

## Seaweed Accelerates the Excretion of Dioxin Stored in Rats

KUNIMASA MORITA<sup>†</sup> AND TAKAHISA NAKANO<sup>\*,‡</sup>Fukuoka Institute of Health and Environmental Sciences, 39 Mukaizano, Dazaifu City,  
Fukuoka 818-0135, Japan, and Riken Vitamin Company Ltd., 2-9-18 Misaki-cho, Chiyoda-ku,  
Tokyo 101-8370, Japan

To prevent health problems of humans exposed to dioxin, it is important to enhance the fecal excretion of dioxin stored in the body. The effects of seaweed such as wakame, hiziki, and kombu on the gastrointestinal absorption and reabsorption of 17 types of polychlorinated dibenzo-*p*-dioxin (PCDD) and polychlorinated dibenzofuran (PCDF) congeners was investigated in Wistar rats. Rats were fed 4 g of the basal diet or a seaweed diet containing PCDD and PCDF standard solution [233 ng of toxic equivalents (TEQ)/kg of body weight] once during the experiment period. In the group fed the 10% wakame diet, the levels of fecal excretion of PCDD and PCDF congeners were higher ( $p < 0.01$ ) from days 1 to 5 by 2.8-fold for 2,3,7,8-TCDD, by 4.0-fold for 1,2,3,7,8-pentaCDD, by 3.4-fold for 1,2,3,4,7,8-hexaCDD, by 3.2-fold for 1,2,3,6,7,8-hexaCDD, by 2.5-fold for 1,2,3,7,8,9-hexaCDD, by 1.7-fold for 1,2,3,4,6,7,8-heptaCDD, by 1.1-fold for 1,2,3,4,6,7,8,9-octaCDD, by 3.0-fold for 2,3,7,8-tetraCDF, by 3.7-fold for 1,2,3,7,8-pentaCDF, by 3.7-fold for 2,3,4,7,8-pentaCDF, by 3.2-fold for 1,2,3,4,7,8-hexaCDF, by 3.0-fold for 1,2,3,6,7,8-hexaCDF, by 3.2-fold for 1,2,3,7,8,9-hexaCDF, by 2.9-fold for 2,3,4,6,7,8-hexaCDF, by 1.6-fold for 1,2,3,4,6,7,8-heptaCDF, by 2.2-fold for 1,2,3,4,7,8,9-heptaCDF, and by 1.2-fold for 1,2,3,4,6,7,8,9-octaCDF than those of the basal group, respectively. Rats were fed 4 g of the basal diet containing PCDD and PCDF standard solution (2991 ng of TEQ/kg of body weight) once on day 1 and then placed on the basal diet for 7 days. After 1 week, the rats were fed either the basal diet or seaweed diet from days 8 to 35. In the group fed the 10% wakame diet, the levels of fecal excretion of PCDD and PCDF congeners were higher ( $p < 0.01$  or  $p < 0.05$ ) during the period from days 8 to 35 by 1.7-fold for 2,3,7,8-TCDD, by 1.8-fold for 1,2,3,7,8-pentaCDD, by 2.0-fold for 1,2,3,4,7,8-hexaCDD, by 1.9-fold for 1,2,3,6,7,8-hexaCDD, by 1.6-fold for 1,2,3,7,8,9-hexaCDD, by 1.5-fold for 1,2,3,4,6,7,8-heptaCDD, by 2.0-fold for 2,3,4,7,8-pentaCDF, by 2.1-fold for 1,2,3,4,7,8-hexaCDF, by 1.9-fold for 1,2,3,6,7,8-hexaCDF, by 1.7-fold for 2,3,4,6,7,8-hexaCDF, by 1.5-fold for 1,2,3,4,6,7,8-heptaCDF, and by 1.9-fold for 1,2,3,4,7,8,9-heptaCDF than those of the basal group, respectively. These findings suggest that the administration of seaweed such as wakame is efficient in preventing the absorption and reabsorption of dioxin from the gastrointestinal tract and might be useful in treatment of humans exposed to dioxin.

**KEYWORDS:** Dioxin; polychlorinated dibenzo-*p*-dioxin; polychlorinated dibenzofuran; seaweed; rats; wakame; hiziki; kombu

## INTRODUCTION

Dioxin is the common name for polychlorinated dibenzo-*p*-dioxin (PCDD) and polychlorinated dibenzofuran (PCDF). The main route of human contamination by dioxins seems to be through foods (1–5).

In many countries, intake of PCDD and PCDF via foods is comparable at levels of ~2 pg of toxic equivalents (TEQ)/kg of body weight per day (5). In Japan, PCDD and PCDF intake has been reduced from 3.75 pg of TEQ/kg of body weight per

day in 1977 to 0.92 pg of TEQ/kg of body weight per day in 1998. PCDD and PCDF levels in breast milk have also reduced (6). In Germany, the amount of PCDD and PCDF excreted from the body (98 pg of TEQ/day) has been shown to exceed the intake (49 pg of TEQ/day) (7). Blood PCDD and PCDF levels were reported to be 42.7 pg of TEQ/g of lipid in 1991 and have been shown to be reduced to 20.7 pg of TEQ/g of lipid in 1996 (8).

Because of the lipophilic nature of PCDD and PCDF, they are absorbed from the digestive tract, transferred thereafter to various tissues from the lymphatic system via blood flow (9), and finally stored in adipose tissues and liver in abundance. In studies of dioxins excretion in human and experimental animals, PCDD and PCDF in which the 2-, 3-, 7-, and 8-positions are

\* Author to whom correspondence should be addressed (telephone +81-3-5275-5118; fax +81-3-3237-1087; e-mail tak\_nakano@rike-vita.co.jp).

<sup>†</sup> Fukuoka Institute of Health and Environmental Sciences.

<sup>‡</sup> Riken Vitamin Co. Ltd.

**Table 1.** Composition of the Experimental Diets

component	diet (g/100 g)					
	basal	2% wakame	10% wakame	2% hiziki	10% hiziki	10% kombu
sucrose	65	63	55	63	55	55
cellulose	5	5	5	5	5	5
casein	20	20	20	20	20	20
corn oil	5	5	5	5	5	5
mineral mixture	4	4	4	4	4	4
vitamin mixture	0.85	0.85	0.85	0.85	0.85	0.85
choline	0.15	0.15	0.15	0.15	0.15	0.15
seaweed	0	2	10	2	10	10

substituted with chlorine show little metabolism and therefore persistence in tissues. The PCDD, PCDF, and polychlorinated biphenyl (PCB) stored in the body are eliminated from the walls of the gastrointestinal tract into the gastrointestinal tract, which results in fecal excretion (10–12).

The biological half-life of 2,3,7,8-tetrachlorodibenzo-*p*-dioxine (TCDD) in rats has been reported to be 31 days (13), with that of 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (pentaCDD) 29.5 days (14) and that of 2,3,4,7,8-pentachlorodibenzofuran (pentaCDF) 64 days (15).

In humans, the biological half-life of TCDD has been reported to be 5.8–9.7 years (16, 17). The biological half-life of TCDD in soldiers in Vietnam was shown to be 11.3 years (18). The half-lives of TCDD, 1,2,3,7,8-pentaCDD, and 2,3,4,7,8-pentaCDF in the workers in a herbicide plant were 7.2, 15.7, and 19.6 years, respectively (19). Furthermore, it has been reported that the half-life of 2,3,4,7,8-pentaCDF in Yusho patients was 13.4 years (20). The elimination of PCDD and PCDF in humans is considerably slower than in rats (21).

It may be possible that a diet that inhibits the absorption of PCDD and PCDF in foods from the digestive tract may prevent the occurrence of health disorders due to PCDD and PCDF in humans. It is important for those who were exposed to PCDD and PCDF to find a method to accelerate the fecal excretion of PCDD and PCDF as well as to reduce their storage in the body. We have so far reported that chlorophyll, dietary fiber, chlorella, and green vegetables show activity that promotes the fecal excretion of PCDD and PCDF (22–27).

Seaweeds are traditional popular foods in Japan. Among the seaweeds, wakame (*Undaria pinnatifida*), hiziki (*Hizikia fusiformis*), and kombu (*Laminaria japonica*) are widely eaten brown seaweeds. They are daily cooked and served in salads, as additives to soup, and as boiled foods. Seaweeds are known to contain large quantities of soluble dietary fiber. Brown seaweeds are rich, in particular, in the polysaccharide group, alginates, having many physiological effects on blood pressure, serum lipids, blood glucose, and insulin.

