Chlorophyll Derived from *Chlorella* Inhibits Dioxin Absorption from the Gastrointestinal Tract and Accelerates Dioxin Excretion in Rats

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We investigated the effects of chlorophyll derived from *Chlorella* on gastrointestinal absorption of seven types of polychlorinated dibenzo-*p*-dioxin (PCDD) and 10 types of polychlorinated dibenzofuran (PCDF) in Wistar rats. Twenty-eight rats were randomly distributed into seven groups (n = 4). After overnight food deprivation, rats were given 4 g of the basal diet or 4 g of the chlorophyll diet containing 0.01–0.5% chlorophyll one time on day 1; each diet also contained 0.2 mL PCDD and PCDF standard solutions. The amounts of fecal excretion of PCDD and PCDF congeners from days 1 to 5 in the group fed 0.01% chlorophyll were 64.8% for 1,2,3,7,8-pentaCDD, 78.6% for 1,2,3,4,7,8-hexaCDD, 73.5% for 1,2,3,6,7,8-hexaCDD, 58.5% for 1,2,3,7,8,9-hexaCDD, 33.3% for 1,2,3,4,6,7,8-heptaCDD, 85.7% for 1,2,3,7,8-pentaCDF, 77.3% for 2,3,4,7,8-pentaCDF, 88.6% for 1,2,3,4,7,8-hexaCDF, 78.0% for 1,2,3,6,7,8-hexaCDF, 62.5% for 1,2,3,7,8,9-hexaCDF, 84.1% for 2,3,4,6,7,8-hexaCDF, 41.7% for 1,2,3,4,6,7,8-heptaCDF, and 40.0% for 1,2,3,4,6,7,8-heptaCDF greater ($p < 0.01$) than those of the control group, respectively. The fecal excretion of PCDD and PCDF congeners was remarkably increased along with the increasing dietary chlorophyll. The amounts of PCDD and PCDF congeners in rats on day 5 administered dioxin mixtures were lower in the 0.01% chlorophyll group than in the control group, ranging from 3.5 to 50.0% for PCDD congeners and from 3.7 to 41.7% lower for PCDF congeners, except for 2,3,7,8-tetrachlorodibenzofuran. The amount of PCDD and PCDF congeners in rats was remarkably decreased along with the increasing dietary chlorophyll. These findings suggest that chlorophyll is effective for preventing dioxin absorption via foods. *Key words*: *Chlorella*, chlorophyll, dioxin, polychlorinated dibenzofurans, polychlorinated dibenzo-*p*-dioxins rats. *Environ Health Perspect* 109:289–294 (2001). [Online 2 March 2001]
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Polychlorinated dibenzo-*p*-dioxin (PCDD) and polychlorinated dibenzofuran (PCDF) congeners are among most toxic synthetic chemicals known. They cause cancer promotion, immunosuppression, hyperkeratosis, hepatotoxicity, and tetratogenicity in experimental animals (*1*).

The main route of human contamination by dioxins seems to be through foods (*2*,*3*). Because of the lipophilic nature of dioxin, it tends to be stored in fat and thus to be present at relatively high concentrations in the fat of animal and fish products. Japanese diets generally include large amounts of seafood, and the percentage of intake of PCDD, PCDF, and coplanar PCB congeners via seafoods is accordingly high—62.4% of the total daily intake [1.4–3.2 pg toxic equivalents (TEQ)/kg body weight per day in Japan] (*4*). Fatty seafoods obtained from along the coast of Japan are highly contaminated with dioxin, whereas seafoods obtained far from the coast of Japan are less contaminated with dioxin (*5*). In contrast, European and American diets generally include the consumption of large quantities of meat, eggs, and dairy products, which are highly contaminated with PCDD, PCDF, and coplanar PCB congeners; the intake of these congeners from all food sources is approximately 0.3–3.0 pg TEQ/kg body weight per day in adult Americans (*2*).

Breast milk is also a source of dioxin exposure in infants. For most of the dioxin congeners, the absorption of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) dissolved in corn oil is > 87% after an overnight fast in human adults (*6*). In rats, the half-life of TCDD in the body is 31 days, and the half-life of 2,3,4,7,8-pentachlorodibenzofuran (pentaCDF) is 64 days (*7*,*8*), but in humans, the reported biological half-life of TCDD in the body is 5.8 years in adults (*6*) or 11.3 years in soldiers who were veterans of the Vietnam war (*9*); moreover, the half-lives of 2,3,4,7,8-pentaCDF and 1,2,3,4,7,8-hexachlorodibenzofuran (hexaCDF) are 13.4 years and 12.0 years, respectively, in patients with Yusho disease who consumed rice oil contaminated with PCDF and polychlorinated biphenyls (PCBs) (*10*). Symptoms of Yusho disease include limb numbness, coughing, expectoration, fever, headache, dizziness, abdominal pain, and swelling in the joints (*11*).

The excretion rate of dioxin stored in the human body is much slower than that of other mammals. The TCDD absorbed in the body of guinea pigs was not detected in either urine or bile, and was eliminated directly to the digestive tract and excreted into the feces (*12*). To prevent health problems caused by dioxin exposure in humans, it is important to capture the dioxin in the digestive tract and prevent its absorption. In the case of humans who have already been exposed to dioxin and accumulated it in their bodies, the goal is to inhibit dioxin reabsorption from the digestive tract, resulting in a decrease of its accumulation in the body. We have reported the effect of dietary fiber on absorption of PCDD and PCDF congeners. In addition, we reported the stimulating effect of dietary fiber on fecal excretion of PCDD and PCDF congeners stored in the body of rats orally administered the rice oil that caused Yusho disease (*13*–*15*).

All plants possess chlorophyll for the purpose of photosynthesis. There is no clear evidence of the metabolism or toxicity of dietary chlorophyll. Baxter (*16*) reported that dietary chlorophyll was not absorbed in the body and that instead it was metabolized and excreted in feces as pheophytin in humans and rats. It was reported that chlorophyllin, a chlorophyll derivative, formed a complex with heterocyclic amines such as 2-amino-3-methylimidazo [4,5-*f*]-quinoline and 2-amino-1-methyl-6-phenylimidazo [4,5-*b*] pyridine (*17*,*18*). The amount of unmetabolized heterocyclic amine excreted in feces was increased by oral administration of chlorophyllin. We have recently reported that *Chlorella* accelerates dioxin excretion in rats (*19*). In this study, to elucidate the effect of chlorophyll derived from *Chlorella*, we examined the fecal excretion of PCDD and PCDF congeners in rats administered a mixture with dioxin.

