



ELSEVIER

Chemosphere 59 (2005) 297–304

CHEMOSPHERE

www.elsevier.com/locate/chemosphere

Effect of *Chlorella pyrenoidosa* on fecal excretion and liver accumulation of polychlorinated dibenzo-*p*-dioxin in mice

Hideo Takekoshi^{a,b}, Go Suzuki^a, Hirofumi Chubachi^b, Masuo Nakano^{a,c,d,*}

^a Department of Bioresource Science, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido 080-8555, Japan

^b Sun Chlorella Crop., 369 Osaku-cho, Karasuma-dori, Gojo, Shimogyo-ku, Kyoto 600-8177, Japan

^c Hokkaido Medicinal Plant Research Institute, 1-4 Shimoaikappu, Ashoro-cho, Ashoro-gun, Hokkaido 089-3707, Japan

^d Rakuno Gakuen University, 582 Bunkyo-dai-Midorimachi, Ebetsu, Hokkaido 069-8501, Japan

Received 18 December 2003; received in revised form 22 October 2004; accepted 12 November 2004

Abstract

The effect of *Chlorella pyrenoidosa* on fecal excretion and liver accumulation of polychlorinated dibenzo-*p*-dioxin in C57BL/6N mice administered dioxin was examined. Mice were administered 2.2 µg of 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin (H₆CDD) dissolved in corn oil once after a period of acclimatization, after which they were fed either a basal diet, a 10% *C. pyrenoidosa* diet, or a 10% *Spinach* diet, for five weeks. Among mice fed the 10% *C. pyrenoidosa* diet, cumulative fecal excretion of H₆CDD over the first week following administration was significantly greater (9.2-fold) than that observed among mice fed the basal diet. Moreover, excretion during the fifth week following administration of H₆CDD was still significantly greater (3.1-fold) among mice fed the 10% *C. pyrenoidosa* diet than among mice fed the basal diet. Five weeks after administration of H₆CDD, liver accumulation of H₆CDD in mice fed the 10% *C. pyrenoidosa* diet was significantly less than that observed among mice fed either the basal diet and the *Spinach* diet (by 27.9% and 34.8%, respectively).

These findings suggest that *C. pyrenoidosa* may be useful in inhibiting the absorption of dioxins via food and the reabsorption of dioxins stored already in the body in the intestinal tract, thus preventing accumulation of dioxins within the body.

© 2004 Elsevier Ltd. All rights reserved.

Keywords: *Chlorella pyrenoidosa*; Dioxin; 1,2,3,4,7,8-Hexachlorodibenzo-*p*-dioxin; Fecal excretion; Hepatic accumulation; Mice

1. Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and co-planar polychlorinated biphenyls (Co-PCBs) are collectively referred to as dioxins. Dioxins are non-intentional by-products produced during industrial processing as a result of combustion reactions, such as those that occur during rubbish incineration and iron manufacturing.

* Corresponding author. Address: Department of Bioresource Science, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido 080-8555, Japan. Tel.: +81 1562 5 5777; fax: +81 1562 5 4706.

E-mail address: htakekoshi@sunchlorella.co.jp (M. Nakano).

They are also produced during the manufacture and use of chloride-containing chemicals (Olie et al., 1983). Dioxins that have been released into the environment can be present in the air, soil, and water. As dioxins display an extremely high chemical stability and are lipid soluble, these chemicals are primarily introduced into the body through food, as a result of biomagnification in the food chain. Approximately 90% of dioxin intake in humans reportedly occurs through food, and in Japan, 60–80% of this intake is through seafood (Takayama et al., 1991; Tsutsumi et al., 2001). Conversely, in Europe and North America, the primary sources of dioxin are meat, eggs, and dairy products (Brimingham et al., 1989; Schecter et al., 1994, 1995; Jensen and Bolger, 2001). Moreover, daily exposure of humans to dioxin via food has been estimated to range from 0.3 to 3.2 pg of the toxic equivalency quantity (TEQ)/kg body weight/day among the above studies. However, it is understood that the intake of dioxins varies according to the local environment.