This study was conducted to elucidate the effect of seaweed on the intestinal absorption and reabsorption of PCDD and PCDF congeners. We investigated the fecal excretion of PCDD and PCDF congeners in rats fed a mixture of diet with dioxin.

## MATERIALS AND METHODS

**Animals.** Male Wister rats were purchased from Seac Yoshitomi Co. Ltd. (Fukuoka, Japan) and kept in the animal facility of the Fukuoka Institute of Health and Environmental Sciences. Rats were raised in metabolic cages with constant humidity and exposed to a 12/12 h light/dark cycle. Water and feed were consumed ad libitum. Rats received the experimental diets shown in **Table 1**. The mineral and vitamin mixtures were purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). Animal care and use conformed to NIH published guidelines.

**Table 2.** Concentrations of PCDD and PCDF Congeners in Dioxin Mixture Dissolved in Corn Oil

dioxin	concn (μg/L)	
	A	B
2,3,7,8-TCDD	50	500
1,2,3,7,8-pentaCDD	50	500
1,2,3,4,7,8-hexaCDD	50	500
1,2,3,6,7,8-hexaCDD	50	500
1,2,3,7,8,9-hexaCDD	50	500
1,2,3,4,6,7,8-heptaCDD	50	500
octaCDD	100	1000
2,3,7,8-TCDF	50	500
1,2,3,7,8-pentaCDF	50	500
2,3,4,7,8-pentaCDF	50	500
1,2,3,4,7,8-hexaCDF	50	500
1,2,3,6,7,8-hexaCDF	50	500
1,2,3,7,8,9-hexaCDF	50	500
2,3,4,6,7,8-hexaCDF	50	500
1,2,3,4,6,7,8-heptaCDF	50	500
1,2,3,4,7,8,9-heptaCDF	50	500
octaCDF	100	1000
total TEQ	169.02	1690.2

**Samples and Reagents.** Wakame (*Undaria pinnatifida*), hiziki (*Hizikia fusiformis*), and kombu (*Laminaria japonica*) were gifts from Riken Vitamin Co., Ltd. (Tokyo, Japan). Native PCDD and PCDF (7 PCDD and 10 PCDF) standard solutions (Wellington Laboratories, Guelph, ON, Canada) were dissolved in corn oil, and then two kinds of solutions (A and B) were prepared as shown in **Table 2**. <sup>13</sup>C-labeled PCDD and PCDF standard solutions (Wellington Laboratories) dissolved in *n*-nonane was used as the internal standard for quantitative analysis of dioxins.

Hexane, acetone, chloroform, methanol, dichloromethane, and Florisil were purchased from Wako Pure Chemical Industries Co., Ltd. (Osaka, Japan). These reagents were of pesticide grade. All other reagents were of analytical grade. Silica gel of silver nitrate was prepared as follows: 10 g of silver nitrate was dissolved in 5 mL of H<sub>2</sub>O by heating; Kieselgel 60 (85 g, 70–230 mesh; Merck & Co., Inc., Darmstadt, Germany) was added to the silver nitrate solution, and the mixture was stirred and left standing overnight.

**Experiment for the Suppression of Dioxin Absorption and Reabsorption from the Gastrointestinal Tract by Seaweed Administration.** To examine the effect of seaweed administration on fecal excretion of PCDD and PCDF congeners, dioxins were orally administered to Wister rats in two experiments. In experiment 1, after 5 days of acclimation period, rats were randomly divided into six groups (*n* = 4). After overnight food deprivation, rats (mean body weight = 145 g) were given 4 g of the basal diet or 4 g of the seaweed-supplemented diet once on day 1, in which each serving contained 0.2 mL of dioxin mixture (**Table 1** and solution A of **Table 2**). Rats of the six groups were given their respective dioxin-free experimental diets for 5 days. The dose of dioxin mixture in experiment 1 was 233 ng of TEQ (28)/kg/day. In experiment 2, after 5 days acclimation period, rats were randomly divided into five groups (*n* = 4). After overnight food deprivation, rats (mean body weight = 113 g) were given 4 g of the basal diet once on day 1, in which each serving contained 0.2 mL of dioxin mixture (**Table 1** and solution B of **Table 2**). Rats of the six groups were given their respective dioxin-free basal diets for 7 days. Then rats were fed the basal diet or the seaweed-supplemented diet from day 8 to day 35. The dose of dioxin mixture in experiment 2 was 2991 ng of TEQ/kg/day. In both experiments 1 and 2, rats were housed individually in metabolic cages designed for the separate collection of feces and urine. Body weight, food intake, and fecal weight were measured. Feces were dried overnight at 70 °C, and fecal weight was measured. After being fed the respective diets for the experimental period, rats were anesthetized with ether and the whole bodies of the rats were homogenized with a vertical cutter mixer (R-3 plus; FMI Co., Osaka Japan). The fresh homogenates were stored at –20 °C until use for analysis of dioxin.

**Analysis of Dioxin.** Fecal samples from each rat were homogenized and quantitatively extracted with 150 mL of chloroform/methanol (2:1, v/v) in a cylindrical glass-fiber filter in a Soxhlet extractor. The individual extract of each sample was concentrated to ~5 mL by evaporation and then diluted with chloroform to a final volume of 50 mL. To analyze the dioxin level in each fecal sample, we put 10–30 mL of extract into a test tube (50 mL); the sample was concentrated and dried. After the addition of 200 pg of stable isotope tracer,  $^{13}\text{C}$ -labeled internal standard of tetra–hepta CDDs and CDFs (Wellington Laboratories), and/or 1000 pg of  $^{13}\text{C}$ -labeled internal standard of octaCDDs and octaCDFs (Wellington Laboratories), we added 10 mL of 1 M KOH in ethanol to each sample; the sample was then hydrolyzed overnight at room temperature. The alkaline hydrolysates of each sample were shaken with 10 mL of hexane plus 5 mL of  $\text{H}_2\text{O}$  and centrifuged at 2500 rpm. The hexane layer was collected. The aqueous layer was extracted two times with 10 mL of hexane. The collected hexane layer was washed with 5 mL of  $\text{H}_2\text{O}$  and concentrated to 20 mL. The hexane extract was washed four times with 10 mL of  $\text{H}_2\text{SO}_4$  and then concentrated to 2 mL. The hexane extract was applied to a 0.8 g silver nitrate column (7 mm diameter) and eluted from the column with 8 mL of hexane, and then the eluate was concentrated to 1 mL. The concentration was applied to a 0.6 g Florisil column (7 mm diameter), and dioxin was eluted with 4 mL of hexane, followed by 8 mL of dichloromethane. The eluates from the column were dried and dissolved in 50  $\mu\text{L}$  of *n*-nonane. We measured the levels of PCDD and PCDF congeners in these samples.

Approximately 10 g of homogenate from the whole body of each rat was put into a test tube (50 mL) for centrifugation; we then added 200 pg of stable isotope tracer,  $^{13}\text{C}$ -labeled internal standard of tetra–hepta CDFs, and/or 1000 pg of  $^{13}\text{C}$ -labeled internal standard of octaCDDs and octaCDFs to the homogenate. We added 10 mL of 1.5 M KOH in ethanol to each sample, and the sample was then hydrolyzed overnight at room temperature. The subsequent procedure was the same as that for the dioxin analysis of fecal samples.

Dioxin analysis was performed using gas chromatography–mass spectrometry (GC-MS; AutoSpec-Ultima, Micromass Ltd., Manchester, U.K.) with a capillary column (0.25 mm  $\times$  60 m, BPX5; SGE Co., Yokohama Japan) and setting the resolution mode at 10000; quantitation was performed in the selected ion monitoring acquisition mode.

**Statistical Analysis.** All values were expressed as means  $\pm$  SD. Student's *t* test was used to check differences of dioxin levels between seaweed and control groups. Statistical significance was considered to be at  $p < 0.05$ . Statistical analyses were performed using the computer program SPSS (SPSS Inc., Chicago, IL).

## RESULTS

**Effect of Seaweeds on Food Consumption, Body Weight, and Fecal Amount.** There was no statistically significant difference in food consumption and body weight gain after administration of the dioxin mixture between the basal diet group and the seaweed group in experiments 1 and 2. The fecal weight of the seaweed food was increased compared by statistically significant differences with the basal diet except for the 2% wakame food with (Tables 3 and 4).

**Effect of Seaweeds on Fecal Excretion and Accumulation in the Body of PCDD and PCDF Congeners (Experiment 1).** Table 5 shows the fecal excretion of the PCDD and PCDF congeners from day 1 to day 5 in rats administered the dioxin mixture.