Materials and Methods

Animals. Male Wistar rats (average 138 g) 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 hr light–dark cycle. Water and feed were consumed *ad libitum*.

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Animals 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 published guidelines (*20*).

Samples and chemicals. Chlorophyll was prepared from *Chlorella* dry powder manufactured by Chlorella Industry Co., Ltd. (Tokyo, Japan). The method of chlorophyll preparation was based on the acetone-dioxane extraction method (*21*). The purity of the chlorophyll was about 86% (chlorophyll a, 73%; chlorophyll b, 13%). Chlorophyll preparations were stored at –20°C until used for experiments. Native PCDD and PCDF standard solution (7 PCDD and 10 PCDF; Wellington Laboratories, Guelph, Ontario, Canada) was given to rats (average 155 g) by feeding them the experimental diet containing 0.2 mL native PCDD and PCDF standard solutions (1.29 mL/kg body weight, 218 ng TEQ/kg body weight) per 4 g of the diet. We used this formulation to prevent diarrhea. ^{13}C-labeled PCDD and PCDF standard solution dissolved in *n*-nonane was used as an internal standard. The concentrations of PCDD and PCDF in the standard solution used in these experiments are shown in Table 2.

Hexane, acetone, chloroform, methanol, dichloromethane, and Florisil were purchased from Wako Pure Chemical Industries Co., Ltd. (Osaka, Japan). These reagents were of the grade required for residual agricultural drug measurements. All other reagents were special grade or better. Silica gel of silver nitrate was prepared as follows. We dissolved 10 g silver nitrate in 5 mL H_2O by heating. We added 8.5 g of Kieselgel 60 (70-230 mesh; Merck & Co., Inc., Darmstadt, Germany) to the silver nitrate solution, mixed it, and left it overnight.

Test for effects of chlorophyll administration on dioxin absorption from the gastrointestinal tract. After a 5-day acclimation period, 28 rats were randomly distributed into 7 groups (n = 4). After overnight food deprivation, rats (mean body weight 155 g) were given 4 g of the basal diet or 4 g of the chlorophyll diet containing 0.01–0.5% chlorophyll one time on day 1, with each serving containing 0.2 mL dioxin mixture (Tables 1 and 2).

The average of total dioxin intake from foodstuffs is 2.4 pg TEQ/kg/day in Japan (*4*). The dose of dioxin mixture in this experiment was 218 ng TEQ/kg/day, which is 90,800-fold higher than the average total dioxin intake by humans in Japan.

We reported previously (*19*) that the 10% *Chlorella* diet inhibited the absorption and accelerated the excretion of dioxins, which suggests that the main effective component was chlorophyll. In this experiment, we studied the effect of chlorophyll on dioxin absorption. In our previous study (*19*) we determined that a 10% *Chlorella* diet contained 0.2% chlorophyll; therefore, the dose we tested in the present study was between 0.01 and 0.5%. A 0.01% chlorophyll diet corresponds to a diet of approximately 10% spinach or 20% seaweed. We chose to add the dioxin mixture to a regular diet rather than dissolving it in corn oil because corn oil tends to cause diarrhea. Rats of the seven groups were each fed the diet containing the dioxin mixture on day 1, and then fed the dioxin-free basal diet or the dioxin-free chlorophyll diet on days 1–5.

Rats were housed individually in metabolic cages designed for the separate collection of feces and urine. Body weight, food intake, and fecal weight (from days 1 to 5) were measured. Feces were dried overnight at 70°C, and the weight of feces was measured. After being fed the respective experimental diets for 5 days, 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 dioxin determination.

Analysis of dioxin. Fecal samples from each rat were homogenized and quantitatively extracted with 150 mL 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 approximately 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 2–10 mL extract into a test tube (10 mL); the sample was then centrifuged, concentrated, and dried. After adding 200 pg stable isotope tracer, ^{13}C-labeled internal standard of tetra–hepta CDDs and tetra–hepta CDFs (Wellington Laboratories), and/or 1,000 pg ^{13}C-labeled internal standard of octaCDDs and octaCDFs (Wellington Laboratories), we added 1 mL 1 M KOH in ethanol to each sample; the sample was then hydrolyzed overnight at room temperature.

The alkali hydrolysates of each sample were shaken with 2 mL hexane plus 0.5 mL H_2O and centrifuged at 2,500 rpm for 10 min. The hexane layer was then collected.

The aqueous layer was extracted 2 times with 2 mL hexane. The collected hexane layer was washed with 2 mL H_2O and concentrated to 2 mL, and the hexane extract was washed 4 times with 2 mL concentrated H_2SO_4.

The hexane extract was applied to a 0.8-g silver nitrate column (7 mm diameter) and eluted from the column with 8 mL hexane, and then the eluate was concentrated to 1 mL. Next, the hexane extract was applied to a 0.6-g Florisil (U.S. Silica Company, New York, NY, USA) column (7 mm diameter) and dioxin was eluted with 4 mL hexane, followed by 8 mL dichloromethane. The eluates from the column were dried and dissolved in 50 µL *n*-nonane. We measured the levels of PCDD and PCDF congeners in these samples.

Approximately 10 g homogenate from the whole body of each rat was put into a test

Table 1. Composition of the experimental diets.

Component	Basal diet	Chlorophyll diet (g/100 g)					
		0.01	0.02	0.05	0.1	0.2	0.5
Sucrose	65	64.99	64.98	64.95	94.9	64.8	64.5
Cellulose	5	5	5	5	5	5	5
Casein	20	20	20	20	20	20	20
Corn oil	5	5	5	5	5	5	5
Mineral mixture[a]	4	4	4	4	4	4	4
Vitamin mixture[a]	0.85	0.85	0.85	0.85	0.85	0.85	0.85
Choline chloride	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Chlorophyll		0.01	0.02	0.05	0.1	0.2	0.5

The basal diet was fed during acclimation, and the basal or chlorophyll diet was fed during study periods; 0.2 mL corn oil containing 7 types of PCDD and 10 types of PCDF congeners were mixed with 4 g of the basal diet or 4 g of the chlorophyll diet and administered to rats once during the experimental period on day 1.
[a]Composition of mineral mixture (g/100 g): CaHPO4 · $2H_2O$, 0.43; KH_2PO_4, 34.31; NaCl, 25.06; Fe-citrate, 0.623; $MgSO_4$ · $7H_2O$, 9.98; $ZnCl_2$, 0.02; $MnSO_4$ · 4- 6 H_2O, 0.121; $CuSO_4$ · $5H_2O$, 0.156; KI, 0.0005; $CaCO_3$, 29.29; $(NH_4)6Mo_7O_24H_2O$, 0.0025. Composition of vitamin mixture (mg/100 g): retinyl acetate, 16; cholecalciferol, 0.6; all-rac-α-tocopheryl acetate, 1,200; menadione, 6; thiamine · HCl, 59; riboflavin, 59; pyridoxine-HCl, 29; cyanocobalamine, 0.2; ascorbic acid, 588; biotin, 1; folic acid, 2; calcium-pantothenate, 235; nicotinic acid, 294; inositol, 1,176.