Dioxins are absorbed from the digestive tract along with lipids in food due to their lipophilic nature. It has been reported that the digestive tract absorption of the tetra- to hexa-congeners in adult humans via food and in infants via breast milk were also 60–90% of intake (Jödicke et al., 1992; Abraham et al., 1996; Schlummer et al., 1998; Moser and McLachlan, 2001). Furthermore, absorption of 50–90% of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in vegetable oil has been reported in rats, guinea pigs and hamsters (Piper et al., 1973; Rose et al., 1976; Nolan et al., 1979). The biological and toxicological effects of dioxins have been extensively examined in experimental animals, wildlife and humans. TCDD causes both acute and chronic toxicity and has carcinogenic, teratogenic, and immunosuppressive effects in animals (Landers and Bunce, 1991). The effects of dioxins on human health has been investigated in cases of known exposure, such as the Yusho incident in Japan in 1968, and the Yu-Cheng incident in Taiwan in 1979, both of which resulted from the consumption of cooking oil contaminated with dioxins (Hsu et al., 1985; Masuda et al., 1985; Yamashita and Hayashi, 1985). Moreover, it has been reported that the IQs and reading ability of children with a history of high dioxin exposure *in utero*, following maternal ingestion of contaminated fish from lake Michigan in the USA, were inferior relative to children with low dioxin exposure *in utero* (Jacobson and Jacobson, 1996). In general, children are more susceptible to dioxin accumulation. Dioxins appear to transfer to fetus via placenta and concentrate within the breast milk, resulting in high pre- and peri-natal exposure. As things stand at the present time, it is difficult for humans to avoid exposure to dioxins, since they are pervasive in the environment. Consequently, in order to prevent health disorders as a consequence of dioxin exposure in humans, dietary measures which both inhi-

bit absorption and promote the excretion of dioxins are a potentially effective means by which to reduce dioxin accumulation. With regard to the promotion of the excretion of lipophilic contaminants, several studies of dietary supplements, such as cholestyramine, mineral oil, hexadecane, and dietary fiber, in laboratory animals have been reported (Boylan et al., 1978; Rozman et al., 1981; Rozman et al., 1982; Morita et al., 1997b). In addition, Moser et al. reported that the enhancing effect of the non-absorbable lipid substitute olestra on the fecal excretion of PCDD/DFs and PCBs in the body in humans (Moser and McLachlan, 1999).

Chlorella pyrenoidosa is one of the supplements or health food widely utilized in Japan, the USA, Europe, and other countries. *C. pyrenoidosa* is a unicellular green algae that grows in fresh water. It has the highest concentration of chlorophyll and protein of any known plant, and also contains high concentrations of certain vitamins, minerals, dietary fiber, as well as nucleic acid. The protein contained within of *C. pyrenoidosa* contains all of the essential amino acids required for normal growth and maintenance of health in humans. This algae has a strong cell wall, so that the nutritive components in *Chlorella* can only be sufficiently digested by humans after breaking the cell wall (Mitsuda et al., 1977). A number of scientific reports have shown that broken cell wall preparations or extracts of *C. pyrenoidosa*, as well as other *Chlorella* species, promote human health, stimulate the immune system, thereby protecting the host from infection, and enhancing anticancer activity, when given orally or injected (Konishi et al., 1985; Komiyama et al., 1986; Tanaka et al., 1986; Miyazawa et al., 1988; Merchant et al., 1990).

The purpose of this study was to investigate the effects of *C. pyrenoidosa* on the fecal excretion and hepatic accumulation of H₆CDD in mice administered H₆CDD, as well as the relation of the fecal excretion of H₆CDD and sterols. The effect of *C. pyrenoidosa* was compared with those of *Spinach*, green vegetable.

2. Materials and methods

2.1. Animals and experimental diets

Male C57BL/6N mice were purchased from Charles River Japan Inc. (Kanagawa, Japan). All animals were housed individually in cages with a 12 h light–dark cycle. Temperature and humidity were controlled at 22 ± 3 °C and 50 ± 10%, respectively. Mice (five weeks old) were divided randomly into three groups of 12 animals each. The composition of each experimental diet is shown in Table 1. Dried *C. pyrenoidosa* (SUN CHLORELLA strain) powder (*C. pyrenoidosa*) of which the cell wall was broke by DYNO-Mill processing (Mitsuda et al., 1977) was provided by Sun Chlorella Corp. (Kyoto,

animal procedures were in accordance with the NIH guidelines (National Research Council, 1985).