In the group fed the 10% wakame diet, the levels of fecal excretion of 17 types of PCDD and PCDF congeners were higher ( $p < 0.01$ ) than in the basal group. The increases ranged from 1.2-fold for 1,2,3,4,6,7,8,9-octachlorodibenzofuran (octaCDF) to 4.0-fold for 1,2,3,7,8-pentaCDD.

In the groups fed the 10% hiziki and kombu diets, the levels of fecal excretion of TCDD, 1,2,3,7,8-pentaCDD, and 2,3,4,7,8-pentaCDF, which are highly toxic, increased 1.8–2.8-fold, 3.0–

**Table 3.** Effect of the Seaweed Diet on Food Intake, Body Weight Gain, and Feces Weight during a Period from Day 1 to Day 5 in Rats Administered the Dioxin Mixture (Experiment 1)<sup>a</sup>

diet	food intake (g/5 days)	body wt gain (g/5 days)	feces wt (g/5 days)
basal	98.2 $\pm$ 5.6	54.4 $\pm$ 3.4	5.0 $\pm$ 0.6
2% wakame	97.7 $\pm$ 4.4	54.9 $\pm$ 2.1	5.3 $\pm$ 0.5
10% wakame	100.7 $\pm$ 3.0	57.3 $\pm$ 5.8	10.9 $\pm$ 0.5**
2% hiziki	104.2 $\pm$ 0.5	55.7 $\pm$ 5.1	6.6 $\pm$ 0.5**
10% hiziki	101.6 $\pm$ 2.8	54.4 $\pm$ 1.2	12.7 $\pm$ 0.5**
10% kombu	99.1 $\pm$ 3.5	57.0 $\pm$ 7.8	13.2 $\pm$ 1.0**

<sup>a</sup> Values represent the mean  $\pm$  SD ( $n = 4$ ). \*\*, significantly different from the basal diet group ( $p < 0.01$ ).

**Table 4.** Effect of the Seaweed Diet on Food Intake, Body Weight Gain, and Feces Weight during a Period from Day 8 to Day 35 in Rats Administered the Dioxin Mixture (Experiment 2)<sup>a</sup>

diet	food intake (g/28 days)	body wt gain (g/28 days)	feces wt (g/28 days)
basal	596.6 $\pm$ 35.0	170.9 $\pm$ 21.7	39.1 $\pm$ 2.6
10% wakame	599.1 $\pm$ 22.1	156.9 $\pm$ 11.8	69.0 $\pm$ 5.3**
10% hiziki	619.1 $\pm$ 5.1	166.9 $\pm$ 6.4	89.5 $\pm$ 4.5**
10% kombu	599.7 $\pm$ 38.5	151.1 $\pm$ 17.3	92.1 $\pm$ 6.7**

<sup>a</sup> Values represent the mean  $\pm$  SD ( $n = 4$ ). \*\*, significantly different from basal group ( $p < 0.01$ ).

3.8-fold, and 2.6–3.3-fold, respectively ( $p < 0.01$  or  $p < 0.05$ ), as compared to the basal diet.

As the dose of hiziki and kombu administered increased from 2 to 10%, the amounts of fecal excretion of PCDD and PCDF were increased.

Thus, it was shown that the seaweed foods were effective in inhibiting absorption of the dioxin mixture via foods and in promoting the fecal excretion thereof.

Table 6 shows the PCDD and PCDF amounts stored in the body 5 days after administration of PCDD and PCDF.

In the group fed the 10% wakame diet, the amounts of PCDD and PCDF congeners stored in the body were decreased compared with the basal diet by 7.6% for TCDD, by 17.9% for 1,2,3,7,8-pentaCDD, by 35.5% for 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin (hexaCDD), by 37.5% for 1,2,3,6,7,8-hexaCDD, by 55.8% for 1,2,3,7,8,9-hexaCDD, by 64.3% for 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin (heptaCDD), by 83.6% for octaCDD, by 27.2% for 1,2,3,7,8-pentaCDF, by 12.1% for 2,3,4,7,8-pentaCDF, by 47.4% for 1,2,3,4,7,8-hexaCDF, by 46.0% for 1,2,3,6,7,8-hexaCDF, by 41.7% for 1,2,3,7,8,9-hexaCDF, by 45.3% for 2,3,4,6,7,8-hexaCDF, by 73.2% for 1,2,3,4,6,7,8-heptachlorodibenzofuran (heptaCDF), by 62.6% for 1,2,3,4,7,8,9-heptaCDF, by 82.5% for 1,2,3,4,6,7,8,9-octachlorodibenzofuran(octaCDF), and by 18.9% for total TEQ (28) ( $p < 0.01$  or  $p < 0.05$ ).

In the groups fed the 10% hiziki and wakame diets, the amounts of the highly toxic TCDD, 1,2,3,7,8-pentaCDD, and 2,3,4,7,8-pentaCDF stored in the body were decreased compared with the basal diet by 1.8–6.2, 12.6–19.0, and 3.4–14.1%, respectively.

As the dose of hiziki and kombu administered increased from 2 to 10%, the amounts of PCDD and PCDF stored in the body were decreased.

Thus, it was shown that the seaweed foods were effective in inhibiting the absorption of PCDD and PCDF via foods and in preventing accumulation thereof in the body.

**Table 5.** Effect of the Seaweed Diet on Fecal Excretion of PCDD and PCDF Congeners in Rats Administered the Dioxin Mixture (Experiment 1)<sup>a</sup>

dioxin	diet					
	basal	2% wakame	10% wakame	2% hiziki	10% hiziki	10% kombu
2,3,7,8-TCDD	2.0 ± 0.4	2.0 ± 0.2 (100.0)	5.7 ± 0.8 (285.0)**	3.5 ± 0.7 (175.0)**	5.6 ± 2.0 (280.0)*	3.7 ± 1.1 (185.0)*
1,2,3,7,8-pentaCDD	4.2 ± 1.3	6.2 ± 0.5 (147.6)*	16.8 ± 1.3 (400.0)**	8.6 ± 1.4 (204.8)**	16.0 ± 6.7 (381.0)*	12.7 ± 3.6 (302.4)**
1,2,3,4,7,8-hexaCDD	11.9 ± 3.7	20.8 ± 1.2 (174.8)**	41.1 ± 2.5 (345.4)**	22.7 ± 2.9 (190.8)**	36.3 ± 11.9 (305.0)**	34.3 ± 6.4 (288.2)**
1,2,3,6,7,8-hexaCDD	13.0 ± 4.3	22.1 ± 1.4 (170.0)**	42.5 ± 1.9 (326.9)**	23.1 ± 2.7 (177.7)**	37.5 ± 11.3 (288.5)**	35.3 ± 5.3 (271.5)**
1,2,3,7,8,9-hexaCDD	24.4 ± 5.7	39.8 ± 2.6 (163.1)**	61.7 ± 1.2 (252.9)**	35.6 ± 3.6 (145.9)*	52.4 ± 9.7 (214.8)*	53.8 ± 5.7 (220.5)**
1,2,3,4,6,7,8-heptaCDD	47.7 ± 6.7	62.7 ± 2.0 (131.4)**	81.8 ± 2.1 (171.5)**	63.6 ± 4.4 (133.3)**	80.7 ± 3.7 (169.2)**	72.7 ± 4.9 (152.4)**
octaCDD	80.3 ± 1.6	92.7 ± 2.2 (115.4)**	93.9 ± 3.9 (116.9)**	90.1 ± 3.9 (112.2)**	101.6 ± 5.5 (126.5)**	91.4 ± 4.5 (113.8)**
2,3,7,8-TCDF	1.2 ± 0.3	1.1 ± 0.2 (91.7)	3.6 ± 0.8 (300.0)**	2.3 ± 0.6 (191.7)*	3.7 ± 1.3 (308.3)**	2.2 ± 0.8 (183.3)
1,2,3,7,8-pentaCDF	6.2 ± 2.1	10.2 ± 0.7 (164.5)*	23.0 ± 1.4 (371.0)**	11.2 ± 1.6 (180.6)**	19.7 ± 4.0 (317.7)*	18.4 ± 4.0 (296.8)**
2,3,4,7,8-pentaCDF	3.8 ± 1.4	5.3 ± 0.5 (139.5)	14.3 ± 1.4 (376.3)**	6.9 ± 1.2 (181.6)*	12.6 ± 5.8 (331.6)*	10.1 ± 3.3 (265.8)*
1,2,3,4,7,8-hexaCDF	17.0 ± 4.9	30.6 ± 2.2 (180.0)**	54.9 ± 2.8 (322.9)**	29.5 ± 3.2 (173.5)**	45.4 ± 12.2 (267.1)**	44.7 ± 5.8 (262.9)**
1,2,3,6,7,8-hexaCDF	16.0 ± 4.1	27.3 ± 1.5 (170.6)**	48.5 ± 2.0 (303.1)**	27.8 ± 3.1 (173.8)**	42.5 ± 11.7 (265.6)**	40.8 ± 5.6 (255.0)**
1,2,3,7,8,9-hexaCDF	15.8 ± 4.6	27.3 ± 1.8 (172.8)**	50.3 ± 1.6 (318.4)**	26.3 ± 2.9 (166.5)**	42.7 ± 12.3 (270.3)**	42.6 ± 5.8 (269.6)**
2,3,4,6,7,8-hexaCDF	18.5 ± 5.0	31.3 ± 1.8 (169.2)**	54.0 ± 2.3 (291.9)**	32.3 ± 3.7 (174.6)**	50.3 ± 13.3 (271.9)**	46.0 ± 5.5 (248.6)**
1,2,3,4,6,7,8-heptaCDF	45.6 ± 5.7	59.3 ± 2.2 (130.0)**	77.1 ± 0.9 (169.1)**	59.4 ± 2.7 (130.3)**	74.9 ± 2.6 (164.3)**	69.0 ± 3.9 (151.3)**
1,2,3,4,7,8,9-heptaCDF	34.0 ± 5.6	53.0 ± 2.1 (155.9)**	74.8 ± 2.2 (220.0)**	49.6 ± 4.0 (145.9)**	66.4 ± 7.9 (195.3)**	64.6 ± 6.7 (190.0)**
octaCDF	73.8 ± 2.2	89.7 ± 2.9 (121.5)**	93.1 ± 3.5 (126.2)**	87.9 ± 3.3 (119.1)*	99.8 ± 7.5 (135.2)**	89.4 ± 4.6 (121.1)**
total TEQ	6.3 ± 1.7	9.8 ± 0.6 (154.5)**	20.4 ± 1.1 (321.5)**	11.2 ± 1.5 (176.8)**	18.4 ± 6.0 (290.5)**	16.1 ± 3.1 (254.2)**

