$$\text{Score } X = \log\left(\frac{M}{Q}\right) \quad (2)$$

where M is the quantity of pesticide sold (mg/year), Q is the annual river flow rate (km^3/year).

2.3.2. Score for pesticide degradation and adsorption

Using the diffuse pollution hydrologic model, sensitivity analyses were previously conducted to evaluate the influence of each pesticide property independently and indicated that adsorption and degradation in soil are the most influential properties and water solubility somewhat affects pesticide runoff (Tani et al., 2010). However, the analyses were not aimed to design score tables. For designing score tables, interdependency of pesticide properties needs to be incorporated in sensitivity analyses. In the current sensitivity analyses, interdependency of soil adsorption coefficient and water solubility are incorporated: highly soluble compounds tend to have low soil adsorption coefficients and vice versa. The following regression equation for the relationship between K_{OC}, the organic-carbon-based soil adsorption coefficient (mL/g), and water solubility (S, mg/L) has been proposed to be applicable for most pesticides (Lyman et al., 1990):

$$0.55\log S = -\log K_{OC} + 3.64 \quad (3)$$

Therefore, we considered two parameters [K_{OC} for adsorption and half-life in soil (HLS) for degradation] in our sensitivity analyses, and water solubility was treated as a subparameter represented by the soil adsorption coefficient according to Eq. (3). Finally, in the sensitivity analyses of this study, input values of HLS, which is an index of soil degradation, were varied between 10^{-2} and 10^3 day, and K_{OC}, an index of organic-carbon-based soil adsorption in soil, was varied between 1 and 10^6 mL/g [the ranges covers all pesticides listed in JDWQG and covers almost all pesticides listed in The e-Pesticide Manual (Tomlin, 2006)]). Because the runoff rates of herbicides and fungicides differ owing to differences in application dates (Tani et al., 2010), sensitivity analyses for herbicides and fungicides were conducted separately. Details of the sensitivity analysis procedure are seen in the paper of Tani et al. (2010).

Fig. 1 shows a graph of the results of the fungicide sensitivity analysis for adsorption and degradation in soil of the Sagami River basin; the vertical axis is the dimensionless simulated pesticide concentration (C/M_V), where C is the average simulated concentration (μg/L) for the 4-month period starting with the date of fungicide application, and M_V is a parameter determined by the quantity of the applied fungicide (mg) divided by the total river flow volume (L) for the 4 months starting from the date of application. Another fungicide sensitivity analysis was conducted for Chikugo River basin and a similar result to Fig. 1 was obtained. Because Score X is designed as such that a score increment of 1 corresponds to a 10-fold increase in pesticide concentration, scores for soil adsorption and soil degradation, defined as Score Y (view A in Table 1), were also designed similarly as Eq. (4) after the C/M_V values for the two rivers were averaged geometrically.

$$\text{Score } Y = \log\left(\frac{C}{M_V}\right) \quad (4)$$

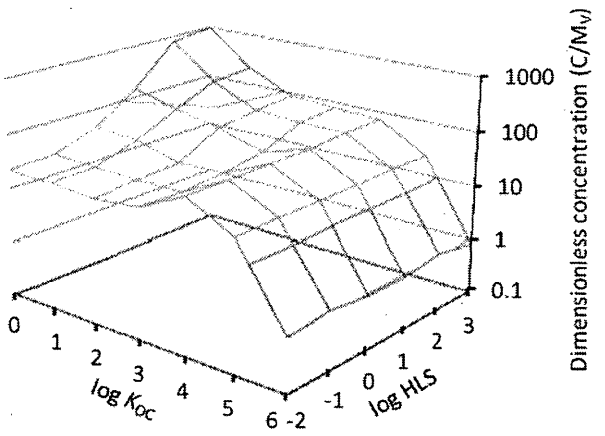

Fig. 1 – Plot of results of sensitivity analysis for adsorption and degradation in soil for fungicides in the Sagami river basin. Dimensionless pesticide concentrations (C/M_V), where C is the average simulated concentration for the 4-month period starting from the date of pesticide application (μg/L), and M_V is a parameter determined by the quantity of the applied fungicide (mg) divided by the total river flow volume (L) for the 4 months starting from the date of application, are plotted against K_{OC} and HLS (half-life with regard to degradation in soil).

Because the concentration is normalized by the quantity of the applied pesticide in Eq. (4), Score Y value is not affected by the quantity of the applied fungicide but it is reflected by HLS and K_{OC} values. A score table for herbicides (view B in Table 1) was determined by means of a process similar to that used for fungicides.

With regard to degradation in water, Tani et al. (2010) reported that pesticide concentrations are influenced and decreased only when the rate constant of degradation in water was large. The sensitivity analyses for degradation in water of the Sagami and Chikugo River basins were conducted in the current study and similar results to Tani et al. (2010) were obtained. Using the results of the sensitivity analyses and Eq. (5), the score tables for values of half-life in water (HLW) were determined in the same way as that of Score Y (Score Z in view C of Table 1).

$$\text{Score } Z = \log\left(\frac{C}{M_V}\right) \quad (5)$$

3. Results and discussion

3.1. Correlation between contamination index and pesticide concentration

Pesticide concentrations observed from 2004 through 2008 in the Mabechi River as well as from 2004 through 2007 in the Chikugo and Sagami Rivers were used for the evaluation of the score tables. Contamination index values, defined as the sum of the scores, were calculated for each of the pesticides used in the Mabechi, Chikugo, and Sagami River basins by means of

Table 1 – New score tables for soil adsorption, soil degradation, and water degradation of fungicides and herbicides. K_{OC} is the organic-carbon-based soil adsorption coefficient (mL/g), and HLS is the half-life (day) with respect to degradation in soil. HLW is the half-life (day) with respect to degradation in water.

A. Values of score Y for fungicides

log HLS	log K_{OC}											
	6.0≥ & >5.5	5.5≥ & >5.0	5.0≥ & >4.5	4.5≥ & >4.0	4.0≥ & >3.5	3.5≥ & >3.0	3.0≥ & >2.5	2.5≥ & >2.0	2.0≥ & >1.5	1.5≥ & >1.0	1.0≥ & >0.5	0.5≥ & >0.0
3.0≥ & >2.5	0.4	1.1	1.5	1.6	1.7	1.8	1.8	1.8	1.9	2.1	2.2	2.5
2.5≥ & >2.0	0.4	1.1	1.5	1.6	1.7	1.7	1.8	1.8	1.9	2.0	2.2	2.5
2.0≥ & >1.5	0.4	1.1	1.5	1.6	1.7	1.7	1.7	1.8	1.8	2.0	2.1	2.3
1.5≥ & >1.0	0.5	1.1	1.5	1.6	1.6	1.6	1.6	1.7	1.8	1.9	2.0	2.1
1.0≥ & >0.5	0.4	1.1	1.5	1.6	1.6	1.5	1.4	1.5	1.6	1.7	1.8	1.9
0.5≥ & > 0.0	0.4	1.1	1.5	1.6	1.6	1.4	1.3	1.3	1.4	1.5	1.6	1.7
0.0≥ & > −0.5	0.4	1.1	1.5	1.6	1.5	1.3	1.2	1.2	1.3	1.4	1.4	1.5
−0.5≥ & >−1.0	0.3	1.0	1.5	1.6	1.5	1.3	1.2	1.1	1.2	1.2	1.3	1.3
−1.0≥ & >−1.5	0.3	1.0	1.5	1.6	1.5	1.3	1.1	1.1	1.1	1.1	1.1	1.2
−1.5≥ & >−2.0	0.4	1.1	1.5	1.6	1.5	1.3	1.1	1.1	1.0	1.1	1.1	1.1

B. Values of score Y for herbicides

log HLS	log K_{OC}											
	6.0≥ & >5.5	5.5≥ & >5.0	5.0≥ & >4.5	4.5≥ & >4.0	4.0≥ & >3.5	3.5≥ & >3.0	3.0≥ & >2.5	2.5≥ & >2.0	2.0≥ & >1.5	1.5≥ & >1.0	1.0≥ & >0.5	0.5≥ & >0.0
3.0≥ & >2.5	0.4	1.1	1.5	1.7	1.7	1.8	1.8	1.8	1.9	2.0	2.1	2.3
2.5≥ & >2.0	0.3	1.1	1.5	1.6	1.7	1.7	1.8	1.8	1.9	2.0	2.1	2.2
2.0≥ & >1.5	0.3	1.1	1.5	1.6	1.7	1.7	1.7	1.7	1.8	1.9	2.0	2.1
1.5≥ & >1.0	0.3	1.0	1.5	1.6	1.6	1.6	1.6	1.7	1.7	1.8	1.9	2.0
1.0≥ & >0.5	0.3	1.0	1.5	1.6	1.6	1.5	1.5	1.5	1.6	1.7	1.7	1.8
0.5≥ & >0.0	0.3	1.0	1.5	1.6	1.5	1.4	1.3	1.4	1.5	1.5	1.6	1.7
0.0≥ & >−0.5	0.3	1.0	1.5	1.5	1.5	1.3	1.3	1.3	1.3	1.4	1.5	1.5
−0.5≥ & >−1.0	0.3	1.0	1.5	1.5	1.5	1.3	1.2	1.2	1.3	1.3	1.3	1.4
−1.0≥ & >−1.5	0.3	1.1	1.5	1.5	1.5	1.3	1.2	1.2	1.2	1.2	1.3	1.3
−1.5≥ & >−2.0	0.3	1.0	1.5	1.6	1.5	1.3	1.2	1.2	1.2	1.2	1.2	1.2

C. Values of score Z for fungicides and herbicides

Log HLW of fungicides	Log HLW of herbicide	Score Z
>1.59	>1.38	3.0
1.59≥ & >0.87	1.38≥ & >0.86	2.9
0.87≥ & >0.55	0.86≥ & >0.62	2.8
0.55≥ & >0.32	0.62≥ & >0.43	2.7
0.32≥ & >0.14	0.43≥ & >0.29	2.6
0.14≥ & >−0.02	0.29≥ & >0.16	2.5
−0.02≥ & >−0.15	0.16≥ & >0.05	2.4
−0.15≥ & >−0.27	0.05≥ & >−0.05	2.3
−0.27≥ & >−0.38	−0.05≥ & >−0.14	2.2
−0.38≥ & >−0.48	−0.14≥ & >−0.23	2.1
−0.48≥ & >−0.57	−0.23≥ & >−0.31	2.0
−0.57≥ & >−0.66	−0.31≥ & >−0.38	1.9
−0.66≥ & >−0.74	−0.38≥ & >−0.45	1.8
−0.74≥ & >−0.82	−0.45≥ & >−0.52	1.7
−0.82≥ & >−0.89	−0.52≥ & >−0.59	1.6
−0.89≥ & >−0.97	−0.59≥ & >−0.65	1.5
−0.97≥ & > −1.03	−0.65≥ & >−0.71	1.4
−1.03≥ & >−1.10	−0.71≥ & >−0.77	1.3
−1.10≥ &>−1.16	−0.77≥ & >−0.83	1.2
−1.16≥ & >−1.22	−0.83≥ & >−0.88	1.1
−1.22≥ & >−1.28	−0.88≥ & >−0.94	1.0
−1.28≥ & >−1.34	−0.94≥ & >−0.99	0.9
−1.34≥ & >−1.39	−0.99≥ & >−1.04	0.8
−1.39≥ &>−1.44	−1.04≥ & >−1.09	0.7
−1.44≥ & >−1.49	−1.09≥ & >−1.14	0.6
−1.49≥ & >−1.55	−1.14≥ & >−1.19	0.5
−1.55≥ & >−1.59	−1.19≥ & >−1.24	0.4
−1.59≥ & >−1.64	−1.24≥ & >−1.28	0.3
−1.64≥ & >−1.69	−1.28≥ & >−1.33	0.2
−1.69≥ & >−1.74	−1.33≥ & >−1.38	0.1
−1.74≥	−1.38≥	0.0