2.2. Analytical procedures

All feces excreted over one week were collected from the mice during the first and fifth weeks after administration of H₆CDD (results are shown in Fig. 1). The weight of the feces excreted over both periods was recorded. After one week or five weeks of the administration of H₆CDD, the mice were killed by ether inhalation and their livers removed and weighed. The feces and livers were stored at –20 °C until use for analysis of dioxin and steroids.

2.3. Chemical analysis

Hexane, methanol, chloroform, dichloromethane, sodium sulfate and sodium chloride were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). These reagents were of the dioxin analysis grade or pesticide grade. All other reagents were of the JIS special grade. The silica gel 60 (0.063–0.200 mm, Merck & Co., Inc., Darmstadt, Germany) used for the multi-layer silica gel column, and the aluminum oxide 90 activity basic (0.063–0.200 mm, Merck & Co., Inc.) used for the alumina column, were column chromatography grade.

Fecal and liver samples (0.5 g) from each mouse were homogenized and quantitatively extracted with 16 ml of chloroform–methanol mixture (2:1, v/v), after being added to 10 ng of ¹³C-labeled H₆CDD standard solution (Wellington Laboratories, Guelph, ON, Canada) dissolved in nonane as the internal standard. The individual extract of each sample was washed with 4 ml of 5% NaCl solution. The chloroform layer was collected by centrifugation at 1000 × g for 15 min. The concentrated chloroform extract was added to 8 ml of 1 M KOH in methanol, and then the sample was hydrolyzed for 2–3 h at room temperature. The alkaline hydrolysates of each sample were shaken with 4 ml of hexane and 8 ml of 5% NaCl solution. The hexane layer was collected by centrifugation at 1000 × g for 15 min. The aqueous layer was twice extracted with 2 ml of hexane. The hexane layer was washed twice with 4 ml of 5% NaCl solution. The hexane extract was concentrated to 2 ml by evaporation. This hexane extract was applied to the multi-layer silica gel column (15 mm id × 300 mm), made from 2% (w/w) KOH–silica gel, 44% (w/w) H₂SO₄–silica gel, 22% (w/w) H₂SO₄–silica gel, and 10% (w/w) AgNO₃–silica gel, in that order, after which the column was eluted with 120 ml of hexane. The eluate was then concentrated to 2 ml. The concentrated eluate was applied to the alumina column (10 mm id × 300 mm), which was filled with 10 g of aluminum oxide 90 (Merck & Co., Inc.), and eluted with 90 ml of dichloromethane–hexane mixture (1:39, v/v), and then

Table 1
Composition of the diets

Component	Diet group		
	Basal diet (g/100 g)	<i>Chlorella</i> diet (g/100 g)	<i>Spinach</i> diet (g/100 g)
Casein	20	20	20
α-Corn starch	15	15	15
Sucrose	55	45	45
Corn oil	5	5	5
Mineral mixture ^a	3.5	3.5	3.5
Vitamin mixture ^b	1	1	1
DL-Methionine	0.3	0.3	0.3
Choline Bitartrate	0.2	0.2	0.2
<i>Chlorella</i> powder	–	10	–
<i>Spinach</i> powder	–	–	10

^a AIN-76 mineral mixture (American Institute of Nutrition, 1977).

^b AIN-76 vitamin mixture (American Institute of Nutrition, 1977).