<sup>a</sup> Each value represents the mean of percent of dose ± SD ( $n = 4$ ) during the period from day 1 to day 5 in rats administered the dioxin mixture; acceleration index of fecal excretion (in parentheses) equals [(the percent fecal excretion of PCDD and PCDF congeners of rats in the seaweed diet)/(the percent fecal excretion of PCDD and PCDF congeners of rats in the basal diet) × 100]. \*, significantly different from the basal diet group ( $p < 0.05$ ). \*\*, significantly different from the basal diet group ( $p < 0.01$ ).

**Table 6.** Effect of the Seaweed Diet on Body Burden of PCDD and PCDF Congeners in Rats Administered the Dioxin Mixture (Experiment 1)<sup>a</sup>

dioxin	diet					
	basal	2% wakame	10% wakame	2% hiziki	10% hiziki	10% kombu
2,3,7,8-TCDD	95.9 ± 1.3	95.7 ± 2.6 (99.8)	88.6 ± 1.7 (92.4)**	95.7 ± 2.8 (99.8)	90.0 ± 2.8 (93.8)**	94.2 ± 1.0 (98.2)
1,2,3,7,8-pentaCDD	94.3 ± 2.5	91.6 ± 1.7 (97.1)	77.4 ± 3.1 (82.1)**	89.2 ± 1.1 (94.6)*	76.4 ± 3.4 (81.0)**	82.4 ± 4.2 (87.4)**
1,2,3,4,7,8-hexaCDD	84.8 ± 4.0	77.3 ± 3.1 (91.2)*	54.7 ± 2.2 (64.5)**	75.2 ± 1.8 (88.7)**	50.2 ± 4.3 (59.2)**	63.8 ± 5.4 (75.2)**
1,2,3,6,7,8-hexaCDD	83.8 ± 3.4	76.6 ± 1.6 (91.4)**	52.4 ± 1.9 (62.5)**	76.0 ± 2.4 (90.7)**	46.3 ± 3.9 (55.3)**	59.9 ± 4.5 (71.5)**
1,2,3,7,8,9-hexaCDD	72.8 ± 5.9	55.2 ± 2.2 (75.8)**	32.2 ± 1.1 (44.2)**	57.0 ± 2.5 (78.3)**	31.0 ± 2.5 (42.6)**	39.7 ± 4.5 (54.5)**
1,2,3,4,6,7,8-heptaCDD	47.9 ± 10.3	36.0 ± 2.1 (75.2)	17.1 ± 0.7 (35.7)**	36.4 ± 3.6 (76.0)	14.6 ± 1.7 (30.5)**	20.3 ± 3.6 (42.4)**
octaCDD	21.4 ± 3.4	11.2 ± 1.4 (52.3)**	3.5 ± 0.2 (16.4)**	12.5 ± 2.5 (58.4)**	3.2 ± 0.3 (15.0)**	4.9 ± 1.2 (22.9)**
2,3,7,8-TCDF	19.6 ± 5.6	18.8 ± 2.7 (95.9)	14.3 ± 1.4 (73.0)	19.5 ± 1.9 (99.5)	16.3 ± 3.4 (83.2)	11.5 ± 1.8 (58.7)*
1,2,3,7,8-pentaCDF	44.5 ± 6.7	45.8 ± 2.3 (102.9)	32.4 ± 5.9 (72.8)*	44.5 ± 4.3 (100.0)	32.3 ± 3.4 (72.6)*	36.1 ± 0.7 (81.1)
2,3,4,7,8-pentaCDF	88.7 ± 5.3	87.1 ± 2.7 (98.2)	78.0 ± 1.0 (87.9)**	86.7 ± 3.2 (97.7)	76.2 ± 4.8 (85.9)*	85.8 ± 4.1 (96.7)
1,2,3,4,7,8-hexaCDF	80.8 ± 6.2	65.9 ± 1.5 (81.6)**	42.5 ± 1.1 (52.6)**	70.5 ± 5.2 (87.3)*	40.5 ± 3.4 (50.1)**	49.9 ± 5.0 (61.8)**
1,2,3,6,7,8-hexaCDF	78.3 ± 5.9	63.5 ± 1.6 (81.1)**	42.3 ± 1.8 (54.0)**	65.2 ± 3.7 (83.3)**	38.8 ± 2.5 (49.6)**	49.4 ± 4.8 (63.1)**
1,2,3,7,8,9-hexaCDF	69.5 ± 5.3	61.1 ± 2.0 (87.9)*	40.5 ± 5.2 (58.3)**	62.6 ± 3.0 (90.1)**	37.7 ± 2.8 (54.2)**	47.2 ± 3.7 (67.9)**
2,3,4,6,7,8-hexaCDF	79.7 ± 6.7	68.8 ± 0.6 (86.3)	43.6 ± 2.1 (54.7)**	65.9 ± 5.2 (82.7)*	34.5 ± 2.7 (43.3)**	49.8 ± 5.9 (62.5)**
1,2,3,4,6,7,8-heptaCDF	41.0 ± 4.8	25.6 ± 1.7 (62.4)**	11.0 ± 0.6 (26.8)**	26.7 ± 3.2 (65.1)**	8.9 ± 1.0 (21.7)**	13.8 ± 2.4 (33.7)**
1,2,3,4,7,8,9-heptaCDF	58.8 ± 5.5	43.3 ± 3.9 (73.6)**	22.0 ± 1.7 (37.4)**	45.8 ± 5.1 (77.9)*	20.7 ± 1.8 (35.2)**	27.7 ± 3.5 (47.1)**
octaCDF	20.0 ± 3.7	11.6 ± 1.9 (58.0)**	3.5 ± 0.3 (17.5)**	12.1 ± 2.5 (60.5)	3.1 ± 0.2 (15.5)**	4.9 ± 1.3 (24.5)**
total TEQ	87.3 ± 2.2	83.7 ± 1.1 (95.9)*	70.8 ± 1.7 (81.1)**	83.1 ± 0.4 (95.2)**	69.8 ± 3.1 (80.0)**	76.6 ± 2.5 (87.7)**

<sup>a</sup> Each value represents the mean of percent of dose ± SD ( $n = 4$ ) on day 5 in rats administered the dioxin mixture; acceleration index of disappearance from the body on day 5 (in parentheses) equals [(the percent body burden of PCDD and PCDF congeners of rats in the seaweed diet)/(the percent body burden of PCDD and PCDF congeners of rats in the basal diet) × 100]. \*, significantly different from the basal diet group ( $p < 0.05$ ). \*\*, significantly different from the basal diet group ( $p < 0.01$ ).