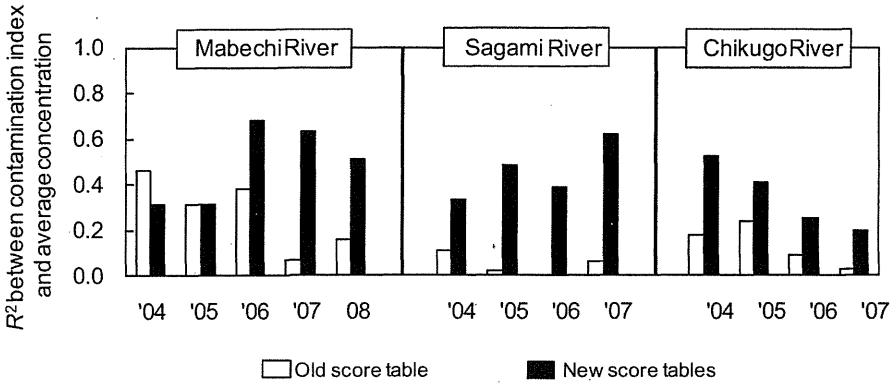

Fig. 2 – Comparison of the coefficients of determination (R^2) for plots of average pesticide concentrations versus contamination index values calculated with the new score tables and the old score table.

the new score tables (Score X was calculated from Eq. (2), and Scores Y and Z were obtained from Table 1) and the old score table (Scores A–C, Table 1S in the supplementary information). Fig. 1S in the supplementary information shows an example of the correlation between the contamination index values and average measured concentrations (sum of the measured concentrations divided by the number of water samples) of pesticides in the Chikugo River in 2004. Higher correlations were obtained with the new score tables than with the old score table. The coefficient of determination R^2 increased from 0.18 to 0.52 when the new score tables were applied (compare views A and B in Fig. 1S). However, the concentrations for any given contamination index value were scattered in a range around the logarithm of 2. The data scattering may have been due to the uncertainty for the application dates and application methods (including drift and adjuvant) for the pesticides; the acquisition of accurate application dates and the accurate evaluation for the effects of application methods were hard (Matsui et al., 2006a) and therefore these factors were not considered in the calculation of the contamination index. It may also be due to the accuracy of Score X values. In evaluating Score X value it is assumed that the quantity of pesticide applied was equal to the amount of the sales in that year. This assumption may, however, not be very accurate because all the purchased pesticides may not necessarily be applied in the agricultural field in a year after the purchase.

Fig. 2 summarizes the R^2 values for the plots of the average concentrations of pesticides against the contamination index values for the three rivers. Overall, the contamination index values calculated with the new score tables were better correlated with pesticide concentrations than the values calculated with the old score table, indicating that the new score tables are effective tools for preliminary ranking or prioritizing pesticides to be monitored.

3.2. *Use of the tables to select pesticides to be monitored*

3.2.1. *Estimating maximum pesticide concentration with the new score tables*

Fig. 3 shows the relationship between the highest observed pesticide concentrations and contamination index values. Since score is defined such that a score increase of 1 corresponds to a 10-fold increase in the pesticide concentration in river water, the relationship between highest observed concentration and contamination index, which is given by a sum of scores, is described, in the ideal situation, by the following equation:

$$\log C_H = \text{Contamination Index} - A \quad (6)$$

where C_H is the highest observed pesticide concentration (μg/L) and A is a constant. The mean A value was 11.6. The A value for the one-sided 95% upper confidence limit was 10.7. Therefore, C_U, the one-sided 95% upper confidence limit of the maximum pesticide concentration (μg/L), is predicted by the following equation:

$$\log C_U = \text{Contamination Index} - 10.7 \quad (7)$$

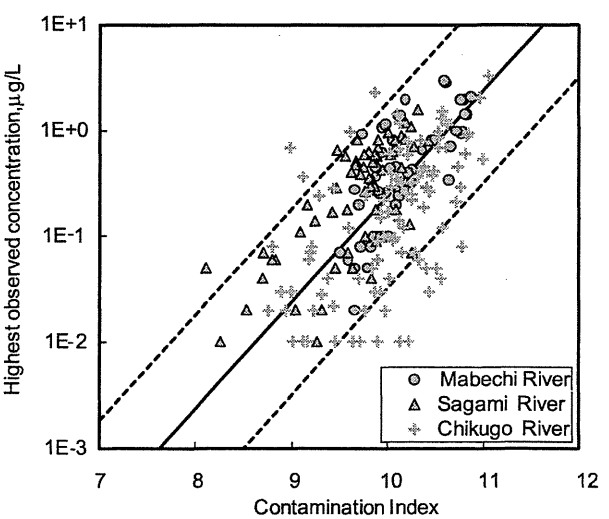

Fig. 3 – Relationship between the highest observed concentrations of pesticides and the contamination index values calculated with the new score tables. The solid line is a plot of the equation log C_H = Contamination index - 11.4. The dashed lines indicate one-sided 95% upper and lower confidence limits.

3.2.2. *Use of possible maximum concentration to select pesticides to be monitored*

JDWQG specifies that *DI* value, defined by Eq. (1), should not exceed 1. However, the calculation of *DI* value still has a potential problem that the value is heavily dependent on the number of monitored pesticides. In the current study, we predicted the maximum possible concentration of pesticide i, PV_i, in river water by using Eq. (7), and we then calculated PV_i/GV_i values. The PV_i/GV_i values were compared with the DV_i/GV_i values for the highest observed concentration.

Fig. 4 shows the DV_i/GV_i and PV_i/GV_i quotients of pesticides applied in the Sagami River basin in 2004. Because the quantification of pesticide concentrations above 1/100 of each GV_i value is recommended in evaluating *DI* value in JDWQG (MHLWJ, 2003b), a PV_i/GV_i quotient of 0.01 can be regarded as a threshold limit in selecting pesticides to be monitored and used for *DI* value calculation. That is, if the PV_i/GV_i value of pesticide *i* is less than 0.01, nonnecessity for monitoring that pesticide is suggested. By applying this threshold limit, we selected 16 pesticides from the 34 pesticides applied in the Sagami River basin for monitoring in the year 2004, as shown in Fig. 4. Among the selected pesticides, seven compounds DV_i/GV_i values exceeded the 0.01 threshold (iprobenfos, molinate, mefenacet, benthiocarb, esprocarb, simetryn, and bromobutide). However, other seven pesticides had DV_i/GV_i values of <0.01 although their PV_i/GV_i values were ≥ 0.01. This is understandable because PV_i is the maximum possible pesticide concentration predicted by the one-sided 95% upper confidence limit, and PV_i can be regarded as a conservative estimate. Two pesticides (diquat monohydrate and cafenstrole) were selected as pesticide candidates, but their concentrations were not actually measured.

The numbers of pesticide candidates for monitoring selected by this method are summarized in Table 2: 16–21 pesticide candidates among the 30–34 in the Mabechi River, 16–18 among the 31–34 in the Sagami River, and 22–25 among the 32–33 in the Chikugo River. The efficiency of the selection method was evaluated by comparison with pesticide monitoring data (columns E–H in Table 2). For all the data in the table, the groups of selected pesticides for which the PV_i/GV_i values were >0.01 included all the pesticide with DV_i/GV_i values of ≥ 0.01. There was no pesticide with a PV_i/GV_i value of <0.01 and a DV_i/GV_i value of ≥ 0.01. As shown in Column H of Table 2, 26–75% of the selected pesticides with PV_i/GV_i values of ≥ 0.01 had DV_i/GV_i values of ≥ 0.01 (the success rate in selecting pesticide candidates for monitoring was 26–75%). We feel these percentages constitute successful first efforts. It is notable that the pesticides with PV_i/GV_i values of <0.01 were all (100% in Column H of Table 2) with DV_i/GV_i values of <0.01 (the success rate in removing pesticides unnecessary for monitoring was 100%).

The three rivers we evaluated in the current study are currently monitored for pesticides selected on the basis of previous experience by the local water supply authorities rather than by means of our proposed method. However, for rivers where pesticides are not monitored or have no basis for determining the necessity of monitoring, our proposed method could save time and expense in identifying monitoring needs. The use of this methodology would also help determining the necessity of monitoring metabolites and degrades of pesticides if reaction pathway and kinetics of pesticide degradation are known and incorporated into the diffuse pollution hydrologic model.

A considerable number of water supply authorities in Japan monitor all or most of the 102 pesticides that are listed in Pesticide Group 1 of JDWQG without conducting any risk assessment to properly select the mentoring pesticides. Under such circumstances, our proposed new score tables and method could assist in the selection of pesticides to be monitored and the determination of which pesticides require no monitoring. No such decision could be reached by means of any other

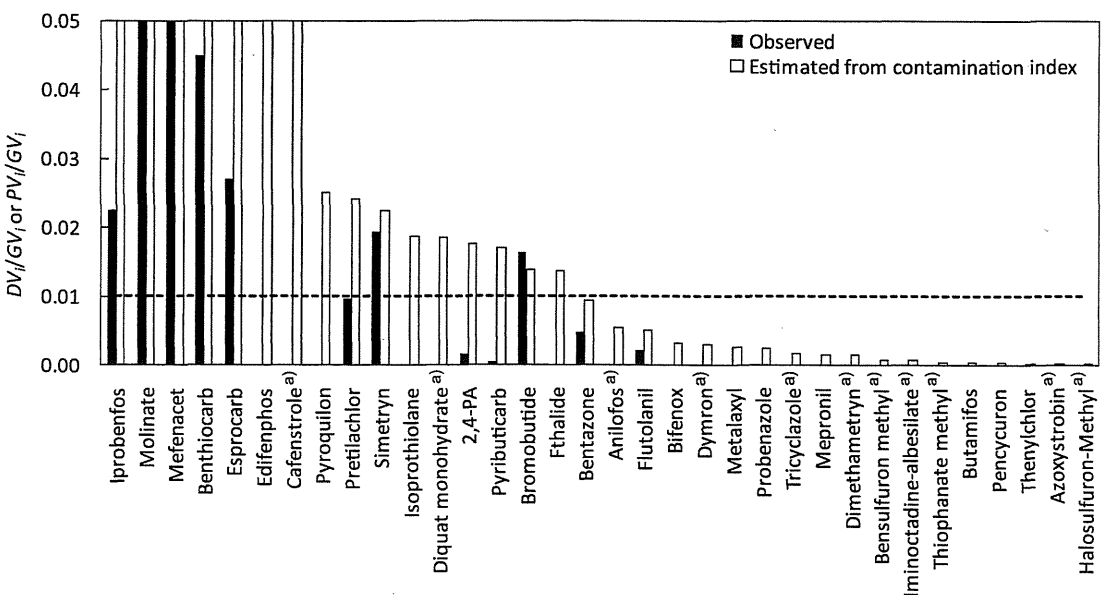

Fig. 4 – PV_i/GV_i (open bars) and DV_i/GV_i (solid bars) values for pesticides in the Sagami river basin in 2004. a) the concentrations were not measured (PV_i is the possible maximum predicted concentrations of pesticide i, GV_i is the reference concentration of pesticide i in JDWQG, and DV_i is the observed concentration of pesticide i).