Japan). Dried *Spinach* (*Spinach oleracea* L.) powder (*Spinach*) was purchased from JA Otofuke Foods Co. Ltd. (Hokkaido, Japan). The experimental groups were fed a diet that contained 10 g/100 g of *C. pyrenoidosa*, or 10 g/100 g of *Spinach*, for either two or six weeks. The *C. pyrenoidosa* and the *Spinach* were composed as follows (g/100 g): moisture, 4.2 and 4.1; chlorophyll, 1.8 and 0.4; total dietary fiber, 9.8 and 6.5; protein (N × 6.25), 66.9 and 27.2; lipid, 11.7 and 2.6; ash, 6.0 and 16.3, respectively. Each mouse was given a single oral dose of 2.2 μg of purified 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin (H₆CDD; Accu Standard Inc., New Haven, CT, USA) dissolved in corn oil after an acclimatization period, during which mice from the three respective groups were fed either the basal diet or the experimental diet for one week (Experimental design shown in Fig. 1). The mice were allowed free access to the experimental diets and water for six weeks. Body weight and food consumption were recorded weekly. All

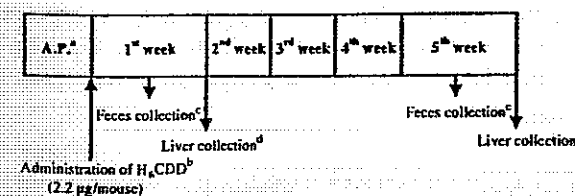


Fig. 1. Experimental design. ^aThe one-week acclimatization period. ^bThe mice were administered 2.2 μg of 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin (H₆CDD) after a one-week acclimatization period, during which they were fed either the basal or the experimental diet. ^cDuring the first and fifth weeks, cumulative feces (produced over one week) were collected from six mice. ^dOne week and five weeks after administration of H₆CDD, livers were collected from six mice within each group.

with 120 ml of dichloromethane–hexane mixture (1:1, v/v). H₆CDD was contained in this second fraction. The second fraction was dried and dissolved in 20 µl of decane. The level of H₆CDD was measured within these samples. H₆CDD was assayed by gas chromatography/mass spectrometry (HRGC/HRMS, HP-6890; Hewlett Packard Com., Palo Alto, CA, USA, Autospec-Ultima; Micromass Ltd., Manchester, UK) with a capillary column (0.32 mm id × 60 m, SP-2331, SUPELCO, Bellefonte, PA, USA), and quantitation was performed in the selected ion monitoring (SIM) mode.

Total lipids were extracted from the feces using a mixture of chloroform/methanol (2:1, v/v) (Folch et al., 1957). The neutral sterol in each total lipid fraction obtained by saponification was acetylated (Matsubara et al., 1990) and analyzed by gas-liquid chromatography (GLC) using a Shimadzu GC-17A (Kyoto, Japan) with a DB-WAX capillary column (0.25 mm id × 30 m; J & W Scientific, Folsom, CA, USA). Acidic sterols in feces were measured by GLC following the method of Grundy et al. (1965).

2.4. Statistical analysis

All data are presented as means ± standard deviations (SD). Statistical analysis was performed by ANOVA. When significance was established, differences among the three groups of data were tested for significance using Tukey's test. Correlation between the excretion of neutral sterols and the excretion of H₆CDD in

feces was evaluated by Pearson product moment analysis. All statistical procedures were performed using SPSS (SPSS Inc., Chicago, IL, USA). The differences were considered significant at $P < 0.05$.

3. Results

3.1. Food intake, mouse growth, and fecal weight

The results are summarized in Table 2. There were no significant differences in food intake or weight gain among all the groups examined. The *C. pyrenoidosa* diet and the *Spinach* diet significantly increased the weight of feces produced over the first and fifth weeks following H₆CDD ingestion, compared to the basal diet.

3.2. Fecal excretion of H₆CDD and lipids

Table 3 shows the amount of H₆CDD excreted in feces during the first and fifth weeks after administration of H₆CDD to mice. The amount of H₆CDD excreted in feces during the first week in the basal diet group, the *C. pyrenoidosa* diet group, and the *Spinach* diet group, was 4.3%, 39.6% and 23.0% of dose, respectively. In mice fed the 10% *C. pyrenoidosa* diet, fecal excretion of H₆CDD during the week following administration was significantly greater than that observed among mice fed the basal diet. Mice fed the 10% *C. pyrenoidosa* diet demonstrated 9.2-times greater fecal excretion of H₆CDD,