Table 2. Concentrations of PCDD and PCDF congeners in dioxin mixture dissolved in corn oil.

Dioxin	Concentration (µg/L)
TCDD	50
1,2,3,7,8-PentaCDD	50
1,2,3,4,7,8-HexaCDD	50
1,2,3,6,7,8-HexaCDD	50
1,2,3,7,8,9-HexaCDD	50
1,2,3,4,6,7,8-HeptaCDD	50
1,2,3,4,6,7,8,9-OctaCDD	100
2,3,7,8-TCDF	50
1,2,3,7,8-PentaCDF	50
2,3,4,7,8-PentaCDF	50
1,2,3,4,7,8-HexaCDF	50
1,2,3,6,7,8-HexaCDF	50
1,2,3,7,8,9-HexaCDF	50
2,3,4,6,7,8-HexaCDF	50
1,2,3,4,6,7,8-HeptaCDF	50
1,2,3,4,7,8,9-HeptaCDF	50
1,2,3,4,6,7,8,9-OctaCDF	100

tube (50 mL) for centrifugation; we then added 200 pg stable isotope tracer, ^{13}C-labeled internal standard of tetra–hepta CDDs and tetra–hepta CDFs, and/or 1,000 pg ^{13}C-labeled internal standard of octaCDDs and octaCDFs to the homogenate. We added 10 mL 1.5 M KOH in ethanol to each sample, and the sample was then hydrolyzed overnight at room temperature. The alkali hydrolysates of each sample were shaken with 10 mL hexane plus 5 mL H_2O and centrifuged at 2,500 rpm for 10 min and hexane layers were collected. The aqueous layer was extracted 2 times with 10 mL hexane. The collected hexane was washed with 5 mL H_2O and concentrated to 20 mL. The subsequent procedures were the same as for the dioxin analysis of fecal samples.

Dioxin analysis was performed using gas chromatography-mass spectrometry (AutoSpec-Ultima; Micromass Ltd., Manchester, England) with a capillary column (0.25 mm × 60 m, BPX5; SGE Co., Yokohama, Japan) and setting the resolution mode at 10,000; quantification was performed in the selected ion monitoring acquisition mode.

We calculated the inhibition of gastrointestinal absorption in the chlorophyll group compared with the control group using the following equation: inhibition of gastrointestinal absorption due to the chlorophyll diet (%) = {[gastrointestinal absorption in the control group (%)] – [gastrointestinal absorption in the chlorophyll group (%)]}/{[gastrointestinal absorption in the control group (%)] × 100}.

Statistics

We tested differences between the control group and the chlorophyll groups by one-way analysis of variance (ANOVA) using StatView for the Macintosh (Brain Power, Calabasas, CA, USA). A p-value of < 0.05 was considered significant.

Results

Effects of chlorophyll on body weight, food intake, and fecal weight. There was no significant difference in body weight gain or food intake between the control group and all of the six chlorophyll groups (Table 3). Total fecal weight during days 1–5 was significantly lower ($p < 0.01$) in the rats fed the 0.1% chlorophyll diet compared with those fed the basal diet and unchanged in the other chlorophyll groups.

Effect of chlorophyll on fecal excretion of PCDD and PCDF congeners. In the control group, the percentage of fecal excretion of PCDD congeners from days 1 to 5 in rats administered the dioxin mixture was 1.4% for TCDD, 3.6% for 1,2,3,7,8-pentaCDD, 9.5% for 1,2,3,4,7,8-hexaCDD, 11.4% for 1,2,3,6,7,8-hexaCDD, 21.7% for 1,2,3,7,8,9-hexaCDD, 41.1% for 1,2,3,4,6,7,8-heptaCDD, and 72.9% for 1,2,3,4,6,7,8,9-octaCDD; that of PCDF congeners was 1.1% for 2,3,7,8-TCDF, 4.7% for 1,2,3,7,8-pentaCDF, 3.0% for 2,3,4,7,8-pentaCDF, 14.9% for 1,2,3,4,7,8-hexaCDF, 14.0% for 1,2,3,6,7,8-hexaCDF, 13.5% for 1,2,3,7,8,9-hexaCDF, 14.9% for 2,3,4,6,7,8-hexaCDF, 41.5% for 1,2,3,4,6,7,8-heptaCDF, 33.1% for 1,2,3,4,7,8,9-heptaCDF, and 65.0% for 1,2,3,4,6,7,8,9-octaCDF (Figure 1). The percentage of fecal excretion of TCDD and 2,3,7,8-TCDF, which are resistant to metabolic degradation and show a tendency to be stored in the body, was lower than that of penta–octa CDD and CDF congeners. In fact, the percentage of fecal excretion of penta–octa CDD and CDF congeners was increased compared to the TCDD and 2,3,7,8-TCDF congeners in the control group. On the other hand, in the 0.01% chlorophyll group, the percentage of fecal excretion of PCDD congeners was

Table 3. Effect of the chlorophyll diet on food intake, body weight gain, and feces weight in rats administered the dioxin mixtures.