Table 2 – Performance of new score tables for selecting pesticides (PV_i is the possible maximum predicted concentrations of pesticide i, GV_i is the reference concentration of pesticide i in JDWQG, and DV_i is the observed concentration of pesticide i).

Column A	Column B	Column C	Column D	Column E	Column F	Column G	Column H
	Year		Applied in rice-paddy	With measured concentration	With $DVi/GVi < 0.01$	With $DVi/GVi \geq 0.01$	Success ratio (%)
Mabechi River	2004	Number of pesticides	34	18	13	5	
		With $PV_i/GV_i \geq 0.01$	21	14	9	5	36[a]
		With $PV_i/GV_i < 0.01$	13	4	4	0	100[b]
	2005	Number of pesticides	34	22	17	5	
		With $PV_i/GV_i \geq 0.01$	20	16	11	5	31[a]
		With $PV_i/GV_i < 0.01$	14	6	6	0	100[b]
	2006	Number of pesticides	33	18	12	6	
		With $PV_i/GV_i \geq 0.01$	19	14	8	6	43[a]
		With $PV_i/GV_i < 0.01$	14	4	4	0	100[b]
	2007	Number of pesticides	30	18	12	6	
		With $PV_i/GV_i \geq 0.01$	18	13	7	6	46[a]
		With $PV_i/GV_i < 0.01$	12	5	5	0	100[b]
	2008	Number of pesticides	30	18	9	9	
		With $PV_i/GV_i \geq 0.01$	16	12	3	9	75[a]
		With $PV_i/GV_i < 0.01$	14	6	6	0	100[b]
Sagami River	2004	Number of pesticides	34	23	16	7	
		With $PV_i/GV_i \geq 0.01$	16	14	7	7	50[a]
		With $PV_i/GV_i < 0.01$	18	9	9	0	100[b]
	2005	Number of pesticides	31	26	20	6	
		With $PV_i/GV_i \geq 0.01$	17	15	9	6	40[a]
		With $PV_i/GV_i < 0.01$	14	11	11	0	100[b]
	2006	Number of pesticides	32	30	23	7	
		With $PV_i/GV_i \geq 0.01$	18	17	10	7	41[a]
		With $PV_i/GV_i < 0.01$	14	13	13	0	100[b]
	2007	Number of pesticides	31	28	22	6	
		With $PV_i/GV_i \geq 0.01$	17	16	10	6	38[a]
		With $PV_i/GV_i < 0.01$	14	12	12	0	100[b]
Chikugo River	2004	Number of pesticides	32	23	16	7	
		With $PV_i/GV_i \geq 0.01$	23	20	13	7	35[a]
		With $PV_i/GV_i < 0.01$	9	3	3	0	100[b]
	2005	Number of pesticides	32	29	23	6	
		With $PV_i/GV_i \geq 0.01$	24	23	17	6	26[a]
		With $PV_i/GV_i < 0.01$	8	6	6	0	100[b]
	2006	Number of pesticides	33	29	23	6	
		With $PV_i/GV_i \geq 0.01$	22	21	15	6	29[a]
		With $PV_i/GV_i < 0.01$	11	8	8	0	100[b]
	2007	Number of pesticides	32	28	21	7	
		With $PV_i/GV_i \geq 0.01$	25	22	15	7	32[a]
		With $PV_i/GV_i < 0.01$	7	6	6	0	100[b]

a Number of pesticides with $DV_i/GV_i \geq 0.01$/number of pesticides with $PV_i/GV_i \geq 0.01$.
b Number of pesticides with $DV_i/GV_i < 0.01$/number of pesticides with $PV_i/GV_i < 0.01$.

ranking tool, such as the old score table, which prioritizes pesticides but does not have an absolute criterion for selection. Pesticide compounds with a similar property can be analyzed with the same multi-residue method. The monitoring costs thus do not simply depend on the mere number of compounds to analyze, but on the number of analytical methods to run and the individual costs of these methods. However, the analysis requires the standard solution and the accuracy control of analysis for each compound, and therefore the proper selection of monitoring pesticides has a strong merit.

4. Conclusions

1) New, improved score tables designed for selecting pesticides on the basis of their properties were prepared by restructuring and refinement based on sensitivity analyses conducted with a pesticide diffuse pollution model. The correlations between observed pesticide concentrations and contamination index values calculated with the new score tables were greatly improved over correlations obtained with the old score table.
2) Possible maximum concentrations of pesticides (PV_i) were estimated from the one-sided 95% upper confidence limit for the regression line for the contamination index. The number of pesticide candidates for monitoring selected on the basis of the threshold PV_i/GV_i quotient of 0.01 was roughly two-thirds of the number of pesticides applied. All the pesticides that actually detected in the river waters with DV_i/GV_i quotients larger than 0.01 were included in the list of selected pesticide candidates. The new score tables give contamination index values and then PV_i/GV_i values,

which can be expected to be useful criteria for determining whether or not a pesticide should be monitored on the basis of a threshold value of 0.01.

Acknowledgments

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Appendix. Supplementary data

Supplementary data related to this article can be found online at doi:10.1016/j.watres.2011.11.036.

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Original Article

Repeated dose and reproductive/developmental toxicity of perfluorooctadecanoic acid in rats

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ABSTRACT — Male and female rats were given perfluorooctadecanoic acid (PFOdA) by gavage at 40, 200 or 1,000 mg/kg/day, and each female was mated with a male in the same dose group after 14-day administration. Males were dosed for 42 days and females were dosed throughout the gestation period until day 5 of lactation. One female given 1,000 mg/kg/day was euthanized on day 18 of gestation due to a moribund condition; however, no other treatment-related clinical signs of toxicity were observed. Body weights fell at 1,000 mg/kg/day from day 28 through the administration period in males and throughout gestation and lactation in females. Red blood cell count, hemoglobin level and hematocrit were decreased at 200 and 1,000 mg/kg/day in males and activated partial thromboplastin time was prolonged at 1,000 mg/kg/day in females. Histopathological examination revealed hepatic changes, such as centrilobular hypertrophy and necrosis, in males given 200 and 1,000 mg/kg/day and in females given 1,000 mg/kg/day. Pancreatic zymogen granule was decreased in both sexes at 1,000 mg/kg/day. As for reproductive and developmental toxicity, there were decreases in the number of corpora lutea, implantation, total number of pups born and the number of live pups on postnatal days 0 and 4 at 1,000 mg/kg/day. At this dose, birth weights of pups were decreased and postnatal body weight gain was inhibited. Based on these findings, the NOAEL of PFOdA was considered to be 40 mg/kg/day for repeated dose toxicity and 200 mg/kg/day for reproductive/developmental toxicity.

Key words: Perfluorooctadecanoic acid, Repeated dose toxicity, Reproductive and developmental toxicity, Screening test, Rat

INTRODUCTION

Perfluoroalkyl acids (PFAAs) consist of a carbon chain surrounded by fluorine atoms and a charged functional moiety (primarily carboxylate, sulfonate or phosphonate) at the end of the carbon chain. Since PFAAs have extremely low surface tension and show a unique hydrophobic and oleophobic nature (Lau *et al.*, 2007), they have been widely used as a surface protectant for paper and packaging products, carpets, leather products and textiles (Hekster *et al.*, 2003; Schultz *et al.*, 2003). They have been also used in industrial surfactants, additives and coatings and in firefighting foam. PFAAs are stable in air at high temperatures, nonflammable, not readily degraded by strong acids, alkalis or oxidizing agents and not subject to photolysis (Lau *et al.*, 2007). Such stability of PFAAs must be favorable for their use, but as they are also considered to be stable in the environment (non-biodegradable and persistent) (OECD, 2002, 2007; Schultz *et al.*, 2003), there is growing concern regarding the impact on the environment and on human health via the environment.

The most well-known PFAAs are the 8-carbon chemicals: perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), which are most effective surfactants among PFAAs (Lau *et al.*, 2007). Although the global production volume of PFOS was as high as 3,500 metric tons in 2000 (Lau *et al.*, 2007), the major manufacturer of PFOS, 3M, expressed its intention to discontinue the production of PFOS and related chem-

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icals in 2000 and completed its phase-out in 2002 (3M year not specified; US EPA, 2000). PFOS and related chemicals were listed as a persistent organic pollutant under the Stockholm Convention in 2009 (Stockholm Convention, 2010), and are now regulated in the United States, European Union, Canada and Japan (Canada, 2010; US EPA, 2008b; EU, 2006; MHLW, ME and NITE year not specified). The phase-out of PFOS and related chemicals increased the production volume of PFOA to 1,200 metric tons per year by 2004 (Lau *et al.*, 2007); however, in 2006, the United States Environmental Protection Agency and the eight major producers of fluoropolymer/telomer launched the PFOA Stewardship Program, in which manufacturers gave their pledge to reduce global facility emissions and product content of PFOA and related chemicals by 95% by 2010, and to work toward eliminating emissions and product content by 2015 (US EPA, 2008a). Although PFOS and PFOA have been reported to be detected in the environmental media, wildlife and human tissues in many different geographic locations throughout the world (Lau *et al.*, 2007), these industrial and regulatory activities must reduce their concentrations in the future. Of current concern is the anticipated growth of the production of other PFAAs as alternative products and the subsequent increase in their environmental concentrations.

Many toxicological researches on PFOS or PFOA have been carried out, and their hepatotoxicity, including carcinogenic effects, and reproductive/developmental toxicity have been shown in laboratory animals (ATSDR, 2009; US EPA, 2005; Lau *et al.*, 2007; OECD, 2002). Data on the toxic properties of other PFAAs are limited, but they have been studied in recent years (Chengelis *et al.*, 2009; Das *et al.*, 2008; Fang *et al.*, 2008; Lieder *et al.*, 2009a, 2009b; Mertens *et al.*, 2010; Shi *et al.*, 2007; Stump *et al.*, 2008; van Otterdijk 2007a, 2007b; Zhang *et al.*, 2008). Available data indicate that PFAAs with a longer carbon chain are eliminated more slowly from the body (Chang *et al.*, 2008; Ohmori *et al.*, 2003), and their toxic potency increases by lengthening the carbon chain (Kudo *et al.*, 2000, 2006; Permadi *et al.*, 1992). Since the bioaccumulation potential of PFAAs also increases depending on their carbon number (Martin *et al.*, 2003), long-chain PFAAs may cause serious environmental and/or human health concerns in the future. As no data are available for the toxicity of PFAAs with a carbon chain length 13 and above, toxicological researches are needed urgently. In order to obtain initial risk information on the toxicity of such long-chain PFAAs, the Ministry of Health, Labour and Welfare, Japan, conducted repeated dose and reproductive/developmental toxicity screening tests for several long-chain perflurocarboxylic acids (carbon chain length C12 to C18) under the Japanese existing chemical safety programme in 2009-2011. The present article reports the result of the study of perfluorooctadecanoic acid [PFOdA (C18); CAS No. 16517-11-6] concluded recently.