Table 2
Food intake, weight gain, and fecal weight of mice fed the basal diet, the *Chlorella* diet, or the *Spinach* diet^a

	Diet group		
	Basal diet	<i>Chlorella</i> diet	<i>Spinach</i> diet
Initial body weight (g)	13.8 ± 1.2	13.5 ± 0.9	12.5 ± 1.4
Food intake (g/five weeks)	100.9 ± 5.4	102.2 ± 4.9	104.5 ± 4.3
Weight gain (g/five weeks)	7.0 ± 1.6	6.2 ± 2.0	7.5 ± 1.5
Fecal weight (g/one week) ^b	0.59 ± 0.05 [#]	1.22 ± 0.18 ^{###}	1.13 ± 0.08 ^{###}
Fecal weight (g/five weeks) ^b	0.86 ± 0.14 [#]	1.53 ± 0.17 ^{###}	1.71 ± 0.32 ^{###}

Values within the same row and not sharing common superscript signs (# and ##) are significantly different at $P < 0.05$.

^a Values represent the mean ± SD of six mice per group.

^b Fecal weight produced over one week in the first and fifth weeks following H₆CDD administration.

Table 3
Fecal excretion of H₆CDD in mice fed the basal diet, the *Chlorella* diet, or the *Spinach* diet^a

	Diet group		
	Basal diet	<i>Chlorella</i> diet	<i>Spinach</i> diet
One week ^b (ng/feces/week)	95.2 ± 39.9 [#]	871.2 ± 62.6 ^{###}	505.8 ± 112.0 ^{###}
Five weeks ^b (ng/feces/week)	9.7 ± 2.5 [#]	29.6 ± 9.5 ^{###}	32.5 ± 7.5 ^{###}

Values within the same row and not sharing common superscript signs (#, ## and ###) are significantly different at $P < 0.05$.

^a Values represent the mean ± SD of six mice per group.

^b Cumulative fecal excretion H₆CDD over one week during the first and fifth weeks post-H₆CDD administration.

Table 4
Bile acids and neutral sterols excretion in mice fed the basal diet, the *Chlorella* diet, or the *Spinach* diet during the fifth week^{a,b}

	Diet group		
	Basal diet	<i>Chlorella</i> diet	<i>Spinach</i> diet
DCA (µmol/feces/week)	0.73 ± 0.21	1.07 ± 0.35	1.20 ± 0.37
LCA (µmol/feces/week)	0.41 ± 0.10	0.81 ± 0.45	0.40 ± 0.21
Cholesterol (µmol/feces/week)	27.11 ± 9.60 [#]	21.10 ± 10.15 ^{###}	12.95 ± 3.92 ^{###}
Coprostanol (µmol/feces/week)	43.03 ± 15.59 [#]	138.47 ± 89.92 ^{###}	330.16 ± 74.64 ^{###}

Values within the same row and not sharing common superscript signs (# and ##) are significantly different at $P < 0.05$.

^a Cumulative bile acids and neutral sterols excretion over the fifth week following H₆CDD administration.

^b Values represent the mean ± SD of six mice per group. DCA; deoxycholic acid. LCA; lithocholic acid.

compared to the basal diet group. Also, fecal excretion of H₆CDD during the first week of the *C. pyrenoidosa* group was significantly greater (1.7-fold) than that of the *Spinach* group. Furthermore, significantly greater cumulative fecal excretion of H₆CDD was observed during the fifth week following H₆CDD administration in the 10% *C. pyrenoidosa* group, compared to the basal diet group. Mice fed the 10% *C. pyrenoidosa* diet had 3.1 times greater excretion of H₆CDD, compared to the basal diet group. Mice fed the 10% *Spinach* diet had 3.4 times greater fecal excretion of H₆CDD during the fifth week, than the basal diet group.

The effect of *C. pyrenoidosa* on fecal excretion of bile acids and neutral sterols during the fifth week post-H₆CDD administration are shown in Table 4. Fecal excretion of deoxycholic acid (DCA) and lithocholic acid (LCA) did not differ among the *C. pyrenoidosa* diet and the basal diet groups. The excretion of coprostanol in mice fed the 10% *Spinach* diet were significantly greater than that excreted by mice on the basal diet.