**Effect of Seaweeds on Fecal Excretion and Accumulation in the Body of PCDD and PCDF Congeners (Experiment 2).** Table 7 shows the fecal excretion of the PCDD and PCDF congeners from day 8 to day 35 in rats administered the dioxin mixture.

2,3,7,8-Tetrachlorodibenzofuran (tetraCDF) and 1,2,3,7,8-pentaCDF were readily metabolized (13, 29). Seven days after administration of the dioxin mixture, the amounts of 2,3,7,8-tetraCDF and 1,2,3,7,8-pentaCDF in the body were reduced by 2.8 and 21.4%, respectively. Because of this, the amounts of the fecal excretion of 2,3,7,8-tetraCDF and 1,2,3,7,8-pentaCDF were lower than the amounts of the fecal excretion of other PCDD and PCDF congeners.

In the group fed the 10% wakame diet, the fecal excretion of PCDD and PCDF congeners was increased compared with the basal diet by 1.7-fold for TCDD, by 1.8-fold for 1,2,3,7,8-pentaCDD, by 2.0-fold for 1,2,3,4,7,8-hexaCDD, by 1.9-fold

for 1,2,3,6,7,8-hexaCDD, by 1.6-fold for 1,2,3,7,8,9-hexaCDD, by 1.5-fold for 1,2,3,4,6,7,8-heptaCDD, by 2.0-fold for 2,3,4,7,8-pentaCDF, by 2.1-fold for 1,2,3,4,7,8-hexaCDF, by 1.9-fold for 1,2,3,6,7,8-hexaCDF, by 1.7-fold for 2,3,4,6,7,8-hexaCDF, by 1.5-fold for 1,2,3,4,6,7,8-heptaCDF, by 1.9-fold for 1,2,3,4,7,8,9-heptaCDF, and by 1.7-fold for total TEQ ( $p < 0.01$  or  $p < 0.05$ ).

In the group fed the 10% hiziki diets, the amounts of fecal excretion of TCDD, 1,2,3,7,8-pentaCDD, and 2,3,4,7,8-pentaCDF increased 2.0–2.2-fold ( $p < 0.01$ ), and in the group fed the 10% kombu diets, the amounts of fecal excretion of 2,3,4,7,8-pentaCDF increased 1.5-fold ( $p < 0.05$ ) compared with the basal diet.

It was thus shown that the seaweed diets were effective in inhibiting the reabsorption of the dioxin mixture congeners excreted in the gut from the gastrointestinal walls as well as in promoting the fecal excretion thereof.

**Table 7.** Effect of the Seaweed Diet on Fecal Excretion of PCDD and PCDF Congeners in Rats Administered the Dioxin Mixture (Experiment 2)<sup>a</sup>

dioxin	diet			
	basal	10% wakame	10% hiziki	10% kombu
2,3,7,8-TCDD	1.19 ± 0.20	2.03 ± 0.65 (170.6)*	2.46 ± 0.65 (206.7)**	1.44 ± 0.46 (121.0)
1,2,3,7,8-pentaCDD	1.13 ± 0.22	2.07 ± 0.45 (183.2)**	2.50 ± 0.54 (221.2)**	1.63 ± 0.35 (144.2)
1,2,3,4,7,8-hexaCDD	1.04 ± 0.13	2.14 ± 0.34 (205.8)**	2.26 ± 0.29 (217.3)**	1.76 ± 0.25 (169.2)**
1,2,3,6,7,8-hexaCDD	1.02 ± 0.14	1.98 ± 0.29 (194.1)**	2.05 ± 0.23 (201.0)**	1.65 ± 0.23 (161.8)**
1,2,3,7,8,9-hexaCDD	1.30 ± 0.13	2.08 ± 0.41 (160.0)*	2.11 ± 0.20 (162.3)**	2.01 ± 0.35 (154.6)*
1,2,3,4,6,7,8-heptaCDD	1.94 ± 0.39	3.00 ± 0.57 (154.6)*	2.56 ± 0.13 (132.0)*	3.06 ± 0.58 (157.7)*
octaCDD	3.24 ± 0.24	3.21 ± 0.56 (99.1)	3.07 ± 0.35 (94.8)	3.71 ± 0.82 (114.5)
2,3,7,8-TCDF	0.007 ± 0.002	0.010 ± 0.002 (142.9)	0.013 ± 0.002 (185.7)**	0.014 ± 0.011 (200.0)
1,2,3,7,8-pentaCDF	0.12 ± 0.03	0.13 ± 0.06 (108.3)	0.20 ± 0.05 (166.7)**	0.16 ± 0.09 (133.3)
2,3,4,7,8-pentaCDF	0.27 ± 0.06	0.54 ± 0.07 (200.0)*	0.59 ± 0.11 (218.5)**	0.42 ± 0.07 (155.6)*
1,2,3,4,7,8-hexaCDF	0.73 ± 0.08	1.55 ± 0.18 (212.3)**	1.45 ± 0.14 (198.6)**	1.31 ± 0.22 (179.5)**
1,2,3,6,7,8-hexaCDF	0.65 ± 0.09	1.29 ± 0.13 (198.5)**	1.24 ± 0.10 (190.8)**	1.14 ± 0.21 (175.4)**
1,2,3,7,8,9-hexaCDF	0.91 ± 0.21	1.04 ± 0.53 (114.3)	1.29 ± 0.42 (141.8)	1.00 ± 0.44 (109.9)
2,3,4,6,7,8-hexaCDF	0.67 ± 0.08	1.15 ± 0.11 (171.6)**	1.18 ± 0.12 (176.1)*	1.17 ± 0.25 (174.6)**
1,2,3,4,6,7,8-heptaCDF	1.40 ± 0.31	2.13 ± 0.33 (152.1)*	1.85 ± 0.12 (132.1)*	2.35 ± 0.54 (167.9)*
1,2,3,4,7,8,9-heptaCDF	1.12 ± 0.16	2.16 ± 0.33 (192.9)**	1.76 ± 0.12 (157.1)**	1.99 ± 0.43 (177.7)**
octaCDF	1.99 ± 0.55	2.29 ± 0.44 (115.1)	2.05 ± 0.29 (103.0)	2.85 ± 0.98 (143.2)
total TEQ	0.93 ± 0.15	1.65 ± 0.37 (177.4)*	1.92 ± 0.39 (206.5)**	1.29 ± 0.28 (138.7)

<sup>a</sup> Each value represents the mean of percent of dose ± SD ( $n = 4$ ) during the period from day 8 to day 35 in rats administered the dioxin mixture; acceleration index of fecal excretion (in parentheses) equals [(the percent fecal excretion of PCDD and PCDF congeners of rats in the seaweed diet)/(the percent fecal excretion of PCDD and PCDF congeners of rats in the basal diet) × 100]. \*, significantly different from the basal diet group ( $p < 0.05$ ). \*\*, significantly different from the basal diet group ( $p < 0.01$ ).

**Table 8.** Effect of the Seaweed Diet on Body Burden of PCDD and PCDF Congeners in Rats Administered the Dioxin Mixture (Experiment 2)<sup>a</sup>