MATERIALS AND METHODS

This study was conducted in accordance with OECD guideline 422 "Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test" (OECD, 1996) at the Safety Research Institute for Chemical Compounds Co., Ltd. (Sapporo, Japan). All procedures involving the use and care of animals complied with the principles for Good Laboratory Practice (ME, METI and MHLW, 2008) and applicable animal welfare regulations ["Act on Welfare and Management of Animals" (Japanese Animal Welfare Law, 2005) and "Standards Relating to the Care, Management of Laboratory Animals and Relief of Pain" (ME 2006)].

Animals and housing conditions

Crl:CD(SD) rats (8 weeks old) were purchased from Atsugi Breeding Center, Charles River Laboratories Japan, Inc. (Kanagawa, Japan). They were acclimated to the laboratory for 13 days, and then rats found to be in good health were divided into each dose group by stratified random sampling based on body weight. For female animals, vaginal smears were prepared daily to examine the estrous cycle for 9 days before assignment to each group, but no abnormalities were found in any animal.

Throughout the study, animals were maintained in an air-conditioned room with controlled temperature (22 ± 3°C) and humidity (50 ± 20%). Light was provided on a 12-hr light/dark cycle (light: 8:00-20:00). The animals were housed individually, except during acclimation, mating and nursing periods, in metal bracket-type cages with wire-mesh floors. From day 17 of gestation to day 4 after delivery, individual dams and litters were reared using wood chips as bedding (White Flake; Charles River Laboratories Japan, Inc.). All animals were fed *ad libitum* with a standard rat diet (CRF-1; Oriental Yeast Co., Ltd., Tokyo, Japan), and had free access to tap water (Sapporo, Japan).

Chemicals and dosing

PFOdA was purchased from Exfluor Research Corporation (Round Rock, TX, USA). The PFOdA (Lot No. 3654) used in this study was 98.9% pure, and was kept at room temperature in a dark place. The test article was suspended in a 0.5% aqueous solution of carboxymethylcel-

lulose sodium (CMC-Na; Maruishi Pharmaceutical Co., Ltd., Osaka, Japan), and administered to the animals by gastric intubation. Control rats received the vehicle alone. The daily application volume (10 ml/kg body weight) was calculated according to the latest body weight.

Before the start of the study, the stability of PFOdA in a 0.5% CMC-Na aqueous solution at concentrations of 1 and 100 mg/ml was confirmed after 8-hr storage at room temperature following 15-day refrigerated storage; therefore, dosing solutions were prepared at least once per 15 days and kept in a cool (2.0-6.7°C) and dark place under airtight conditions until dosing. The concentrations of PFOdA in the formulations were analyzed at the first and last preparation using high-performance liquid chromatography-tandem mass spectrometry, and were confirmed to be 85.0 to 115% of the target.

Experimental design

Twelve male rats and twelve female rats were daily administered PFOdA at 0 (vehicle control), 40, 200 or 1,000 mg per kilogram of body weight per day (mg/kg/day). The dose levels were determined based on the previous dose-finding study in which male and female rats were given PFOdA by gavage at 0, 30, 100, 300 or 1,000 mg/kg/day for 14 days. In that study, inhibition of body weight gain, grossly enlarged liver with an increase in the organ weight and various changes in blood biochemical parameters were observed in the 1,000 mg/kg/day group. Increased liver weight was also found in males given 300 mg/kg/day, but there were no treatment-related changes in any examined parameter at 30 and 100 mg/kg/day.

After 14-day administration, each female was mated with a male rat in the same dose group, and pregnant females were allowed to deliver spontaneously and nurse their pups. Males were dosed for 42 days, including the mating period, and seven males in the control and 1,000 mg/kg/day groups and all males in the 40 and 200 mg/kg/day groups were necropsied on the day after the last treatment (main group). The remaining 5 males in the control and 1,000 mg/kg/day groups were further maintained for 2 weeks without PFOdA treatment and then necropsied (recovery group). For females, administration of PFOdA was continued throughout the mating and gestation periods until day 5 of lactation (total administration period: 42-56 days), and all twelve females were necropsied on the day after the last treatment (main group). Aside from these animals, 5 females were given 0 or 1,000 mg/kg/day PFOdA for 42 days without mating and were necropsied after the 14-day recovery period (recovery group). The first day of dosing was designated as day 1 of administration and the day after the final dose as day 1 of the recovery period.

Observation and examinations

Repeated dose toxicity data

Throughout the study, all animals were observed twice daily for general appearance and behavior during the administration period (before and after the administration) and during the recovery period (morning and afternoon). In addition, detailed clinical observations were conducted using a standardized scoring system for all of the animals once a week throughout the administration and recovery periods. Evaluations included observations in the home cage, during handling and outside the home cage in an open field.

Body weight was measured on days 1, 3, 5, 7, 10, 14, 21, 28, 35 and 42 of the dosing period and on days 7 and 14 of the recovery period. For females exhibiting evidence of successful mating (main group), body weight was recorded on days 0, 1, 3, 5, 7, 10, 14, 17 and 20 of gestation and on days 0, 1 and 4 of lactation. Food consumption was measured at a similar interval throughout the study, except during cohabitation.

At the end of the administration and recovery period, five males and five females per dose were subjected to the following functional observations: (i) sensory reactivity to visual, tactile, auditory, pain and proprioceptive stimuli was assessed on an examination table, (ii) forelimb and hindlimb grip strength was measured three times with a CPU gauge (Aikoh Engineering Co., Ltd., Osaka, Japan), and (iii) spontaneous motor activity was recorded for 1 hr at intervals of 10 min using an automated activity monitor system [SUPERMEX and CompAct AMS (Muromachi Kikai Co., Ltd., Tokyo, Japan)].

Urinalysis was performed at the end of the administration and recovery periods for five males per dose selected for functional observations and for five females per dose in the recovery group. Urine was collected for 3 hr in the metabolism cage, and examined for color and dipstick parameters, such as occult blood, pH, protein, glucose, ketone bodies, bilirubin and urobilinogen. A 21-hr urine sample was also collected to measure the volume of the urine and the specific gravity.

For hematology and blood biochemistry, blood samples were collected from the abdominal aorta of five males and females per dose each in the main and recovery groups at necropsy under ether anesthesia after overnight starvation. One portion of the blood was treated with ethylenediaminetetraacetic acid dipotassium (EDTA-2K) and examined for the red blood cell count, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin

concentration (MCHC), white blood cell count, platelet count, reticulocyte count and differential count of white blood cells. Prothrombin time (PT) and activated partial thromboplastin time (APTT) were measured using plasma separated from another blood sample treated with 3.8% sodium citrate. Further, serum or plasma prepared from the remaining portions of blood was analyzed for total protein, albumin, protein fraction ratio, glucose, total cholesterol, triglyceride, total bilirubin, urea nitrogen (BUN), creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) γ-glutamyltranspeptidase (γ-GTP), calcium, inorganic phosphorus, sodium, potassium and chloride.

All surviving animals were euthanized by exsanguination under deep ether anesthesia on the day after the final administration (main group) or after completion of the recovery period (recovery group). The external surfaces and cavity of the body and the organs and tissues of the entire body were examined macroscopically. The brain, thymus, heart, liver, spleen, kidney, adrenal gland, testis, epididymis and ovary were isolated and weighed. Further, the spinal cord, pituitary gland, thyroid, esophagus, stomach, pancreas, duodenum, jejunum, ileum, cecum, colon, rectum, trachea, lung (after tracheal infusion fixation), urinary bladder, prostate, seminal vesicle, uterus, eyeball, Harderian gland, mammary gland, femur (including bone marrow), lymph nodes (mesenteric and mandibular), sciatic nerve as well as grossly abnormal tissues were removed. The eyeball and Harderian gland were fixed and preserved with Davidson' fixative solution. The testis and epididymis were fixed with Bouin's solution and preserved in 70% ethanol. The other organs were stored in 10% neutral-buffered formalin. Histopathological evaluations were performed for five males and females in the control and 1,000 mg/kg/day group. All preserved organs were sectioned, stained with hematoxylin–eosin, and examined histopathologically under a light microscope. Since histopathological changes suggestive of fatty changes of the liver and hemosiderin deposit in the liver and spleen were observed, additional specimens of the liver stained with Berlin blue or with oil red O and specimens of the spleen stained with Berlin blue were prepared and examined microscopically to confirm the observed findings. If treatment-related histopathological changes were found, the same tissues from the remaining animals were then examined.

Reproductive/developmental toxicity data

For each female, daily vaginal lavage samples were evaluated for estrous cyclicity from the first day of administration until evidence of copulation was detected in the main group and until the necropsy day in the recovery group. Females having repeated 4-6 day estrous cycles were judged to have normal estrous cycles.

In the main group, each female was transferred to the home cage of a randomly chosen male from the same exposure group on day 14 of administration, and cohabited on a 1:1 basis until successful copulation occurred or the mating period of 2 weeks had elapsed. The presence of sperm in the vaginal smear and/or a vaginal plug was considered as evidence of successful mating, and the day of successful mating was designated as day 0 of gestation. Females that did not mate successfully during the 2-week mating period were cohabited with another male from the same group who had been proven to copulate. Following confirmation of mating, females were returned to their home cages and allowed to deliver spontaneously and nurse their pups. They were checked at least three times daily on days 21-25 of gestation, and the day on which dams held their pups under the abdomen in the nest by 9:00 was designated as day 0 of lactation or postnatal day (PND) 0. Precoital interval and gestational length were recorded, and the copulation index, fertility index and gestation index were computed for each dose group.

On PND 0, all live and dead pups born were counted, and live pups were sexed and examined grossly. They were observed daily for general appearance and behavior, and the body weight of live pups was recorded on PNDs 0, 1 and 4. On PND 4, the pups were euthanized by inhalation of carbon dioxide and subjected to a gross external and internal observation. For the pups with gross abnormalities, the whole body was stored in 10% neutral-buffered formalin. At necropsy of maternal animals, the numbers of corpora lutea and implantation in the uterus were recorded, and the implantation index, delivery index, live birth index and viability index were calculated for each group.