Group	Food intake (g/5 days)	Body weight gain (g/5 days)	Feces weight (g/5 days)
Basal diet	105.4 ± 1.0	52.8 ± 2.8	5.4 ± 0.2
0.01% chlorophyll	103.6 ± 2.0	53.0 ± 0.9	5.4 ± 0.5
0.02% chlorophyll	104.3 ± 1.3	53.3 ± 2.8	5.3 ± 0.2
0.05% chlorophyll	102.9 ± 3.2	52.8 ± 3.5	5.0 ± 0.3
0.1% chlorophyll	101.5 ± 8.2	57.6 ± 4.9	4.5 ± 0.2*
0.2% chlorophyll	102.8 ± 4.0	56.2 ± 3.5	5.3 ± 0.8
0.5% chlorophyll	103.7 ± 4.6	53.0 ± 3.4	5.8 ± 0.3

Values represent the mean ± SD ($n = 4$). Rats consumed 0.2 mL corn oil containing 7 types of PCDD and 10 types of PCDF congeners.
*Significantly different from basal group ($p < 0.01$).

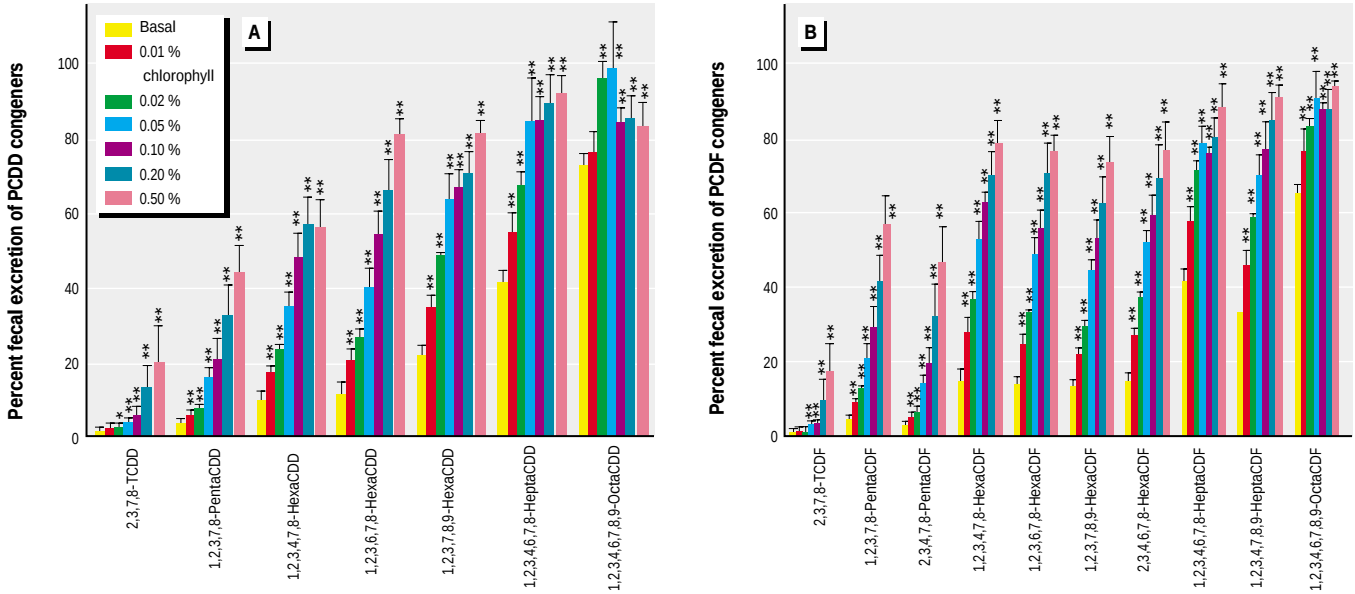

Figure 1. Effect of the chlorophyll diet on fecal excretion of PCDD and PCDF congeners in rats administered the dioxin mixture. Values are mean ± SD ($n = 4$).
*$p < 0.05$ and **$p < 0.01$ as compared to the control group was estimated using one-way ANOVA.

2.3–76.4% and that of PCDF congeners was 1.7–76.7%. The 0.01% chlorophyll diet significantly accelerated the fecal excretion of five types of PCDD and eight types of PCDF congeners, except TCDD, 1,2,3,4,6,7,8,9-octaCDD, 2,3,7,8-TCDF, and 1,2,3,4,6,7,8,9-octaCDF.

The fecal excretion of TCDD was significantly increased in the 0.02–0.5% chlorophyll group, and the fecal excretion of 2,3,7,8-TCDF was significantly increased in the 0.05–0.5% chlorophyll group compared to the control group. The fecal excretion of penta–octa CDD and CDF congeners, which show a tendency to be excreted from the body, were significantly increased in the 0.01–0.5% chlorophyll group compared to the control group. In rats fed the 0.02–0.5% chlorophyll diets, the fecal excretions of TCDD, 1,2,3,7,8-pentaCDD, and 2,3,4,7,8-pentaCDF, which are highly toxic (*33*), were 72.1–1265.1%, 107.6–1148.6%, and 127.3–1470.5% greater, respectively ($p <$ 0.05 or $p < 0.01$), than those of the control group. In rats fed 0.02–0.5% chlorophyll diets, the amounts of fecal excretion of 1,2,3,4,6,7,8,9-octaCDD and 1,2,3,4,6,7,8,9-octaCDF, which are less toxic, were 14.0–32.6% and 25.6–41.0% greater, respectively ($p < 0.01$), than those of the control group. The percent inhibition of absorption in rats fed the 0.01–0.5% chlorophyll diets was 0.9–18.7%, 2.4–42.2%, and 2.4–45.1% for the highly toxic TCDD, 1,2,3,7,8-pentaCDD, and 2,3,4,7,8-pentaCDF congeners, respectively. On the other hand, the percent inhibition of absorption in rats fed the 0.01–0.5% chlorophyll diets was 12.9–95.6% and 33.4–82.1% for the less toxic 1,2,3,4,6,7,8,9-octaCDD and 1,2,3,4,6,7,8,9-octaCDF, respectively. Overall, the gastrointestinal absorption of these compounds was strikingly inhibited in rats fed the chlorophyll diet. The fecal excretion of PCDD and PCDF congeners was increased in proportion to the chlorophyll content of the diet from 0.01% to 0.5% chlorophyll. These results imply that all of the compounds are resistant to metabolism, and the results for the fecal excretion of PCDD and PCDF congeners show that the percentage found in the feces clearly increased with chlorophyll and log P/lipophilicity of the specific congeners.