In general, mice with greater neutral sterols excretion also excreted more H₆CDD in their feces than those demonstrating lower levels of neutral sterols excretion (Fig. 2). The correlation coefficient was 0.646, with a significance level of less than 1%.

3.3. Hepatic accumulation of H₆CDD

Table 5 shows hepatic accumulation of H₆CDD one and five weeks after H₆CDD administration to mice. After one week, hepatic accumulation of H₆CDD in

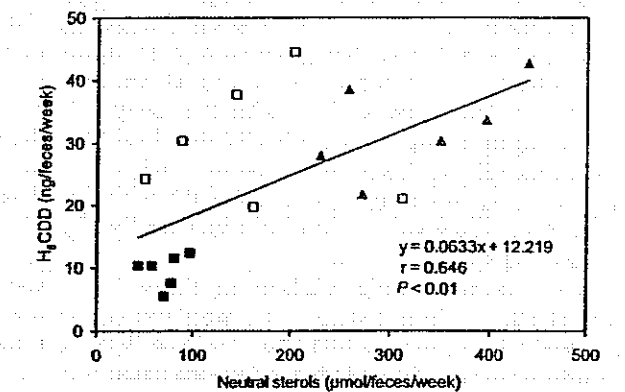


Fig. 2. Correlation between the cumulative fecal excretion of neutral sterols and H₆CDD during the fifth week (The data points represent the amount of neutral sterols or 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin (H₆CDD) excreted in feces during the fifth week post-H₆CDD administration in each mouse. Symbols: (■) Basal diet ($n = 6$); (□) *Chlorella* diet ($n = 6$); (▲) *Spinach* diet ($n = 6$).

mice fed the *C. pyrenoidosa* diet was significantly less than that observed in mice fed the basal diet (by 45.6%). However, a significant difference in hepatic accumulation of H₆CDD was not observed among mice fed the *Spinach* diet, compared to those in the basal diet group. After the fifth week, hepatic accumulation of H₆CDD was significantly lower in the *C. pyrenoidosa* diet group, compared to the basal diet group (72.1% lower). In addition, significantly less hepatic

Table 5
Liver accumulation of H₆CDD in mice fed the basal diet, the *Chlorella* diet, or the *Spinach* diet^a

	Diet group		
	Basal diet	<i>Chlorella</i> diet	<i>Spinach</i> diet
One week ^b (ng/liver)	1019.2 ± 118.9 [#]	465.2 ± 56.1 ^{###}	813.7 ± 103.8 ^{###}
Five weeks ^b (ng/liver)	678.5 ± 145.1 [#]	189.5 ± 40.3 ^{###}	544.6 ± 131.8 ^{###}

Values within the same row and not sharing common superscript signs (#, ## and ###) are significantly different at $P < 0.05$.

^a Values represent the mean ± SD of six mice per group.

^b Feeding period after the administration of H₆CDD.

accumulation of H₆CDD was observed in the 10% *C. pyrenoidosa* diet group (65.2% less), than the 10% *Spinach* diet group, after the fifth week.

4. Discussion

The main route of human exposure by dioxins seems to be through food. Moser and McLachlan (2001) have estimated the gastrointestinal absorption of H₆CDD in human to be approximately 80% of intake. The gastrointestinal absorption of H₆CDD was estimated to be 95% in this study, based on the fecal excretion of H₆CDD and dose in mice fed the basal diet. It is thought that the gastrointestinal absorption of dioxins is very high, regardless of the animal species being studied. Health disorders as a result of dioxins ingestion in humans may be prevented by inhibiting the absorption of dioxins via food from the digestive tract and by promoting the fecal excretion of dioxins stored within the body, thereby reducing the dioxins body burden. In this study, we evaluated the effect of *C. pyrenoidosa* on the elimination of dioxin from the body by examining the fecal excretion and the hepatic accumulation of dioxin in mice administered oral dioxin.