Statistical analysis

Body weight, food consumption, grip strength, spontaneous motor activity, urine volume, hematological and blood biochemical findings, organ weights, length of estrous cycle, precoital interval, the number of corpora lutea and implantation, implantation index, gestational length, the number of pups born and live pups on PNDs 0 and 4 and delivery index were evaluated by Bartlett's test for homogeneity of variances ($p \leq 0.05$). The live birth index, neonatal sex ratio, viability index and body weight of male and female pups were similarly analyzed using the litter as the experimental unit. When homogeneity was recognized, one-way analysis of variance was applied ($p \leq 0.10$). If a significant difference was found, Dunnett's

test was used for pairwise comparisons between control and individual treatment groups ($p \leq 0.01$ or 0.05). Data without homogeneity were subjected to the Kruskal-Wallis test ($p \leq 0.10$), and if significant differences were detected, the Mann-Whitney U test was used to compare PFOdA-treated groups with the control group ($p \leq 0.01$ or 0.05).

For detailed clinical and functional observations, qualitative parameters of urinalysis, specific gravity of urine and histopathological findings with multiple grades, the trend for each group was evaluated by the Kruskal-Wallis test ($p \leq 0.10$). If significant differences were found, the data were compared between the control and each dosage group using Mann-Whitney U test ($p \leq 0.01$ or 0.05). The incidence of females with normal estrous cycles, copulation, fertility and gestation index and histopathological findings with single grade were analyzed using chi-square test or Fisher's exact test ($p \leq 0.01$ or 0.05).

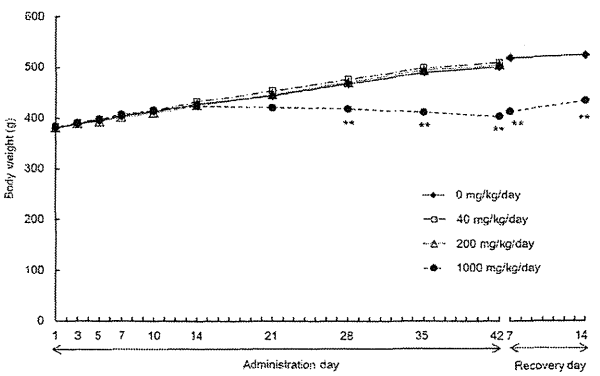

Fig. 1. Body weight changes of male rats dosed orally with PFOdA. **: Significantly different from the control, $p \leq 0.01$.

RESULTS

Clinical and functional observations

One female given 1,000 mg/kg/day in the main group was euthanized on day 18 of gestation due to a moribund condition with bleeding from the vagina, hypothermia, decreased locomotor activity and pale skin. Histopathological examination did not identify the cause of the moribund condition. No treatment-related clinical signs of toxicity were observed in other animals. Detailed clinical observation also revealed no abnormalities during the administration and recovery periods.

In the functional observation, a significant decrease in forelimb grip strength was found at 1,000 mg/kg/day at the end of the administration period (day 4 of lactation) in the female main group (860.4 ± 74.8 g, compared with 1065.2 ± 104.7 g in control, $p \leq 0.05$). Such effects were not observed in males and in the female recovery group at the end of either the administration or recovery period (data not shown). No other treatment-related changes were found in functional observations at the end of the administration and recovery period.

Body weight and food consumption

In males, body weight was significantly lower at 1,000 mg/kg/day than in the control from day 28 through the administration and recovery periods (Fig. 1). Body weight of females showed a significantly lower value at 1,000 mg/kg/day from day 14 of administration through the gestation and lactation periods in the main group and from day 14 through the administration and recovery periods in the recovery group (Fig. 2).

Food consumption was significantly decreased on days 35 and 42 of administration in males given 1,000

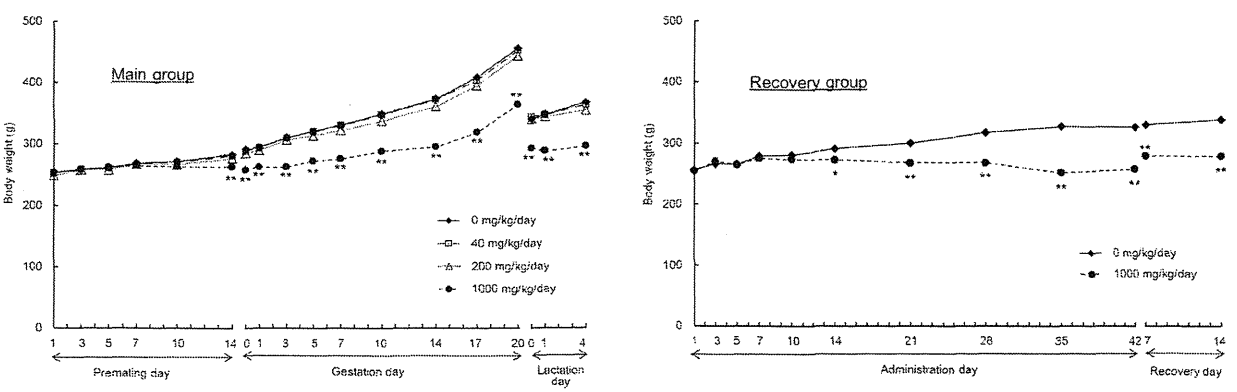

Fig. 2. Body weight changes of female rats dosed orally with PFOdA. *: Significantly different from the control, $p \leq 0.05$, **: Significantly different from the control, $p \leq 0.01$.

mg/kg/day (Fig. 3). In females, a significant decrease in food consumption was found on day 5 of gestation and on day 4 of lactation at 200 mg/kg/day and from day 7 through the administration period, except for days 1 and 20 of gestation at 1,000 mg/kg/day in the main group, and from days 14 to 35 of administration at 1,000 mg/kg/day in the recovery group (Fig. 4). There were no significant changes in food consumption during the recovery period in either sex.

Urinalysis

No significant difference was seen in any urinalysis parameters between the control and PFOdA-treated groups either at the end of the administration period or at the end of the recovery period (data not shown).

Hematology

Red blood cell count, hemoglobin level and hematocrit were significantly decreased in males given 200 mg/kg/day and above at the end of the administration period (Table 1). These changes remained significant at the end of the recovery group. In males given 1,000 mg/kg/day, the reticulocyte ratio was significantly lower at the end of the administration period, but it was significantly higher than that of the control after the recovery period. At the end of the recovery period, APTT was significantly prolonged at 1,000 mg/kg/day in males.

In the female main group, the differential leukocyte count revealed a significant increase in basophils in the 1,000 mg/kg/day group (Table 1). There was also a significant reduction of PT at 200 and 1,000 mg/kg/day and a significant prolongation of APTT at 1,000 mg/kg/day in females in the main group. In the female recovery group, a significant reduction of PT, a significant prolongation of APTT, significant increases in MCHC, total white blood cell count and differential lymphocyte count, and a significant decrease in MCV were found at 1,000 mg/kg/day.

Blood biochemistry

At the completion of the administration period, total protein was significantly lower at 1,000 mg/kg/day than in the control in males (Table 2). In the protein fraction, the albumin ratio was significantly increased at 200 mg/kg/day and above, and there was also a significant decrease in the α_1-globulin ratio and increase in the γ-globulin ratio at 1,000 mg/kg/day in males. Further, significantly higher values in ALT, ALP, total bilirubin and BUN were found at 1,000 mg/kg/day in the male main group. Significant increases in the albumin fraction and ALP and significant decreases in total protein and the α_1-globulin fraction remained after the recovery period in males given 1,000 mg/kg/day. There was also a significant decrease in the

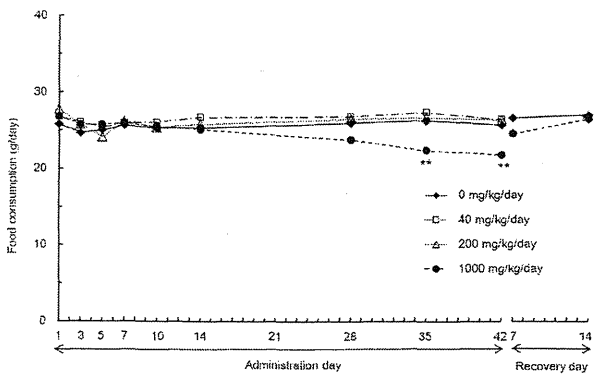

Fig. 3. Food consumption of male rats dosed orally with PFOdA. *: Significantly different from the control, $p \leq 0.05$, **: Significantly different from the control, $p \leq 0.01$.

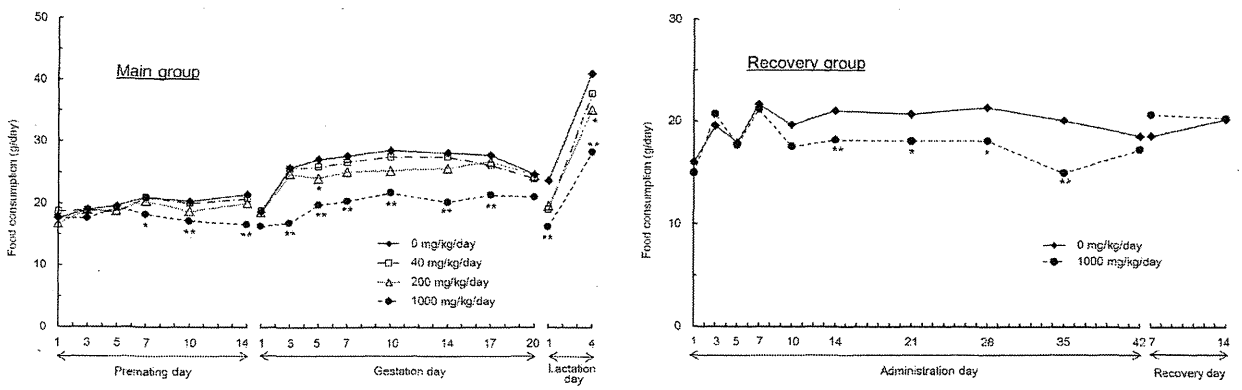

Fig. 4. Food consumption of female rats dosed orally with PFOdA. *: Significantly different from the control, $p \leq 0.05$, **: Significantly different from the control, $p \leq 0.01$.