Effect of chlorophyll on the level of dioxin stored in rats' bodies. The amounts of PCDD and PCDF congeners stored in the bodies of rats administered the dioxin mixture on day 5 are shown in Figure 2. In the control group, the amounts of PCDD congeners stored in the bodies of rats administered the dioxin mixture were 22.7–94.2%, and those of PCDF congeners were 20.4–95.6%. The amounts of hepta–octa CDD and CDF congeners stored in the body were lower than the amounts of tetra–hexa CDD and CDF congeners, except 2,3,7,8-TCDF and 1,2,3,7,8-pentaCDF, in the control group. The amounts of 2,3,7,8-TCDF and 1,2,3,7,8-pentaCDF stored in the body were lowered, although the fecal excretion of these PCDF congeners was lower. These inconsistent results imply that 2,3,7,8-TCDF and 1,2,3,7,8-pentaCDF absorbed in the body were catabolized and metabolized to other metabolites. These data are consistent with those of Brewster and Birnbaum (*22*,*23*). On the other hand, in the 0.01% chlorophyll group, the amounts of PCDD congeners stored in the body were 12.3–89.8%, and the amounts of PCDF congeners were 11.3–92.1%. The 0.01% chlorophyll diet significantly lowered the amounts of three types of PCDD congeners compared to the control group (72.8% vs. 84.5% for 1,2,3,6,7,8-hexaCDD, 60.6% vs. 75.6% for 1,2,3,7,8,9-hexaCDD, and 36.3% vs. 57.4% for 1,2,3,4,6,7,8-heptaCDD) and four types of PCDF congeners (68.9% vs. 86.2% for 1,2,3,4,7,8-hexaCDF, 70.6% vs. 83.5% for 1,2,3,6,7,8-hexaCDF, 27.9% vs. 43.6% for 1,2,3,4,6,7,8-heptaCDF, and 44.8% vs. 62.7% for 1,2,3,4,7,8,9-heptaCDF) stored in the body. The amounts of TCDD stored in the body were lowered significantly in the 0.1–0.5% chlorophyll group compared to levels in the control group, whereas the amounts of 2,3,7,8-TCDF stored in the body were unchanged in all the chlorophyll groups compared with the control group. Thus, chlorophyll administration markedly accelerated the excretion of PCDD and PCDF congeners in the feces of rats administered the dioxin mixture. When the dioxin mixture was administered to rats fed the chlorophyll diet, the amounts of PCDD and PCDF congeners in the body were decreased in proportion to the chlorophyll content of the chlorophyll diet from 0.01% to 0.5%.

Discussion

We previously reported the effect of several types of dietary fiber derived from rice bran, spinach, cabbage, corn, and carrots on dioxin excretion in feces (*15*). The amounts of excretion of PCDD and PCDF congeners

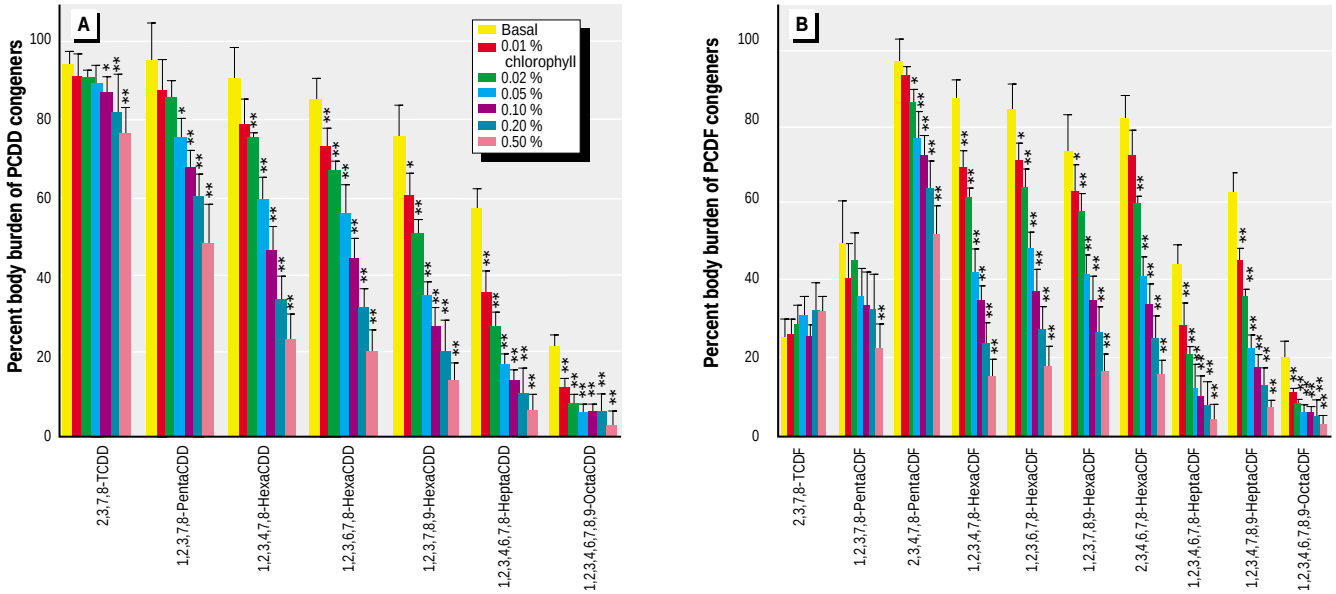

Figure 2. Effect of the chlorophyll diet on total body burden of PCDD and PCDF congeners in rats administered the dioxin mixture. Values are mean ± SD ($n = 4$). *$p < 0.05$ and **$p < 0.01$ as compared to the control group was estimated using one-way ANOVA.

into the feces in rats fed a 10% rice bran fiber diet were 60–370% and 40–1,040% greater than those in rats fed a control diet, respectively. We have more recently reported that *Chlorella* enhanced dioxin excretion more markedly than rice bran fiber. The amounts of excretion of PCDD and PCDF congeners in feces of rats fed a 10% *Chlorella* diet were 20–1,130% and 30–1,280% greater than those in rats fed a control diet, respectively (*19*). Moreover, we have reported that green vegetables such as spinach, perilla, and mitsuba augmented the fecal excretion of dioxin compared with vegetables such as cabbage, onion, and celery (*24*).

