of 2009.

Kudo, N., Suzuki, E., Katakura, M., Ohmori, K., Noshiro, R. and Kawashima, Y. (2001): Comparison of the elimination between perfluorinated fatty acids with different carbon chain length in rats. Chem. Biol. Interact., **134**, 203-216.

Kuklenyik, Z., Reich, J.A., Tully, J.S., Needham, L.L. and Calafat, A.M. (2004): Automated solid-phase extraction and measurement of perfluorinated organic acids and amides in human serum and milk. Environ. Sci. Technol., **38**, 3698-3704.

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

Loveless, S.E., Slezak, B., Serex, T., Lewis, J., Mukerji, P., O'Connor, J.C., Donner, E.M., Frame, S.R., Korzeniowski, S.H. and Buck, R.C. (2009): Toxicological evaluation of sodium perfluorohexanoate. Toxicology, **264**, 32-44.

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

MOE, METI and MHLW (2008): Partial amendments of the standard concerning testing laboratories implementing tests for new chemical substances etc. Joint notification by director generals of Environmental Policy Bureau, Japan, Ministry of the Environment (MOE) (Kanpokihatsu No 080704001) and Manufacturing Industries Bureau, Ministry of Economy, Trade and Industry (METI) (Seikyokuhatsu No 2), dated June 30, 2008 and by director general of Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare (MHLW) (Yakusyokuhatsu No 0704001), dated July 4, 2008.

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

Peters, J.M. and Gonzalez, F.J. (2011): Why toxic equivalency factors are not suitable for perfluoroalkyl chemicals. Chem. Res. Toxicol., **24**, 1601-1609.

So, M.K., Yamashita, N., Taniyasu, S., Jiang, Q., Giesy, J.P., Chen, K. and Lam, P.K. (2006): Health risks in infants associated with exposure to perfluorinated compounds in human breast milk from Zhoushan, China. Environ. Sci. Technol., **40**, 2924-2929.

Vanden Heuvel, J.P., Kuslikis, B.I., Shrago, E. and Peterson, R.E. (1991a): Inhibition of long-chain acyl-CoA synthetase by the peroxisome proliferator perfluorodecanoic acid in rat hepatocytes. Biochem. Pharmacol., **42**, 295-302.

Vanden Heuvel, J.P., Kuslikis, B.I., Van Rafelghem, M.J. and Peterson, R.E. (1991b): Disposition of perfluorodecanoic acid in male and female rats. Toxicol. Appl. Pharmacol., **107**, 450-459.

Vestergren, R., Berger, U., Glynn, A. and Cousins, I.T. (2012): Dietary exposure to perfluoroalkyl acids for the Swedish population in 1999, 2005 and 2010. Environ. Int., **49**, 120-127.

Wolf, C.J., Schmid, J.E., Lau, C. and Abbott, B.D. (2012): Activation of mouse and human peroxisome proliferator-activated receptor-alpha (PPARα) by perfluoroalkyl acids (PFAAs): further investigation of C4-C12 compounds. Reprod. Toxicol., **33**, 546-551.