dioxin	on day 8 <sup>a</sup>	diet			
		basal <sup>b</sup>	10% wakame <sup>b</sup>	10% hiziki <sup>b</sup>	10% kombu <sup>b</sup>
2,3,7,8-TCDD	84.8 ± 8.2	52.1 ± 7.7	26.2 ± 6.9 (50.3)**	33.6 ± 11.4 (64.5)*	31.5 ± 12.6 (60.5)*
1,2,3,7,8-pentaCDD	89.3 ± 7.6	65.3 ± 4.3	42.8 ± 9.1 (65.5)**	48.9 ± 10.7 (74.9)*	51.5 ± 15.4 (78.9)
1,2,3,4,7,8-hexaCDD	79.4 ± 4.0	63.7 ± 3.4	47.1 ± 6.4 (73.9)**	56.9 ± 9.5 (89.3)	54.6 ± 10.8 (85.7)
1,2,3,6,7,8-hexaCDD	83.2 ± 3.7	76.8 ± 4.7	69.8 ± 3.7 (90.9)	76.7 ± 5.0 (99.9)	75.1 ± 8.1 (97.8)
1,2,3,7,8,9-hexaCDD	62.9 ± 7.5	48.0 ± 4.8	38.4 ± 4.8 (80.0)*	45.8 ± 8.5 (95.4)	44.8 ± 9.3 (93.3)
1,2,3,4,6,7,8-heptaCDD	52.6 ± 8.5	46.1 ± 5.4	40.5 ± 3.3 (87.9)	45.2 ± 7.7 (98.0)	45.0 ± 4.8 (97.6)
octaCDD	21.6 ± 4.3	15.6 ± 2.4	12.8 ± 1.2 (82.1)	15.9 ± 3.2 (101.9)	15.6 ± 2.0 (100.0)
2,3,7,8-TCDF	2.8 ± 0.2	0.68 ± 0.07	0.45 ± 0.05 (66.2)**	0.51 ± 0.06 (75.0)*	0.42 ± 0.07 (61.8)**
1,2,3,7,8-pentaCDF	21.4 ± 5.7	3.6 ± 0.1	1.0 ± 0.4 (27.8)**	1.7 ± 0.7 (47.2)*	1.5 ± 0.8 (41.7)*
2,3,4,7,8-pentaCDF	86.2 ± 3.6	79.6 ± 2.6	72.9 ± 5.6 (91.6)	75.0 ± 8.0 (94.2)	75.6 ± 7.0 (95.0)
1,2,3,4,7,8-hexaCDF	76.9 ± 6.4	73.5 ± 6.1	68.2 ± 4.1 (92.8)	73.0 ± 6.7 (99.3)	72.7 ± 2.9 (98.9)
1,2,3,6,7,8-hexaCDF	74.4 ± 6.6	71.1 ± 3.2	65.7 ± 3.0 (92.4)*	70.7 ± 7.1 (99.4)	70.6 ± 6.2 (99.3)
1,2,3,7,8,9-hexaCDF	58.6 ± 11.1	25.7 ± 3.5	8.0 ± 7.0 (31.1)**	14.8 ± 9.6 (57.6)	13.2 ± 10.4 (51.4)
2,3,4,6,7,8-hexaCDF	75.1 ± 7.4	68.9 ± 5.7	58.5 ± 6.0 (84.9)*	67.3 ± 8.1 (97.7)	66.2 ± 9.1 (96.1)
1,2,3,4,6,7,8-heptaCDF	37.1 ± 6.3	34.5 ± 5.8	32.8 ± 2.6 (95.1)	34.7 ± 3.7 (100.6)	35.4 ± 3.7 (102.6)
1,2,3,4,7,8,9-heptaCDF	56.3 ± 7.4	50.9 ± 6.4	43.8 ± 2.8 (86.1)	50.7 ± 7.3 (99.6)	48.5 ± 2.9 (95.3)
octaCDF	20.8 ± 3.6	11.5 ± 2.2	10.7 ± 1.0 (93.0)	11.9 ± 2.8 (103.5)	13.4 ± 2.1 (116.5)
total TEQ	80.1 ± 6.2	59.6 ± 4.2	42.1 ± 6.3 (70.6)**	47.9 ± 9.3 (80.4)	47.9 ± 10.9 (80.4)

<sup>a</sup> Each value represents the mean of percent of dose ± SD ( $n = 4$ ) on day 8 in rats administered the dioxin mixture. <sup>b</sup> Each value represents the mean of percent of dose ± SD ( $n = 4$ ) on day 35 in rats administered the dioxin mixture; acceleration index of disappearance from the body on day 35 (in parentheses) equals [(the percent body burden of PCDD and PCDF congeners of rats in the seaweed diet)/(the percent body burden of PCDD and PCDF congeners of rats in the basal diet) × 100]. \*\*, significantly different from the basal diet group ( $p < 0.05$ ). \*\*, significantly different from the basal diet group ( $p < 0.01$ ).

In the group fed the basal diet and seaweed diets, the percent excretions of PCDD and PCDF congeners from day 8 to day 35 were small, <3.71%, for all PCDD and PCDF congeners. We inferred from this result that the PCDD and PCDF amounts stored in the body 35 days after administration of the dioxin mixture make little difference between the basal diet and the seaweed diets. However, the 10% wakame diet significantly lowered ( $p < 0.01$  or  $p < 0.05$ ) the levels of four types of PCDD congeners and five types of PCDF congeners compared with the control group by 49.7% for TCDD, by 34.5% for 1,2,3,7,8-pentaCDD, by 26.1% for 1,2,3,4,7,8-hexaCDD, by 20.0% for 1,2,3,7,8,9-hexaCDD, by 33.8% for 2,3,7,8-TCDF, by 72.2% for 1,2,3,7,8-pentaCDF, by 7.6% for 1,2,3,6,7,8-hexaCDF, by 68.9% for 1,2,3,7,8,9-hexaCDF, and by 15.1% for 2,3,4,6,7,8-hexaCDF (Table 8). In the group fed the 10% hiziki diets, the

levels of body burden of PCDD and PCDF congeners were significantly lower ( $p < 0.01$  or  $p < 0.05$ ) by 35.5% for TCDD, by 25.1% for 1,2,3,7,8-pentaCDD, by 25.0% for 2,3,7,8-TCDF, and by 52.8% for 1,2,3,7,8-pentaCDF than those of the basal group, respectively. In the group fed the 10% kombu diets, the levels of body burden of PCDD and PCDF congeners were significantly lower ( $p < 0.01$  or  $p < 0.05$ ) by 39.5% for TCDD, by 38.2% for 2,3,7,8-TCDF, and by 58.3% for 1,2,3,7,8-pentaCDF than those of the basal group, respectively.

Table 9 shows the whole body half-life calculated by one compartment model from the mean amounts of PCDD and PCDF stored in the body 8 and 35 days after administration of the dioxin mixture in this study. The half-lives of TCDD, 1,2,3,7,8-pentaCDD, and 2,3,4,7,8-pentaCDF were 40, 62, and 244 days with the basal diet, respectively, and were significantly

**Table 9.** Effect of the Seaweed Diet on Whole Body Half-Life of PCDD and PCDF Congeners in Rats Administered the Dioxin Mixture (Experiment 2)<sup>a</sup>

dioxin	whole body half-life of PCDD and PCDF congeners during the period from day 8 to day 35 in rats administered the dioxin mixture (days)			
	basal diet	10% wakame diet	10% hiziki diet	10% kombu diet
2,3,7,8-TCDD	40	17	21	20
1,2,3,7,8-pentaCDD	62	26	32	35
1,2,3,4,7,8-hexaCDD	88	37	58	52
1,2,3,6,7,8-hexaCDD	242	111	239	189
1,2,3,7,8,9-hexaCDD	72	39	61	57
1,2,3,4,6,7,8-heptaCDD	147	74	128	124
octaCDD	60	37	63	60
2,3,7,8-TCDF	14	11	11	10
1,2,3,7,8-pentaCDF	11	6	8	7
2,3,4,7,8-pentaCDF	244	116	139	148
1,2,3,4,7,8-hexaCDF	429	162	373	346
1,2,3,6,7,8-hexaCDF	428	156	380	370
1,2,3,7,8,9-hexaCDF	24	10	14	13
2,3,4,6,7,8-hexaCDF	225	78	177	154
1,2,3,4,6,7,8-heptaCDF	258	154	279	391
1,2,3,4,7,8,9-heptaCDF	192	77	185	130
octaCDF	33	29	35	44
total TEQ	66	30	38	38

<sup>a</sup> Values represent the whole body half-life of PCDD and PCDF congeners. Each value was recalculated to obtain the average of body burden of PCDD and PCDF congeners on days 8 and 35; whole body half-life of PCDD and PCDF congeners equals  $\ln 2 / [(\log \text{ average of body burden of PCDD and PCDF congeners on day 8}) - (\log \text{ average of body burden of PCDD and PCDF congeners on day 35})] / 28 \text{ days}$ .

reduced as follows: (1) 17, 26, and 116 days with the wakame diet; (2) 21, 32, and 139 days with the hiziki diet; and (3) 20, 35, and 148 days with the kombu diet.

The reduction of the amounts of PCDD and PCDF stored in the body was more prominent with the wakame diet than with the hiziki and kombu diets.

## DISCUSSION

Health disorders in humans due to PCDD and PCDF may be prevented by inhibition of the absorption of PCDD and PCDF via foods in the digestive tract, increase of the fecal excretion of PCDD and PCDF, and reduction of the absorption of PCDD and PCDF in the body. Because the biological half-lives of PCDD and PCDF are extremely long, it may be important to provide the persons who were exposed to PCDD and PCDF with a dietary method that effectively inhibits reabsorption, enhances elimination from the body into the digestive tract, increases the fecal excretion, and reduces the stored amount in the body of PCDD and PCDF (22–27).