Table 1. Hematological findings in male and female rats given PFOdA

Dose (mg/kg/day)	Main Group				Recovery Group	
	0 (control)	40	200	1000	0 (control)	1000
MALE						
Number of animals examined	5	5	5	5	5	5
Red blood cells (10^4/μl)	941 ± 26	907 ± 25	865 ± 30**	829 ± 51**	927 ± 27	804 ± 28**
Hemoglobin (g/dl)	16.3 ± 0.6	16.3 ± 0.5	15.2 ± 0.5*	14.7 ± 0.9**	16.1 ± 0.4	13.9 ± 0.7**
Hematocrit (%)	46.0 ± 1.7	46.0 ± 1.6	42.6 ± 1.0*	40.6 ± 2.0**	44.8 ± 1.3	39.4 ± 2.2**
MCV (fl)	48.9 ± 2.0	50.8 ± 2.0	49.3 ± 0.7	49.3 ± 0.7	48.4 ± 1.5	49.0 ± 1.7
MCH (pg)	17.3 ± 0.6	18.0 ± 0.7	17.6 ± 0.3	17.7 ± 0.7	17.4 ± 0.3	17.2 ± 0.4
MCHC (g/dl)	35.5 ± 0.3	35.5 ± 0.4	35.7 ± 0.4	36.1 ± 0.5	36.0 ± 0.6	35.2 ± 0.6
Reticulolocyte (%)	3.29 ± 0.39	2.71 ± 0.51	3.09 ± 0.66	2.24 ± 0.46*	3.35 ± 0.38	4.98 ± 0.80**
Platelet (10^4/μl)	117.3 ± 20.7	99.3 ± 17.3	114.1 ± 14.1	123.8 ± 8.5	120.8 ± 20.4	111.7 ± 6.1
White blood cells (10^2/μl)	94.6 ± 20.4	89.2 ± 16.0	92.8 ± 25.5	120.5 ± 33.6	118.5 ± 25.3	136.0 ± 28.9
Neutrophil (10^2/μl)	15.8 ± 5.2	18.9 ± 8.3	13.6 ± 6.6	20.2 ± 11.6	16.3 ± 4.3	13.9 ± 2.2
Lymphocyte (10^2/μl)	74.0 ± 15.5	66.1 ± 9.9	74.9 ± 18.1	95.3 ± 32.5	95.1 ± 24.3	116.6 ± 27.6
Monocyte (10^2/μl)	3.66 ± 1.84	2.84 ± 0.83	3.00 ± 1.23	3.32 ± 1.85	5.10 ± 0.99	4.22 ± 0.90
Eosinophil (10^2/μl)	1.14 ± 0.71	1.42 ± 0.74	1.22 ± 0.67	1.64 ± 1.21	1.98 ± 1.12	1.24 ± 0.31
Basophil (10^2/μl)	0.02 ± 0.04	0.02 ± 0.04	0.00 ± 0.00	0.06 ± 0.05	0.04 ± 0.05	0.02 ± 0.04
PT (sec)	20.2 ± 3.3	19.9 ± 2.2	20.5 ± 2.6	21.0 ± 2.7	17.8 ± 0.8	19.3 ± 1.8
APTT (sec)	27.7 ± 3.4	26.8 ± 2.7	26.0 ± 2.9	29.2 ± 1.7	25.6 ± 2.0	28.8 ± 1.0*
FEMALE						
Number of animals examined	5	5	5	5	5	5
Red blood cells (10^4/μl)	809 ± 18	815 ± 30	820 ± 25	814 ± 24	846 ± 20	819 ± 60
Hemoglobin (g/dl)	15.0 ± 0.3	15.7 ± 0.5*	15.5 ± 0.4	14.8 ± 0.4	15.4 ± 0.5	14.4 ± 1.0
Hematocrit (%)	42.6 ± 0.8	45.1 ± 1.4*	44.5 ± 1.5	42.0 ± 1.6	43.3 ± 1.7	40.0 ± 2.7
MCV (fl)	52.6 ± 1.7	55.5 ± 2.8	54.4 ± 1.5	51.6 ± 1.5	51.1 ± 1.4	48.9 ± 1.5*
MCH (pg)	18.5 ± 0.6	19.3 ± 0.7	18.9 ± 0.4	18.2 ± 0.4	18.1 ± 0.4	17.6 ± 0.6
MCHC (g/dl)	35.1 ± 0.4	34.9 ± 1.0	34.8 ± 0.4	35.4 ± 0.5	35.5 ± 0.2	35.9 ± 0.2*
Reticulocyte (%)	8.63 ± 1.15	9.35 ± 3.39	8.24 ± 2.08	6.23 ± 0.48	3.23 ± 0.20	3.06 ± 1.52
Platelet (10^4/μl)	141.5 ± 34.1	111.0 ± 13.9	137.6 ± 12.3	135.5 ± 12.7	119.7 ± 11.4	125.4 ± 28.2
White blood cells (10^2/μl)	102.8 ± 17.6	106.6 ± 25.1	99.6 ± 24.9	144.7 ± 53.6	68.0 ± 12.2	91.9 ± 16.1*
Neutrophil (10^2/μl)	23.2 ± 6.6	25.8 ± 12.1	14.1 ± 4.0	27.9 ± 16.3	11.9 ± 2.9	11.2 ± 7.1
Lymphocyte (10^2/μl)	72.0 ± 19.2	71.6 ± 15.2	79.4 ± 21.8	107.5 ± 37.6	52.2 ± 9.0	75.1 ± 12.3*
Monocyte (10^2/μl)	6.42 ± 1.45	7.26 ± 1.09	4.70 ± 1.56	7.10 ± 1.58	2.78 ± 1.01	4.38 ± 3.11
Eosinophil (10^2/μl)	1.22 ± 0.22	1.90 ± 0.73	1.40 ± 0.66	2.02 ± 0.88	1.10 ± 0.44	1.22 ± 0.45
Basophil (10^2/μl)	0.02 ± 0.04	0.02 ± 0.04	0.00 ± 0.00	0.14 ± 0.05*	0.00 ± 0.00	0.06 ± 0.05
PT (sec)	18.2 ± 0.3	17.4 ± 0.8	16.3 ± 0.4**	16.1 ± 0.9**	16.9 ± 0.4	15.1 ± 1.3*
APTT (sec)	19.6 ± 1.0	20.8 ± 1.5	20.1 ± 0.9	22.2 ± 2.20*	20.4 ± 0.9	27.0 ± 4.4**

Values are given as the mean ± S.D.

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

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

Table 2. Blood biochemical findings in male and female rats given PFOdA

Dose (mg/kg/day)	Main Group				Recovery Group	
	0 (control)	40	200	1000	0 (control)	1000
MALES						
Number of animals examined	5	5	5	5	5	5
Total protein (g/dl)	5.68 ± 0.22	5.34 ± 0.29	5.50 ± 0.19	5.10 ± 0.19**	5.68 ± 0.15	5.40 ± 0.21*
Albumin (g/dl)	2.79 ± 0.07	2.75 ± 0.14	2.90 ± 0.08	2.88 ± 0.07	2.81 ± 0.05	3.14 ± 0.16**
Protein fraction (%)						
Albumin	49.2 ± 0.7	51.6 ± 1.6	52.8 ± 2.2*	56.7 ± 2.3**	49.4 ± 2.2	58.1 ± 1.5**
Globulin α_1	23.1 ± 2.5	19.8 ± 2.3	19.6 ± 2.2	13.7 ± 1.3**	21.6 ± 2.7	15.7 ± 1.5**
Globulin α_2	7.24 ± 0.94	7.94 ± 0.98	7.24 ± 0.43	7.24 ± 1.01	7.14 ± 0.46	7.20 ± 0.70
Globulin β	15.8 ± 0.9	16.0 ± 1.5	15.5 ± 1.2	14.5 ± 1.1	17.0 ± 0.6	14.1 ± 0.7**
Globulin γ	4.72 ± 0.58	4.76 ± 0.56	4.84 ± 0.39	7.92 ± 1.85**	4.82 ± 0.59	4.88 ± 0.75
AST (IU/l)	67.4 ± 8.0	73.6 ± 7.3	72.8 ± 6.9	83.4 ± 16.8	67.4 ± 6.9	74.0 ± 6.5
ALT (IU/l)	30.4 ± 3.2	35.4 ± 8.4	35.0 ± 7.1	46.4 ± 9.7*	34.6 ± 3.1	43.0 ± 9.4
ALP (IU/l)	373 ± 46	387 ± 32	475 ± 74	703 ± 90**	325 ± 93	539 ± 112*
γ-GTP (IU/l)	0.70 ± 0.12	0.76 ± 0.17	0.48 ± 0.04**	0.50 ± 0.30	0.56 ± 0.21	0.50 ± 0.19
Total bilirubin (mg/dl)	0.048 ± 0.016	0.042 ± 0.004	0.030 ± 0.007	0.128 ± 0.027**	0.076 ± 0.015	0.070 ± 0.007
Glucose (mg/dl)	153 ± 16	159 ± 26	155 ± 14	139 ± 10	169 ± 11	163 ± 26
Total cholesterol (mg/dl)	48.4 ± 10.7	44.4 ± 15.4	42.2 ± 16.8	55.2 ± 12.2	57.4 ± 9.8	75.8 ± 23.8
Triglyceride (mg/dl)	33.4 ± 23.7	28.2 ± 30.3	26.4 ± 9.8	17.0 ± 5.8	49.6 ± 9.8	16.2 ± 4.3**
BUN (mg/dl)	16.0 ± 1.2	15.7 ± 0.6	15.3 ± 1.2	22.6 ± 1.9**	16.7 ± 1.8	18.7 ± 2.0
FEMALES						
Number of animals examined	5	5	5	5	5	5
Total protein (g/dl)	6.26 ± 0.21	6.02 ± 0.24	6.34 ± 0.35	6.04 ± 0.24	6.92 ± 0.44	5.98 ± 0.79*
Albumin (g/dl)	2.99 ± 0.17	2.97 ± 0.20	3.28 ± 0.29	3.12 ± 0.30	3.87 ± 0.30	3.64 ± 0.38
Protein fraction (%)						
Albumin	47.8 ± 1.6	49.3 ± 1.6	51.6 ± 2.9	51.6 ± 3.9	55.9 ± 1.5	61.1 ± 3.2*
Globulin α_1	21.2 ± 2.0	18.2 ± 2.5	17.7 ± 1.8*	16.4 ± 1.7**	17.4 ± 1.3	12.8 ± 2.5**
Globulin α_2	8.12 ± 0.96	8.32 ± 0.86	7.58 ± 0.71	8.32 ± 1.43	5.96 ± 0.30	6.68 ± 0.86
Globulin β	18.5 ± 0.7	18.9 ± 1.5	18.2 ± 2.3	16.6 ± 1.5	14.3 ± 0.6	12.9 ± 0.7**
Globulin γ	4.40 ± 1.24	5.22 ± 0.83	4.84 ± 1.00	6.98 ± 0.93**	6.42 ± 0.90	6.52 ± 1.69
AST (IU/l)	82.6 ± 23.7	74.4 ± 11.1	81.6 ± 16.3	98.8 ± 36.9	120.2 ± 97.0	124.0 ± 134.3
ALT (IU/l)	29.8 ± 5.3	29.0 ± 2.1	31.2 ± 5.8	35.8 ± 5.8	58.2 ± 52.9	72.4 ± 87.2
ALP (IU/l)	176 ± 24	228 ± 63	209 ± 38	303 ± 97*	151 ± 23	225 ± 116
γ-GTP (IU/l)	0.76 ± 0.19	0.68 ± 0.08	0.64 ± 0.15	0.98 ± 0.22	0.78 ± 0.16	3.28 ± 5.33
Total bilirubin (mg/dl)	0.068 ± 0.011	0.062 ± 0.008	0.058 ± 0.011	0.058 ± 0.008	0.104 ± 0.021	0.340 ± 0.649
Glucose (mg/dl)	158 ± 10	154 ± 19	153 ± 7	144 ± 10	155 ± 11	150 ± 22
Total cholesterol (mg/dl)	65.2 ± 6.5	58.4 ± 10.5	62.4 ± 11.5	49.2 ± 4.8*	72.4 ± 14.6	66.4 ± 14.8
Triglyceride (mg/dl)	29.2 ± 14.6	39.8 ± 17.6	45.6 ± 10.5	21.6 ± 7.4	26.0 ± 21.1	22.8 ± 15.2
BUN (mg/dl)	22.2 ± 2.5	23.2 ± 2.9	21.6 ± 2.3	31.4 ± 3.4**	15.9 ± 2.0	15.5 ± 3.3

Values are given as the mean ± S.D.