In this study, we found that the percent absorptions of TCDD, 1,2,3,7,8-pentaCDD, and 2,3,4,7,8-pentaCDF were 95.3–98.6% in rats, as shown in Figure 1. We thus obtained evidence that the percent absorptions of PCDD and PCDF congeners in rats resembled those in breast-fed infants. This finding is in accordance with the reports of Abraham et al. (*25*), Mclachlan (*26*), and Pluim et al. (*27*). However, there is a disagreement as to the percent absorption of hexa–octa CDD and CDF congeners between our findings in this study and those presented in these reports. The percent absorptions in breast-fed infants appears to be > 95% for almost all congeners (*27*); therefore, in the case of breast-fed infants exposed to a lower dose of PCDD and PCDF congeners, the percent absorption of hexa–octa CDD and CDF congeners appears to be higher than that in rats exposed to a higher dose of PCDD and PCDF congeners. The present results have been recalculated to obtain nanogram TEQ values, shown in Tables 4 and 5. The amounts of fecal excretion of PCDD and PCDF congeners and the amounts of body burden of PCDD and PCDF congeners in rats fed a chlorophyll diet were greater than those of rats fed a control diet in a dose-dependent manner. The preferential effects of chlorophyll on low TEQ compounds such as 1,2,3,4,6,7,8,9-octaCDD and 1,2,3,4,6,7,8,9-octaCDF may indicate a slighter detoxification effect than that on high TEQ compounds such as TCDD, 1,2,3,7,8-pentaCDD, and 2,3,4,7,8-pentaCDF. These results are critical for evaluating the efficacy of chlorophyll as an agent for decreasing intake of toxic PCDD and PCDF congeners. Chlorophyll administration enhanced the fecal excretion of PCDD and PCDF congeners and reduced PCDD and PCDF congeners absorption in rats administered the dioxin mixture. However, the doses of PCDD and PCDF congeners used in this study are high, and some question remains whether chlorophyll has the same effect at low doses, which is similar to the natural

Table 4. Effect of the chlorophyll diet on fecal excretion of PCDD and PCDF congeners in rats administered the dioxin mixture.

		Chlorophyll diet (%)						
Dioxins	ng TEQ	0 (Basal diet)[a]	0.01[a]	0.02[a]	0.05[a]	0.1[a]	0.2[a]	0.5[a]
TCDD	10	0.43 ± 0.14	0.67 ± 0.17 (155.8)	0.74 ± 0.15 (172.1)**	0.99 ± 0.20 (230.2)*	1.69 ± 0.51 (393.0)*	3.90 ± 1.67 (907.0)*	5.87 ± 2.48 (1365.1)*
1,2,3,7,8-PentaCDD	10	1.05 ± 0.28	1.73 ± 0.12 (164.8)*	2.18 ± 0.25(207.6)*	4.14 ± 0.77 (394.3)*	6.14 ± 1.50 (584.8)*	9.60 ± 2.31 (914.3)*	13.11 ± 2.09 (1248.6)*
1,2,3,4,7,8-HexaCDD	1	0.28 ± 0.06	0.50 ± 0.06 (178.6)*	0.69 ± 0.04(246.4)*	0.95 ± 0.13 (339.3)*	1.42 ± 0.18 (507.1)*	1.68 ± 0.21 (600.0)*	1.95 ± 0.19 (696.4)*
1,2,3,6,7,8-HexaCDD	1	0.34 ± 0.07	0.59 ± 0.07 (173.5)*	0.78 ± 0.05(229.4)*	1.08 ± 0.15 (317.6)*	1.60 ± 0.17 (470.6)*	1.95 ± 0.22 (573.5)*	2.39 ± 0.12 (702.9)*
1,2,3,7,8,9-HexaCDD	1	0.65 ± 0.09	1.03 ± 0.12 (158.5)*	1.43 ± 0.04 (220.0)*	1.71 ± 0.21 (263.1)*	1.98 ± 0.13 (304.6)*	2.09 ± 0.19 (321.5)*	2.41 ± 0.10 (370.8)*
1,2,3,4,6,7,8-HeptaCDD	0.1	0.12 ± 0.01	0.16 ± 0.01 (133.3)*	0.20 ± 0.01 (166.7)*	0.22 ± 0.03 (183.3)*	0.25 ± 0.02 (208.3)*	0.27 ± 0.02 (225.0)*	0.27 ± 0.01 (225.0)*
1,2,3,4,6,7,8,9-OctaCDD	0.002	0.0043 ± 0.0002	0.0045 ± 0.0003 (104.7)	0.0057 ± 0.0003 (132.6)*	0.0052 ± 0.0008 (120.9)	0.0050 ± 0.0003 (116.3)	0.0051 ± 0.0003 (118.6)	0.0049 ± 0.0004 (114.0)
2,3,7,8-TCDF	1	0.03 ± 0.01	0.05 ± 0.01 (166.7)	0.05 ± 0.01 (166.7)	0.08 ± 0.02 (266.7)*	0.11 ± 0.04 (366.7)*	0.29 ± 0.17 (966.7)*	0.52 ± 0.23 (1773.3)*
1,2,3,7,8-PentaCDF	0.5	0.07 ± 0.02	0.13 ± 0.02 (185.7)*	0.19 ± 0.02 (271.4)*	0.26 ± 0.05 (371.4)*	0.43 ± 0.08 (614.3)*	0.62 ± 0.11 (885.7)*	0.84 ± 0.12 (1200.0)*
2,3,4,7,8-PentaCDF	5	0.44 ± 0.13	0.78 ± 0.08 (177.3)*	1.00 ± 0.14 (227.3)*	1.81 ± 0.39 (411.4)*	2.86 ± 0.69 (650.0)*	4.76 ± 1.30 (1081.8)*	6.91 ± 1.44 (1570.5)*
1,2,3,4,7,8-HexaCDF	1	0.44 ± 0.07	0.83 ± 0.11 (188.6)*	1.09 ± 0.05 (247.7)*	1.45 ± 0.15 (329.5)*	1.86 ± 0.09 (422.7)*	2.07 ± 0.21 (470.5)*	2.33 ± 0.19 (529.5)*
1,2,3,6,7,8-HexaCDF	1	0.41 ± 0.06	0.73 ± 0.08 (178.0)*	0.98 ± 0.05 (239.0)*	1.38 ± 0.10 (336.6)*	1.65 ± 0.15 (402.4)*	2.09 ± 0.27 (509.8)*	2.27 ± 0.13 (553.7)*
1,2,3,7,8,9-HexaCDF	1	0.40 ± 0.06	0.65 ± 0.07 (162.5)*	0.88 ± 0.04 (220.0)*	1.25 ± 0.09 (312.5)*	1.57 ± 0.15 (392.5)*	1.86 ± 0.24 (465.0)*	2.17 ± 0.21 (542.5)*
2,3,4,6,7,8-HexaCDF	1	0.44 ± 0.06	0.81 ± 0.09 (184.1)*	1.11 ± 0.03 (252.3)*	1.47 ± 0.12 (334.1)*	1.76 ± 0.15 (400.0)*	2.05 ± 0.20 (465.9)*	2.27 ± 0.16 (515.9)*
1,2,3,4,6,7,8-HeptaCDF	0.1	0.12 ± 0.01	0.17 ± 0.01 (141.7)*	0.21 ± 0.01 (175.0)*	0.22 ± 0.01 (183.3)*	0.23 ± 0.01 (191.7)*	0.24 ± 0.01 (200.0)*	0.26 ± 0.02 (216.7)*
1,2,3,4,7,8,9-HeptaCDF	0.1	0.10 ± 0.01	0.14 ± 0.01 (140.0)*	0.17 ± 0.00 (170.0)*	0.19 ± 0.02 (190.0)*	0.23 ± 0.02 (230.0)*	0.25 ± 0.02 (250.0)*	0.27 ± 0.01 (270.0)*
1,2,3,4,6,7,8,9-OctaCDF	0.002	0.0039 ± 0.0002	0.0046 ± 0.0004 (117.9)	0.0049 ± 0.0001(125.6)*	0.0050 ± 0.0005 (128.2)*	0.0052 ± 0.0001 (133.3)*	0.0052 ± 0.0003 (133.3)*	0.0055 ± 0.0001 (141)*
Total	33.804	5.32 ± 1.01	8.97 ± 0.48 (168.6)*	11.69 ± 0.56 (219.7)*	17.22 ± 2.38 (323.7)[a]	23.77 ± 3.78 (446.8)*	33.71 ± 6.93 (633.6)*	43.83 ± 6.63 (823.9)*