We previously reported the effect of chlorophyll derived from *Chlorella* on dioxin excretion in rats (22). In the group fed the 0.5% chlorophyll diet, the levels of fecal excretion of PCDD and PCDF congeners were higher by 13.6-fold for TCDD, by 12.4-fold for 1,2,3,7,8-pentaCDD, and by 15.7-fold for 2,3,4,7,8-pentaCDF than those of the control diet. Moreover, we reported the effect of several types of dietary fiber derived from rice bran, spinach, cabbage, and carrots on dioxin excretion (24, 25). The levels of excretion of 1,2,3,7,8-pentaCDD and 2,3,4,7,8-pentaCDF into the feces in rats fed the 10% rice bran fiber diet were 3.9- and 4.0-fold higher than those in rats fed the control diet, respectively. Chlorophyll and dietary fiber administration

enhanced dioxin excretion and reduced dioxin absorption in rats administered the dioxin mixture. Wakame, hiziki, and kombu contain 0.02–0.5% chlorophyll, 30–60% dietary fiber, and large amounts of mineral and vitamins.

In this study we examined the effects of the seaweed diets on inhibition of the gastrointestinal absorption of PCDD and PCDF via foods and promotion of the fecal excretion thereof (Table 5). The fecal excretion of highly toxic TCDD, 1,2,3,7,8-pentaCDD, and 2,3,4,7,8-pentaCDF in rats fed the 10% wakame, hiziki, and kombu diets was increased 1.8–2.8-, 3.0–4.0-, and 2.6–3.7-fold over animals fed the basal diet, respectively ( $p < 0.01$  or  $p < 0.05$ ).

In addition, we examine the effects of the seaweed diets that inhibit the reabsorption of PCDD and PCDF eliminated in the gut from the gastrointestinal walls (10, 11) by using rats in which PCDD and PCDF were stored in the body (Table 7). In these experiments the fecal excretion of highly toxic TCDD, 1,2,3,7,8-pentaCDD, and 2,3,4,7,8-pentaCDF in rats fed the 10% wakame and hiziki diets was increased by 1.7–2.0-, 1.8–2.2-, and 2.0–2.1-fold more than in the animals fed the basal diet, respectively ( $p < 0.01$  or  $p < 0.05$ ).

It has been shown that the seaweed diets have the ability to inhibit the absorption of PCDD and PCDF from foods in the digestive tract and the ability to inhibit the reabsorption of PCDD and PCDF congeners eliminated into the gut from the body.

The most effective components in seaweed for enhancement of the fecal excretion of dioxin may be chlorophyll and dietary fiber. However, the effectiveness of chlorophyll and dietary fiber in seaweed on dioxin excretion has not been investigated. In a future study using both components derived from wakame, hiziki, and kombu, we will attempt to experiment on animals.

The decisive factor for application of the abilities of the seaweed to promote the elimination of PCDD and PCDF from the body in rats to humans will be human metabolism and excretion of PCDD and PCDF.

In rats, PCDD and PCDF are metabolized into polar metabolites and excreted into feces and urine. Urinary excretion of PCDD and PCDF is much lower than fecal excretion, which is the main elimination route of PCDD and PCDF (14, 15, 29–31).

In rats, the percent of elimination of the parent compound (nonmetabolite) to the total elimination for PCDD and PCDF was 9.3% for TCDD, 1.0% for 2,3,7,8-TCDF, and 9.9% for 2,3,4,7,8-pentaCDF (21). Thus, the elimination of the parent compound was very small compared to elimination of metabolites.

In humans, elimination of PCDD and PCDF is not completely elucidated. It has been reported that the percent of fecal excretion of the unchanged compounds to the total excretion of the PCDD and PCDF congeners from the body was 37–90% (12). In this experiment in which TCDD dissolved in corn oil was administered to humans, the percent of the fecal excretion of the parent compound to the total amount of TCDD excreted in the feces was ~50% (32). Fecal elimination of the parent compounds is the main route of excretion of PCDD and PCDF in humans.

Blood PCDD and PCDF levels in the German population have decreased (8). In Germany the excretion of PCDD and PCDF became twice as large as the intake (7). Furthermore, as the intake of PCDD and PCDF was reduced after weaning in infants fed breast milk, the mass balance of 1,2,3,6,7,8-hexaCDD and octaCDD is in the state of excretion, which is greater than intake (33).

It has been reported that Olestra (66 g/day), a sucrose polyester, accelerated patients' intestinal excretion of TCDD by 8–10-fold (34).

From our study, the fecal excretion of TCDD (parent compound) during the period from day 8 to day 35 of administration of the dioxin mixture was, compared with the basal diet, increased by 1.7-fold ( $p < 0.05$ ) for wakame, by 2.0-fold ( $p < 0.01$ ) for hiziki, and by 1.2-fold for kombu. The fecal excretion of 1,2,3,7,8-pentaCDD was, compared with the basal diet, increased by 1.8-fold ( $p < 0.01$ ) for wakame, by 2.2-fold ( $p < 0.01$ ) for hiziki, and by 1.4-fold for kombu. The fecal excretion of 2,3,4,7,8-pentaCDF was, compared with the basal diet, increased by 2.0-fold ( $p < 0.01$ ) for wakame, by 2.1-fold ( $p < 0.01$ ) for hiziki, and by 1.5-fold ( $p < 0.05$ ) for kombu. The results suggest that if the fecal excretion of the parent compound of PCDD and PCDF in humans is 50% of the total elimination, the biological half-life of TCDD (7.5 years) could be shortened to 5.5 years with wakame, to 5.0 years with hiziki, and to 6.8 years with kombu, whereas that of 1,2,3,7,8-pentaCDD (15.7 years) could be shortened to 11.2 years with wakame, to 9.8 years with hiziki, and to 13.0 years with kombu, and that of 2,3,4,7,8-pentaCDF (19.6 years) could be shortened to 13.0 years with wakame, to 12.6 years with hiziki, and to 15.6 years with kombu.

The excretion of the TCDD metabolites during the period from day 8 to day 35 of administration of the dioxin mixture was 31.5% for the basal diet, 56.6% for wakame, 48.7% for hiziki, and 51.9% for kombu. As compared with the basal diet, this excretion increased by 1.8-fold ( $p < 0.01$ ) with wakame, by 1.5-fold with hiziki, and by 1.6-fold with kombu ( $p < 0.05$ ). The excretion of the 1,2,3,7,8-pentaCDD metabolites was 22.9% for the basal diet, 44.4% for wakame, 37.9% for hiziki, and 36.2% for kombu. As compared with the basal diet, this excretion increased by 1.9-fold ( $p < 0.01$ ) with wakame, by 1.7-fold with hiziki ( $p < 0.05$ ), and by 1.6-fold with kombu. The excretion of the 2,3,4,7,8-pentaCDF metabolites was 6.3% for the basal diet, 12.8% for wakame, 10.6% for hiziki, and 10.2% for kombu. As compared with the basal diet, this excretion of the 2,3,4,7,8-pentaCDD increased by 2.0-fold with wakame, by 1.7-fold with hiziki, and by 1.6-fold with kombu. This indicates the wakame, hiziki, and kombu diets may have the effect of promoting the metabolism of PCDD and PCDF in the body as well as promoting the fecal excretion of PCDD and PCDF metabolites.

These studies show that a diet containing chlorophyll and dietary fiber from seaweed such as wakame, hiziki, and kombu may prevent health disorders in humans due to the absorption in the digestive tract of PCDD and PCDF.

Furthermore, it is important to inhibit the reabsorption of PCDD and PCDF eliminated in the digestive tract from the body as well as to promote the fecal excretion of the metabolites of PCDD and PCDF. Wakame, hiziki, and kombu may be effective in accelerating the elimination of PCDD and PCDF stored in the body.

Food intake of the Japanese population is 426 g/day as dry mass and that of the German population is 440 g/day (7). The 2 and 10% seaweed foods are equivalent to 8.5–8.8 and 42.0–44.0 g/day (dry weight) when they are converted to the dry mass quantity of food, respectively.