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

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

β-globulin fraction and triglyceride, and an increase in the absolute albumin level at 1,000 mg/kg/day in the male recovery group.

In the female main group, no significant change was found in total protein, but in the protein fraction, the α_1-globulin ratio was significantly lower at 200 and 1,000 mg/kg/day, and the γ-globulin ratio was significantly higher at 1,000 mg/kg/day (Table 2). There were also significant increases in ALP and BUN, and a significant decrease in total cholesterol at 1,000 mg/kg/day in the female main group. In the female recovery group, total protein and α_1- and β-globulin fractions were significantly lower and the albumin fraction was significantly higher at 1,000 mg/kg/day than in the control.

Organ weight

In males, the absolute and relative liver weight was significantly higher at 200 mg/kg/day and above at the end of the administration period (Table 3). The absolute weight of the heart, spleen and thymus was decreased significantly in males given 1,000 mg/kg/day, but the relative weights of these organs was not changed significantly. In this 1,000 mg/kg/day group, the relative weights of the brain, kidney and testis were significantly increased without significant changes in the absolute weights. At the end of the recovery period, increased liver weight remained significant at 1,000 mg/kg/day. There was also a significant decrease in the absolute heart weight and increase in the relative brain and kidney weight in males given 1,000 mg/kg/day in the recovery group.

In the female main group, there were significant increases in the absolute liver weight at 1,000 mg/kg/day and in the relative liver weight at 200 mg/kg/day and above (Table 3). In the 1,000 mg/kg/day group, the absolute heart weight was decreased and the relative brain weight was increased significantly. In the female recovery group, significant increases in the absolute and relative liver weight and in the relative kidney weight, and a decrease in the absolute heart weight were found at 1,000 mg/kg/day.

Histopathological findings

Histopathological findings of scheduled-sacrifice animals in each group are shown in Table 4. Centrilobular hypertrophy of hepatocytes was observed in males given 200 mg/kg/day and above and centrilobular degeneration/necrosis of hepatocytes in males given 1,000 mg/kg/day at the end of the administration period. For these hepatic changes, the increase in the incidence was statistically significant. In the male main group, there was also focal necrosis at 200 mg/kg/day and above and hemosiderin deposit (mainly in Kupffer cells) at 1,000 mg/kg/day in the liver, although the incidence was not significantly different from that in the control group. Further, centrilobular fatty change was observed in two males at 200 mg/kg/day, but there was no dose-dependency in the incidence. In the pancreas, the incidence of decreased zymogen granules showed an increasing tendency (not significant) in males given 1,000 mg/kg/day. In the thymus, cortex atrophy was observed in one male in the main group. Centrilobular hypertrophy of hepatocytes, focal necrosis and hemosiderin deposit in the liver and decreased zymogen granules in the pancreas remained after the recovery period in males given 1,000 mg/kg/day. The incidence of centrilobular hepatocyte hypertrophy in the liver and decreased zymogen granules in the pancreas was significantly higher than in the control. A significant increase in the incidence of microgranuloma was also found in the liver in the male recovery group at 1,000 mg/kg/day.

In the female main group, similar histopathological changes were observed in the liver, pancreas and thymus at 1,000 mg/kg/day. The incidence of centrilobular hepatocyte hypertrophy, focal necrosis and microgranuloma in the liver and decreased pancreatic zymogen granules was significantly higher than in the control. In the main group, hemosiderin deposit was observed in the spleen in all females in all dose groups, but the grade was significantly increased at 1,000 mg/kg/day. In addition, periportal fatty change was observed in the control, 40 mg/kg/day and 1,000 mg/kg/day groups, but the incidence did not increase dose-dependently. In the female recovery group, the incidence of centrilobular hypertrophy of hepatocytes and hemosiderin deposit in the liver was significantly higher in the 1,000 mg/kg bw/group.

Reproductive and developmental findings

In the main group, persistent diestrus was found in one female each in the control and 1,000 mg/kg/day group; however, there were no significant deviations in the mean estrous cycle and in the incidence of females with a normal estrous cycle between the control and PFOdA groups either in the main group or in the recovery group (data not shown).

Reproductive performance and developmental findings are summarized in Table 5. During the cohabitation period, copulation was not observed in two males given 1,000 mg/kg/day. In the 1,000 mg/kg/day group, one female with successful copulation was not impregnated, and one pregnant female did not deliver live pups (all pups stillborn). There were, however, no significant differences in the copulation, fertility or gestation index, and the precoital interval or gestation length between the control and

Table 3. Organ weights of male and female rats given PFOdA

Dose (mg/kg/day)		Main Group				Recovery Group	
		0 (control)	40	200	1000	0 (control)	1000
MALES							
Number of animals examined		5	5	5	5	5	5
Brain	(g)	2.15 ± 0.08	2.17 ± 0.09	2.18 ± 0.11	2.14 ± 0.06	2.16 ± 0.11	2.16 ± 0.10
	(%)[a]	0.470 ± 0.024	0.478 ± 0.031	0.466 ± 0.050	0.586 ± 0.038**	0.436 ± 0.030	0.528 ± 0.026**
Thymus	(mg)	297 ± 85	271 ± 84	335 ± 53	178 ± 51*	253 ± 33	274 ± 94
	(10^{-3}%)[a]	64.5 ± 15.1	59.6 ± 17.6	71.6 ± 11.8	48.4 ± 10.0	50.8 ± 6.8	67.2 ± 23.3
Heart	(g)	1.376 ± 0.084	1.398 ± 0.162	1.408 ± 0.187	1.056 ± 0.100**	1.460 ± 0.070	1.164 ± 0.139**
	(%)[a]	0.300 ± 0.019	0.306 ± 0.021	0.298 ± 0.016	0.288 ± 0.018	0.294 ± 0.017	0.284 ± 0.030
Liver	(g)	10.9 ± 1.8	11.3 ± 1.6	15.8 ± 1.8**	18.2 ± 1.2**	11.9 ± 0.3	18.3 ± 2.1**
	(%)[a]	2.36 ± 0.28	2.48 ± 0.25	3.35 ± 0.14**	5.00 ± 0.13**	2.40 ± 0.06	4.46 ± 0.42**
Spleen	(g)	0.738 ± 0.091	0.704 ± 0.059	0.656 ± 0.087	0.580 ± 0.103*	0.698 ± 0.055	0.682 ± 0.094
	(%)[a]	0.160 ± 0.020	0.156 ± 0.005	0.140 ± 0.012	0.160 ± 0.022	0.140 ± 0.010	0.168 ± 0.027
Kidney[b]	(g)	2.96 ± 0.15	2.90 ± 0.22	3.08 ± 0.28	2.94 ± 0.26	2.93 ± 0.10	2.94 ± 0.19
	(%)[a]	0.648 ± 0.047	0.636 ± 0.044	0.660 ± 0.076	0.806 ± 0.043**	0.590 ± 0.019	0.718 ± 0.030**
Testis[b, c]	(g)	3.28 ± 0.38	3.38 ± 0.470	3.320 ± 0.227	3.421 ± 0.251	3.468 ± 0.190	3.270 ± 0.362
	(%)[a]	0.697 ± 0.124	0.702 ± 0.097	0.697 ± 0.071	0.906 ± 0.066**	0.698 ± 0.041	0.802 ± 0.096
FEMALES							
Number of animals examined		5	5	5	5	5	5
Brain	(g)	2.05 ± 0.06	2.08 ± 0.11	2.09 ± 0.06	2.01 ± 0.07	2.04 ± 0.06	2.04 ± 0.11
	(%)[a]	0.652 ± 0.019	0.638 ± 0.039	0.680 ± 0.061	0.738 ± 0.066*	0.640 ± 0.034	0.788 ± 0.106
Thymus	(mg)	268 ± 29	322 ± 31*	305 ± 27	245 ± 34	274 ± 24	250 ± 86
	(10^{-3}%)[a]	85.1 ± 6.3	98.9 ± 14.4	99.3 ± 11.1	90.6 ± 17.1	86.2 ± 11.5	94.5 ± 27.4
Heart	(g)	1.076 ± 0.048	1.104 ± 0.065	1.048 ± 0.094	0.844 ± 0.056**	1.056 ± 0.054	0.890 ± 0.071**
	(%)[a]	0.342 ± 0.016	0.340 ± 0.028	0.340 ± 0.024	0.310 ± 0.016	0.330 ± 0.012	0.344 ± 0.040
Liver	(g)	10.2 ± 0.5	10.6 ± 0.8	11.7 ± 1.0	13.2 ± 1.6**	7.8 ± 0.2	10.3 ± 1.2**
	(%)[a]	3.23 ± 0.16	3.23 ± 0.11	3.80 ± 0.10**	4.82 ± 0.49**	2.4 ± 0.1	3.96 ± 0.53**
Spleen	(g)	0.710 ± 0.056	0.766 ± 0.161	0.666 ± 0.098	0.570 ± 0.045	0.556 ± 0.098	0.494 ± 0.070
	(%)[a]	0.224 ± 0.015	0.232 ± 0.046	0.218 ± 0.024	0.212 ± 0.015	0.174 ± 0.030	0.186 ± 0.018
Kidney[b]	(g)	2.05 ± 0.20	2.04 ± 0.25	2.01 ± 0.28	1.97 ± 0.10	2.00 ± 0.05	1.94 ± 0.10
	(%)[a]	0.650 ± 0.062	0.624 ± 0.061	0.648 ± 0.054	0.724 ± 0.049	0.628 ± 0.030	0.752 ± 0.120**
Ovary[b]	(mg)	113.8 ± 8.0	112.4 ± 9.4	102.0 ± 20.4	105.2 ± 7.9	106.2 ± 12.5	81.0 ± 21.7
	(10^{-3}%) [a]	35.8 ± 1.5	34.3 ± 2.2	32.8 ± 4.4	38.7 ± 4.7	33.3 ± 4.5	30.5 ± 5.0

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 1,000 mg/kg/day and 12 at 40 and 200 mg/kg/day in the main group and 5 at 0 and 1000 mg/kg/day in the recovery group)

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

Table 4. Histopathological findings in male and female rats given PFOdA

	Grade	Main Group				Recovery Group	
		0 [a]	40 [a]	200 [a]	1000 [a]	0 [a]	1000 [a]
MALES							
Number of animals examined		7	12	12	7	5	5
Liver							
Centrilobular hypertrophy of hepatocytes	+	0	0	12]**	0]**	0	4]**
	++	0	0	0	7	0	1
Centrilobular degeneration/necrosis of hepatocytes	+	0	0	0	4]**	0	0
	++	0	0	0	2	0	0
Focal necrosis	+	0	0	1	2	0	2
Microgranuloma	+	5	9	7	6	2	2]*
	++	0	0	0	0	0	3
Hemosiderin deposit	+	0	0	0	4	0	2
Centrilobular fatty change	+	0	0	2	0	0	0
Pancreas							
Decrease in zymogen granule	+	1	0	0	4	0	4]*
	++	0	0	0	0	0	1
Spleen							
Hemosiderin deposit	+	5	8	8	2	5	5
	++	2	4	4	5	0	0
Thymus							
Atrophy of cortex	+	0	0	0	1	0	0
FEMALES							
Number of animals examined		12	12	12	9[b]	5	5
Liver							
Centrilobular hypertrophy of hepatocytes	+	0	0	0	8]**	0	4 *
	++	0	0	0	1	0	0
Focal necrosis	+	0	0	0	4*	0	0
Microgranuloma	+	4	4	5	2]**	1	3
	++	0	0	0	7	0	1
Hemosiderin deposit	+	0	0	0	1	0	5 **
Periportal fatty change	+	3	1	0	2	0	0
	++	0	0	0	0	0	1
Pancreas							
Decrease in zymogen granule	+	0	0	0	8]**	0	1
	++	0	0	0	1	0	2
Spleen							
Hemosiderin deposit	+	11	12	10	3]*	0	0
	++	1	0	2	6	5	5
Thymus							
Atrophy of cortex	+	0	0	0	1	0	1

Values are the number of animals with findings.
Grade: +: slight change, ++: moderate change.
*: Significantly different from the control at $p \leq 0.05$.
**: Significantly different from the control at $p \leq 0.01$.
[a]: Dose (mg/kg/day).
[b]: Among 12 females in the main group, three females each were euthanized on day 18 of gestation because of a moribund condition, on day 26 of gestation because of non-pregnancy and on day 1 of lactation because of abnormal delivery (stillbirth).