The cumulative excretion of H₆CDD during the week following H₆CDD administration was significantly greater (9.2-fold) in mice fed the 10% *C. pyrenoidosa* diet than in those fed the basal diet. Also, the fecal excretion of H₆CDD during the first week of the *C. pyrenoidosa* group was significantly greater (1.7-fold) than that of the *Spinach* group. This result demonstrates that *C. pyrenoidosa* has an inhibitory effect on absorption of H₆CDD from the digestive tract, thereby promoting H₆CDD excretion into feces. Busbee et al. (1985) have examined the absorption and distribution of Aroclor 1242, a mixture containing Co-PCB, in dogs. They have observed that the absorption of dioxins occurs across the microvilli of epithelial cells of the small intestine by simple diffusion, and then incorporation into chylomicrons in the presence of lipid from food, after which they enter blood from the lymphatic system, travel to the liver via the portal vein, and are stored in adipose tissue or liver. Some of that which is stored within tissue is later excreted with bile into the intestinal tract, after which it may be reabsorbed by the mucosal cells of the intestinal tract. With regard to reabsorption of dioxins, Weber et al. (1982) have described a study in rats in which bile was removed by bile duct cannulation. The authors observed more rapid excretion of TCDD among cannulated rats than among rats in whom bile was allowed to enter the gastrointestinal tract. Moreover, Rozman et al. (1982) reported that intestinal-wall passage in the large intestine is an important route of elimination of hexachlorobenzene, a lipophilic chemical, from the body in rats. These findings suggest that diox-

ins accumulated within the body were eliminated into the intestinal tract by the biliary excretion or the intestinal-wall passage, and then reabsorbed from the intestinal tract. Thus, the amount of dioxins stored within the body might be decreased by promoting fecal excretion of dioxins through inhibition of reabsorption from the intestinal tract. It seems reasonable to suppose that the acceleratory effect of *C. pyrenoidosa* and *Spinach* on elimination of dioxin stored in the body is possible to evaluate by examining the fecal excretion of H₆CDD over the fifth week following oral ingestion of H₆CDD in mice. A significant increase in fecal excretion of H₆CDD over one week was observed in the fifth week following H₆CDD administration in mice fed the 10% *Chlorella* diet and the 10% *Spinach* diet, compared to those fed the basal diet (3.1- and 3.4-fold increases were observed, respectively). This result suggests that *C. pyrenoidosa* and *Spinach* inhibited the reabsorption of H₆CDD excreted into the intestinal tract, thereby promoting the fecal excretion of H₆CDD stored within the body. The mechanism by which *C. pyrenoidosa* has inhibitory effects on absorption and reabsorption of dioxins in the intestinal tract has not been clarified. However, there are several possible factors. Morita et al. (1997a,b) suggest that chlorophyll and dietary fiber contained within *Chlorella* cells might have an important role in inhibiting absorption and reabsorption of dioxins from the intestinal tract. Chlorophyll and chlorophyllin, a copper derivative of chlorophyll, form complexes with heterocyclic amines (Arimoto et al., 1980; Negishi et al., 1989; Dashwood, 1992). It is possible that the chlorophyll or chlorophyll derivatives contained within *C. pyrenoidosa* form complexes with dioxin congeners that have a planar structure, thereby inhibiting their absorption and reabsorption within the digestive tract. The chlorophyll content of *C. pyrenoidosa* used in this study was about 2 g (1100 g dried powder). Absorption of chlorophyll within the digestive tract of rats fed freeze-dried *Chlorella* cells was found to be about 50% (Hayami and Shino, 1964). It is not clear if chlorophyll is absorbed or metabolized by the body, however, it is likely excreted in feces without accumulating in the body. With regard to the effects of dietary fiber, Morita et al. (1997b) have reported that several types of dietary fiber bind dioxins and promote the excretion of dioxin congeners. In this study, there was no significant difference in the fecal excretion of coprostanol, a bacterial metabolite of cholesterol in the intestine, between the *C. pyrenoidosa* diet group and the basal diet group. However, we found that a positive correlation between the amounts of neutral sterols and H₆CDD excreted during the fifth week following administration of H₆CDD to mice. The mechanism of biliary and intestinal excretion of cholesterol, and thereafter metabolism of cholesterol into coprostanol by intestinal microflora may be involved in the fecal excretion of dioxins stored

in the body. It can be seen that the increase in the fecal excretion of coprostanol became an index of the activation of the intestinal microflora (Arjmandi et al., 1992). Therefore, the potential function of *C. pyrenoidosa* as a prebiotic should be considered.