Values represent the mean ± SD ($n = 4$).
[a]Values represent the percent fecal excretion of PCDD and PCDF congeners from days 1 to 5 against total nanograms of TEQ values in rats administered PCDD and PCDF congeners; the acceleration index of fecal excretion (shown in parentheses) equals (the percent fecal excretion of PCDD and PCDF congeners of rats in the chlorophyll diet)/(the percent fecal excretion of PCDD and PCDF congeners of rats in the control diet) × 100. *Significantly different from basal group ($p < 0.01$). **Significantly different from basal group ($p < 0.05$).

Table 5. Effect of body burden of PCDD and PCDF congeners in rats administered the dioxin mixture.

		Chlorophyll diet						
Dioxins	ng TEQ	0 (Basal diet)[a]	0.01[a]	0.02[a]	0.05[a]	0.1[a]	0.2[a]	0.5[a]
TCDD	10	27.52 ± 0.81	26.57 ± 1.67 (96.5)	26.67 ± 0.44 (96.9)	26.16 ± 1.59 (95.1)	25.46 ± 1.26 (92.5)**	24.09 ± 2.52 (87.5)**	22.31 ± 1.69 (81.1)*
1,2,3,7,8-PentaCDD	10	27.86 ± 2.64	25.69 ± 2.32 (92.2)	25.13 ± 1.27 (90.2)	22.21 ± 1.53 (79.7)*	19.93 ± 1.17 (71.5)*	17.77 ± 1.84 (63.8)*	14.19 ± 2.57 (50.9)*
1,2,3,4,7,8-HexaCDD	1	2.65 ± 0.22	2.32 ± 0.16 (87.5)	2.22 ± 0.04 (83.8)*	1.75 ± 0.18 (66.0)*	1.37 ± 0.19 (51.7)*	1.01 ± 0.19 (38.1)*	0.71 ± 0.19 (26.8)*
1,2,3,6,7,8-HexaCDD	1	2.50 ± 0.08	2.15 ± 0.10 (86.0)*	1.98 ± 0.05 (79.2)*	1.65 ± 0.22 (66.0)*	1.32 ± 0.16 (52.8)*	0.96 ± 0.15 (38.4)*	0.62 ± 0.11 (24.8)*
1,2,3,7,8,9-HexaCDD	1	2.23 ± 0.23	1.79 ± 0.16 (80.3)**	1.51 ± 0.08 (67.7)*	1.05 ± 0.09 (47.1)*	0.81 ± 0.15 (36.3)*	0.63 ± 0.19 (28.3)*	0.41 ± 0.13 (18.4)*
1,2,3,4,6,7,8-HeptaCDD	0.1	0.17 ± 0.01	0.11 ± 0.02 (64.7)*	0.08 ± 0.00 (47.1)*	0.05 ± 0.01 (29.4)*	0.04 ± 0.01 (23.5)*	0.03 ± 0.01 (17.6)*	0.02 ± 0.01 (11.8)*
1,2,3,4,6,7,8,9-OctaCDD	0.002	0.0014 ± 0.0002	0.0007 ± 0.0001 (50.0)	0.0005 ± 0.0001 (35.7)	0.0003 ± 0.0001 (21.4)**	0.0004 ± 0.0001 (28.6)*	0.0004 ± 0.0003 (28.6)*	0.0002 ± 0.0001 (14.3)*
2,3,7,8-TCDF	1	0.73 ± 0.16	0.76 ± 0.11 (104.1)	0.83 ± 0.16 (113.7)	0.90 ± 0.16 (123.3)	0.73 ± 0.08 (100.0)	0.94 ± 0.20 (128.8)	0.93 ± 0.12 (127.4)
1,2,3,7,8-PentaCDF	0.5	0.73 ± 0.16	0.59 ± 0.14 (80.8)	0.66 ± 0.11 (90.4)	0.52 ± 0.11 (71.2)	0.48 ± 0.13 (65.8)	0.47 ± 0.14 (64.4)	0.33 ± 0.10 (45.2)*
2,3,4,7,8-PentaCDF	5	14.14 ± 0.83	13.62 ± 0.29 (96.3)	12.54 ± 0.58 (88.7)**	11.25 ± 1.03 (79.6)*	10.57 ± 0.66 (74.8)*	9.31 ± 1.07 (65.8)*	7.61 ± 0.96 (53.8)*
1,2,3,4,7,8-HexaCDF	1	2.55 ± 0.15	2.04 ± 0.13 (80.0)*	1.81 ± 0.05 (71.0)*	1.23 ± 0.13 (48.2)*	1.01 ± 0.12 (39.6)*	0.69 ± 0.14 (27.1)*	0.44 ± 0.12 (17.3)*
1,2,3,6,7,8-HexaCDF	1	2.47 ± 0.19	2.09 ± 0.12 (84.6)**	1.88 ± 0.10 (76.1)*	1.40 ± 0.12 (56.7)*	1.08 ± 0.17 (43.7)*	0.80 ± 0.19 (32.4)*	0.51 ± 0.13 (20.6)*
1,2,3,7,8,9-HexaCDF	1	2.16 ± 0.28	1.85 ± 0.21 (85.6)	1.70 ± 0.16 (78.7)**	1.21 ± 0.13 (56.0)*	1.02 ± 0.18 (47.2)*	0.78 ± 0.20 (36.1)*	0.48 ± 0.13 (22.2)*
2,3,4,6,7,8-HexaCDF	1	2.41 ± 0.16	2.13 ± 0.17 (88.4)	1.76 ± 0.05 (73.0)*	1.20 ± 0.14 (49.8)*	0.99 ± 0.15 (41.1)*	0.73 ± 0.16 (30.3)*	0.46 ± 0.13 (19.1)*
1,2,3,4,6,7,8-HeptaCDF	0.1	0.13 ± 0.02	0.08 ± 0.01 (61.5)*	0.06 ± 0.00 (46.2)*	0.04 ± 0.01 (30.8)*	0.03 ± 0.01 (23.1)*	0.02 ± 0.01 (15.4)*	0.01 ± 0.01 (7.7)*
1,2,3,4,7,8,9-HeptaCDF	0.1	0.19 ± 0.01	0.13 ± 0.01 (68.4)*	0.11 ± 0.00 (57.9)*	0.07 ± 0.01 (36.8)*	0.05 ± 0.01 (26.3)*	0.04 ± 0.02 (21.1)*	0.02 ± 0.01 (10.5)*
1,2,3,4,6,7,8,9-OctaCDF	0.002	0.0012 ± 0.0002	0.0007 ± 0.0001 (58.3)	0.0005 ± 0.0000 (41.7)	0.0003 ± 0.0002 (25.0)*	0.0004 ± 0.0001 (33.3)*	0.0003 ± 0.0003 (25.0)**	0.0001 ± 0.0001 (8.3)*
Total	33.804	88.44 ± 5.33	81.91 ± 3.86 (92.6)	78.91 ± 2.55 (89.2)**	70.68 ± 3.73 (79.9)*	64.88 ± 3.86 (73.4)*	58.25 ± 6.41 (65.9)*	49.04 ± 5.84 (55.5)*