PFOdA-treated groups.

The number of corpora lutea and implantation sites was significantly lower at 1,000 mg/kg/day than in the control. No significant changes were found in the implantation index. The total number of pups born and the number of live pups on PNDs 0 and 4 were significantly lower at 1,000 mg/kg/day. There was no significant difference in the delivery, live birth and viability indices

Table 5. Reproductive performance and developmental findings in rats given PFOdA

Dose (mg/kg/day)	0 (control)	40	200	1000
Number of pairs	12	12	12	12
Copulation index (%) [a] Male	100	100	100	83.3
Female	100	100	100	100
Precoital interval (day)[b]	2.8 ± 0.9	2.5 ± 1.2	2.8 ± 0.9	1.9 ± 1.1
Fertility index (%) [c]	100	100	100	91.7
Gestation index (%) [d]	100	100	100	90
Gestation length (days) [b]	22.4 ± 0.5	22.2 ± 0.4	22.2 ± 0.4	22.1 ± 0.3
Number of pregnant females	12	12	12	10
Number of corpora lutea [b]	16.6 ± 1.4	17.2 ± 1.8	15.3 ± 2.0	14.0 ± 2.4**
Number of implantation [b]	16.4 ± 1.3	16.6 ± 1.7	15.0 ± 2.0	14.0 ± 2.4*
Implantation index (%) [b, e]	99.1 ± 2.2	96.8 ± 5.5	98.4 ± 2.8	100.0 ± 0.0
Number of litters	12	12	12	9
Total number of pups born [b]	15.5 ± 1.2	15.3 ± 1.6	14.3 ± 2.1	11.2 ± 3.6**
Delivery index (%) [b, f]	94.6 ± 5.8	92.7 ± 8.2	95.4 ± 4.5	80.4 ± 22.0
Sex ratio of pups born [b, g]	0.524 ± 0.135	0.450 ± 0.092	0.438 ± 0.136	0.410 ± 0.105
Number of live pups on PND 0 [b]	15.5 ± 1.2	15.3 ± 1.6	14.3 ± 2.1	10.0 ± 5.0**
Live birth index (%) [b, h]	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	84.2 ± 32.6
Number of live pups on PND 4 [b]	15.4 ± 1.2	14.8 ± 1.5	13.8 ± 2.3	10.6 ± 3.5**
Viability index (%) [b, i]	99.4 ± 1.9	96.9 ± 5.0	96.1 ± 9.2	94.8 ± 8.7
Male pups				
Body weight (g) on PND 0 [b]	7.03 ± 0.70	6.63 ± 0.57	6.66 ± 0.51	5.39 ± 0.87**
on PND 1 [b]	7.72 ± 0.80	7.22 ± 0.65	7.32 ± 0.61	5.60 ± 0.86**
on PND 4 [b]	11.08 ± 1.24	10.53 ± 0.99	10.73 ± 1.02	7.96 ± 1.19**
Body weight gain (g) on PNDs 0-4 [b]	4.05 ± 0.62	3.90 ± 0.58	4.08 ± 0.88	2.57 ± 0.68**
Female pups				
Body weight (g) on PND 0 [b]	6.63 ± 0.76	6.18 ± 0.48	6.38 ± 0.51	5.11 ± 0.86**
on PND 1 [b]	7.26 ± 0.92	6.78 ± 0.62	6.94 ± 0.58	5.34 ± 0.84**
on PND 4 [b]	10.50 ± 1.31	9.88 ± 1.06	10.12 ± 0.86	7.76 ± 1.17**
Body weight gain (g) on PNDs 0-4 [b]	3.88 ± 0.67	3.70 ± 0.71	3.74 ± 0.63	2.64 ± 0.75**

[a] Copulation index (%) = (no. of animals with successful copulation/no. of animals mated) × 100
[b] Values are given as the mean ± S.D.
[c] Fertility index (%) = (no. of pregnant females/no. of females with successful copulation) × 100
[d] Gestation index (%) = (no. of females that delivered live pups/no. of pregnant females) × 100
[e] Implantation index = (no. of implantation sites/no. of corpora lutea) × 100
[f] Delivery index = (no. of pups born/no. of implantation sites) × 100
[g] Sex ratio of pups born = (no. of male pups born/no. of female pups born)
[h] Live birth index = (no. of live pups on PND 0/no. of pups born) × 100
[i] Viability index = (no. of live pups on PND 4/no. of live pups on PND 0) × 100
*: Significantly different from the control at $p \leq 0.05$
**: Significantly different from the control at $p \leq 0.01$

between the control and PFOdA-treated groups, but these indices indicated a decreasing tendency at 1,000 mg/kg/day. In the 1,000 mg/kg/day group, the body weight of male and female pups was significantly lower than in the control on PNDs 0, 1 and 4. Body weight gain for the 4 days after birth was significantly decreased in males and females given 1,000 mg/kg/day. Gross external and internal observation did not reveal any treatment-related alterations either in pups that survived until PND 4 or pups found dead during the postnatal period (data not shown).

DISCUSSION

PFAAs are known to affect the liver, causing hypertrophy and in some cases necrosis, in rodents (Chengelis *et al.*, 2009; Goldenthal, 1978; Griffith and Long, 1980; Kawashima *et al.*, 1995; Kennedy, 1987; Lieder *et al.*, 2009b; Mertens *et al.*, 2010; van Otterdijk, 2007a, 2007b; Zhang *et al.*, 2008). Hepatocyte hypertrophy was observed at as low as 0.064~0.23 mg/kg/day in a 2-year dietary study of PFOS (Thomford, 2002) and 0.64 mg/kg/day in a 13-week dietary study of PFOA (Perkins *et al.*, 2004) in rats. Such hepatic effects of PFAAs are considered to be exerted via the peroxisome proliferator-activated receptor alpha (PPARα), but other mechanisms have also been suggested (Lau *et al.*, 2007). Kudo *et al.* (2006) reported that intraperitoneal administration of PFAAs with six- to nine-carbon length increased the liver weight and hepatic peroxisomal β-oxidation activity in mice, and the potency was in the order of perfluorononanonic acid (PFNA; C9) ≈ PFOA (C8) > perfluoroheptanoic acid (PFHpA; C7) > perfluorohexanoic acid (PFHxA; C6). In the other study, PFOA (C8) and perfluorodecanoic acid (PFDeA; C10) considerably increased the liver weight, and hepatic peroxisomal palmitoyl- and lauroyl-CoA oxidase activities, but the responses to perfluorobutanoic acid (PFBA; C4) were lower and perfluoroacetic acid (C2) was inactive in male mice fed a diet containing PFAAs (Permadi *et al.*, 1993). These data suggest that the toxic potency of PFAAs increased with lengthening of the carbon chain, at least up to C9. Such carbon chain length dependency is considered to be associated with the difference in the elimination rate of PFAAs: the elimination half-life was reported to be 6.38 hr for PFBA (C4), 2.4 hr for PFHpA (C7), 135-185 hr for PFOA (C8), 710 hr for PFNA (C9) and 958 hr for PFDeA (C10) in male rats administered intravenously with PFAAs (Chang *et al.*, 2008; Kemper 2003; Kudo *et al.*, 2002; Ohmori *et al.*, 2003).

The present study demonstrated that the major target of the toxicity of PFOdA (C18) was the liver as with the other PFAAs. Centrilobular hepatocyte hypertrophy and necrosis were observed in males given 200 and 1,000 mg/kg/day and in females given 1,000 mg/kg/day. Increased incidence of microgranuloma was considered to be a reparative response to the hepatic injury. These histopathological changes were accompanied with increased liver weight and leaking out of hepatic enzymes. Such hepatic changes were not observed in the 40 mg/kg/day group, indicating that the toxic potency of PFOdA was unexpectedly low. Differences in the toxic potency of PFOdA and the other PFAAs with shorter carbon chain might come from differences in the elimination rate from the body, as suggested for the other PFAAs. However, since hepatic effects of PFOdA as well as other toxic effects observed at the end of the administration period were detected even after the 14-day recovery period in the present study, the elimination rate of PFOdA was considered to be slow as with PFOA, PFNA and PFDeA (Kemper, 2003; Kudo *et al.*, 2002; Ohmori *et al.*, 2003). Intestinal absorption or distribution in the body might differ between PFOdA and other PFAAs. Other possible factors included toxicodynamic determinants, such as PPARα agonistic activity. In the present study, hepatocyte hypertrophy was not accompanied with eosinophilic granular cytoplasm, which is a characteristic change observed in rodents administered with peroxisome proliferators. In addition, no clear effects on lipid metabolism were found in blood biochemical examination, except for decreased serum cholesterol observed in females given 1,000 mg/kg/day. Therefore, the peroxisome proliferative activity of PFOdA was considered weak, if any, and there is the possibility that a different mechanism from peroxisome proliferation is involved in the hepatotoxicity of PFOdA. Further study is needed to clarify the cause of the difference in the toxic potency of PFOdA and the other PFAAs.

In the present study, one female given 1,000 mg/kg/day was found moribund on day 18 of gestation. General appearance and behavior suggest that the moribund state might be associated with anemia caused by bleeding from vagina. Although hematological examination was not performed for this animal, the effect of PFOdA on blood coagulation (discussed below) might be involved in the vaginal bleeding during late pregnancy. In other animals, no clinical signs of toxicity were found. In the female main group, forelimb grip strength was reduced on day 4 of lactation at 1,000 mg/kg/day. No such effect was detected at the end of the 42-day administration in males and in the female recovery group. This result might be due to the higher maternal body burden of PFOdA during pregnancy in comparison to males and non-pregnant females in the same dose group, because the dose level for pregnant females was determined based on the weight