#### LITERATURE CITED

- Beck, H.; Dross, A.; Mather, W. PCDD and PCDF exposure and levels in humans in Germany. *Environ. Health Perspect.* **1994**, *102*, 173–175.
- Schechter, A.; Startin, J.; Wright, C.; Kelly, M.; Papke, O.; Lis, A.; Ball, M.; Olson, J. Dioxins in U.S. food and estimated daily intake. *Chemosphere* **1994**, *29*, 2261–2265.
- Hallikainen, A.; Vartiainen, T. Food control surveys of polychlorinated dibenzo-*p*-dioxins and dibenzofurans and intake estimates. *Food Addit. Contam.* **1997**, *14*, 355–366.
- Mclachlan, M. S. Bioaccumulation of hydrophobic chemicals in agricultural food chains. *Environ. Sci. Technol.* **1996**, *30*, 252–259.
- Liem, A. K.; Forst, P.; Pappé, C. Exposure of populations to dioxins and related compounds. *Food Addit. Contam.* **2000**, *17*, 241–259.
- Toyoda, M.; Uchibe, H.; Yanagi, T.; Kono, Y.; Hori, T.; Iida, T. Decreased daily intake of PCDDs, PCDFs and Co-PCBs from foods in Japan 1977 to 1998. *J. Food Hyg. Soc. Jpn.* **1999**, *40*, 494–499.
- Schrey, P.; Wittsiepe, J.; Mackrodt, P.; Selenka, F. Human fecal PCDD/F-excretion exceeds the dietary intake. *Chemosphere* **1998**, *37*, 1825–1831.
- Wittsiepe, J.; Schrey, P.; Ewers, U.; Wilhelm, M.; Selenka, F. Decrease of PCDD/F level in human blood—Trend analysis for the German population, 1991–1996. *Environ. Res. Sect. A* **2000**, *83*, 46–53.
- Lakshmanan, M. R.; Campbell, B. S.; Chirtel, S. J.; Ekarohita, N.; Ezekiel, M. Studies on the mechanism of absorption and distribution of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the rat. *J. Pharmacol. Exp. Ther.* **1986**, *239*, 673–677.
- Yoshimura, T.; Yamamoto, H. A novel route of excretion of 2,4,3',4'-tetrachlorobiphenyl in rats. *Bull. Environ. Contam. Toxicol.* **1975**, *13*, 681–688.
- Olson, J. R. Metabolism and disposition of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in guinea pigs. *Toxicol. Appl. Pharmacol.* **1986**, *85*, 263–273.
- Rohde, S.; Moser, A.; Papke, O.; Mclachlan, M. S. Clearance of PCDD/Fs via the gastrointestinal tract in occupationally exposed persons. *Chemosphere* **1999**, *38*, 3397–3410.
- Rose, J. Q.; Ramsey, J. C.; Wentzler, T. H.; Hummel, R. A.; Gehring, P. J. The fate of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin following single and repeated oral doses to the rat. *Toxicol. Appl. Pharmacol.* **1976**, *36*, 209–226.
- Wacker, R.; Poiger, H.; Schlatter, C. Pharmacokinetics and metabolism of 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin in the rat. *Chemosphere* **1986**, *15*, 1473–1476.
- Brewster, D. W.; Birnbaum, L. S. Disposition and excretion of 2,3,4,7,8-pentachlorodibenzofuran in the rat. *Toxicol. Appl. Pharmacol.* **1987**, *90*, 243–252.
- Poiger, H.; Schlatter, C. Pharmacokinetics of 2,3,7,8-TCDD in man. *Chemosphere* **1986**, *5*, 1489–1494.
- Schechter, C. Data on kinetics of PCDDs and PCDFs as a prerequisite for human risk assessment. *Banbury Report 35*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 1991; pp 215–228.
- Wolfe, W. H.; Michalek, J. E.; Miner, J. C.; Pirkle, J. L.; Caudill, S. P.; Patterson, D. G.; Needham, L. L. Determinants of TCDD half-life in veterans of Operation Ranch Hand. *J. Toxicol. Environ. Health* **1994**, *41*, 481–488.
- Flesch-Janys, D.; Becher, H.; Gurn, P.; Jung, D.; Konietzko, J.; Manz, A.; Rapke, O. Elimination of polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans in occupationally exposed persons. *J. Toxicol. Environ. Health* **1996**, *47*, 363–378.
- Masuda, Y. Approach to risk assessment of chlorinated dioxins from Yusho PCB poisoning. *Chemosphere* **1996**, *32*, 583–594.
- Vandenbergh, M.; Jongh, J. D.; Poiger, H.; Olson, J. R. The toxicokinetics and metabolism of polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) and their relevance for toxicity. *Crit. Rev. Toxicol.* **1994**, *24*, 1–74.
- Morita, K.; Ogata, M.; Hasegawa, T. Chlorophyll derived from *Chlorella* inhibits dioxin absorption from the gastrointestinal tract and accelerates dioxin excretion in rats. *Environ. Health Perspect.* **2001**, *109*, 289–294.

- (23) Morita, K.; Hirakawa, H.; Matsueda, T.; Iida, T.; Tokiwa, H. Stimulating effect of dietary fiber on fecal excretion of polychlorinated dibenzofuran (PCDF) and polychlorinated dibenzo-*p*-dioxins (PCDD) in rats. *Fukuoka Igaku Zasshi* **1993**, *84*, 273–281.
- (24) Morita, K.; Matsueda, T.; Iida, T. Effect of dietary fiber on fecal excretion and distribution of PCDF in rats. *Fukuoka Igaku Zasshi* **1995**, *86*, 218–225.
- (25) Morita, K.; Matsueda, T.; Iida, T. Effect of dietary fiber on fecal excretion of polychlorinated dibenzo-*p*-dioxins in rats. *Jpn. J. Toxicol. Environ. Health* **1997**, *43*, 35–41.
- (26) Morita, K.; Matsueda, T.; Iida, T.; Hasegawa, T. Chlorella accelerates dioxin excretion in rats. *J. Nutr.* **1999**, *129*, 1731–1736.
- (27) Morita, K.; Matsueda, T.; Iida, T. Effect of green vegetable on digestive tract absorption of polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans in rats. *Fukuoka Igaku Zasshi* **1999**, *90*, 171–183.
- (28) Vandenberg, M.; Birnbaum, L.; Bosveld, A. T.; Brrunstrom, B.; Cook, P. Feeley, M.; Giesy, J. P.; Hanberg, A.; Hasegawa, R.; Kennedy, S. W.; Kubiak, T.; Larsen, J. C.; vanLeeuwen, F. X.; Liem, A. K.; Nolt, C.; Peterson, R. E.; Poellinger, L.; Safe, S.; Schrenk, D.; Tillitt, D.; Tysklind, M.; Younes, M.; Waern, F.; Zacharewski, T. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. *Environ. Health Perspect.* **1998**, *106*, 775–792.
- (29) Brewster, D. W.; Birnbaum, L. S. Disposition of 1,2,3,7,8-pentachlorodibenzofuran in the rat. *Toxicol. Appl. Pharmacol.* **1988**, *95*, 490–498.
- (30) Pohjanvirta, R.; Vartiainen, T.; Uusi-Rauva, A.; Monkkonen, J.; Tuomisto, J. Tissue distribution, metabolism, and excretion of <sup>14</sup>C-TCDD in a TCDD-susceptible and a TCDD-resistant rat strain. *Pharmacol. Toxicol.* **1990**, *66*, 93–100.
- (31) Birnbaum, L. S.; Decad, G. M.; Matthews, H. B. Disposition and excretion of 2,3,7,8-tetrachlorodibenzofuran in the rat. *Toxicol. Appl. Pharmacol.* **1980**, *55*, 342–352.
- (32) Wendling, J. M.; Orth, R. G. Determination of [<sup>3</sup>H]-2,3,7,8-tetrachlorodibenzo-*p*-dioxin in human feces to ascertain its relative metabolism in man. *Anal. Chem.* **1990**, *62*, 796–800.
- (33) Abraham, K.; Knoll, A.; Ende, M.; Papke, O.; Helge, H. Intake, fecal excretion, and body burden of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in breast-fed and formula-fed infants. *Pediatr. Res.* **1996**, *40*, 671–679.
- (34) Geusau, A.; Tschachler, E.; Meixner, M.; Sandermann, S.; Papke, O.; Wolf, C.; Valic, E.; Stingl, G.; Mclachlan, M. Olestra increases fecal excretion of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Lancet* **1999**, *354*, 1266–1267.

---

Received for review September 7, 2001. Revised manuscript received November 6, 2001. Accepted November 12, 2001. This study was supported by grants to K.M. from the Ministry of Health, Labor and Welfare in Japan.

JF0111920