Values represent the mean ± SD ($n = 4$).
[a]Each value represents percent body burden of PCDD and PCDF congeners against total nanograms of TEQ values in rats administered PCDD and PCDF congeners; acceleration index of disappearance from the body (shown in parentheses) equals (percent body burden of PCDD and PCDF congeners of rats fed the chlorophyll diet)/(percent body burden of PCDD and PCDF congeners of rats fed the control diet) × 100. *Significantly different from basal group ($p < 0.01$). **Significantly different from basal group ($p < 0.05$).

background exposure to these contaminants in most countries.

Chlorella and green vegetables contain large amounts of chlorophyll. The most effective compound in *Chlorella* cells and green vegetables for promoting the fecal excretion of dioxin is likely to be chlorophyll. In this study, the amounts of fecal excretion of TCDD, 1,2,3,7,8-pentaCDD, and 2,3,4,7,8-pentaCDF, which are highly toxic, in rats fed the 0.2% chlorophyll diet were 807%, 814%, and 982% greater than those of the control group, respectively. The amounts of fecal excretion of TCDD, 1,2,3,7,8-pentaCDD, and 2,3,4,7,8-pentaCDF in rats fed a 10% *Chlorella* diet were 1,130%, 560%, and 800% greater than those of the control group, respectively (*19*).

The chlorophyll content of the 10% *Chlorella* diet was 0.2% because the content of chlorophyll in the *Chlorella* cells used in the diet preparation was 2%. These data imply that the effective substance for suppressing the dioxin absorption in *Chlorella* cells is chlorophyll. A 10% *Chlorella* diet augmented the fecal excretion of TCDD, 1,2,3,7,8-pentaCDD, and 2,3,4,7,8-pentaCDF already absorbed in tissues by 350%, 180%, and 250%, respectively, compared to control rats. In future studies using chlorophyll derived from *Chlorella*, we will attempt to obtain data on the excretion of dioxin stored in the body. Moreover, it will be necessary to study the effectiveness of chlorophyll derived from green vegetables instead of from *Chlorella* on the fecal excretion of dioxin. Chlorophyll is considered to have the therapeutic potential of inhibiting dioxin reabsorption similarly to *Chlorella* administration, and we hypothesize that chlorophyll will augment dioxin excretion from the body as a result of accelerating fecal dioxin excretion.

The mechanism by which chlorophyll stimulates dioxin excretion from the body is unclear. Sassa et al. (*28*) reported that the addition of hemin (10^{-5} M) to liver cell cultures completely abolished the induction of uroporphyrin formation by 3,3´,4,4´-tetrachlorobiphenyl (TCB), suggesting that the uroporphyrin induction response may be regulated by the availability of heme in the liver cell. These results represent the structure–activity relationship of the dioxin in relation to porphyrin formation and provide support for the existence of the specific structure–activity relationships that allow this response to occur. It was reported that chlorophyllin, a chlorophyll derivative, formed complexes with heterocyclic amines (*17,18*) and exhibited potent antimutagenic activity. Chlorophyllin may form a complex with dioxins with a planar structure, leading to reduced biotoxicity. The toxicity of dioxin involves reacting with the aryl hydrocarbon (Ah) receptor. TCDD causes transcriptional activation of the cytochrome P450 genes via their interaction with the Ah receptor (*29,30*). It was reported that the chlorophyll from vegetables inhibited cytochrome P450-dependent monooxygenases (*31*). Eating foods such as *Chlorella*, green vegetables, and seaweeds containing chlorophyll and dietary fiber seems to protect against health disorders caused by dioxin exposure in humans by capturing dioxin in the digestive tract and by diminishing the amount of dioxin absorption. These foods serve as a first line of defense against dioxins by acting as interceptor molecules. *Chlorella*, green vegetables, and seaweeds may be useful in promoting the excretion of dioxin from the body.

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