Most dioxins absorbed from the intestinal tract accumulate in liver and adipose tissue in humans and animals (Piper et al., 1973; Gasiewicz et al., 1983; Leung et al., 1990). In this study, we measured hepatic accumulation of H₆CDD as an index of dioxin accumulation within the body. Hepatic accumulation of H₆CDD after the fifth week following administration of H₆CDD was significantly less among mice fed the 10% *C. pyrenoidosa* diet than among mice fed the basal diet (by 27.9%), and likewise than among mice fed the *Spinach* diet (by 34.8%). This result demonstrates that *C. pyrenoidosa* is effective in preventing hepatic accumulation of dioxins through inhibition of absorption and reabsorption of dioxins within the digestive tract. In addition, we calculated the ratio of the hepatic accumulation amount of H₆CDD after the fifth week for the total amount of H₆CDD absorbed by the body. Results for mice fed the basal diet, the 10% *C. pyrenoidosa* diet, and the 10% *Spinach* diet, were 32.2%, 14.3%, and 32.1%, respectively. Accordingly, *C. pyrenoidosa* might accelerate catabolism of H₆CDD within the liver, thereby preventing accumulation of H₆CDD in the liver.

In conclusion, our data suggest that daily intake of *C. pyrenoidosa* may be useful in promoting the excretion of dioxin from the body. Additional studies are required to define the enhancing mechanism of dioxins excretion by *C. pyrenoidosa* including the promotion of catabolism of dioxins in the liver.

References

- Abraham, K., Knoll, A., Ende, M., Pöpke, O., Helge, H., 1996. Intake, fecal excretion, and body burden of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in breast-fed and formula-fed infants. *Pediatr. Res.* 40, 671–679.
- Arimoto, S., Ohara, Y., Namba, T., Negishi, T., Hayatsu, H., 1980. Inhibition of the mutagenicity of amino acid pyrolysis products by hemin and other biological pyrrole pigments. *Biochem. Biophys. Res. Commun.* 92, 662–668.
- Arjmandi, B.H., Ahn, J., Nathani, S., Reeves, R.D., 1992. Dietary soluble fiber and cholesterol affect serum cholesterol concentration, hepatic portal venous short-chain fatty acid concentration and fecal sterol excretion in rats. *J. Nutr.* 122, 246–253.
- Boylan, J.J., Egle, J.L., Guzelian, P.S., 1978. Cholestyramine: Use as a new therapeutic approach for chlordecone (Kepone) poisoning. *Science* 199, 893–895.
- Brimingham, B., Gilman, A., Grant, D., Salminen, J., Boddington, M., Thorpe, B., Wile, I., Toft, P., Armstrong, V., 1989. PCDD/PCDF multimedia exposure analysis for the Canadian population: detailed exposure estimation. *Chemosphere* 19, 637–642.
- Busbee, D.L., Yoo, J.S.H., Norman, J.O., Joe, C.O., 1985. Polychlorinated biphenyl uptake and transport by lymph and plasma components. *Proc. Soc. Exp. Bio. Med.* 179, 116–122.
- Dashwood, R.H., 1992. Protection by chlorophyllin against the covalent binding of 2-amino-3-methylimidazo [4,5-f] quinoline (IQ) to rat liver DNA. *Carcinogenesis* 13, 113–118.
- Folch, J., Lees, M., Sloane-Stanley, G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 226, 497–509.
- Gasiewicz, T.A., Geiger, L.E., Rucci, G., Neal, R.A., 1983. Distribution, excretion, and metabolism of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in C57BL/6J, DBA/2J, and B6D2F₁/J mice. *Drug Metab. Dispos.* 11, 397–403.
- Grundy, S.M., Ahrens Jr., E.H., Miettinen, T.A., 1965. Quantitative isolation and gas-liquid chromatographic analysis of total fecal bile acids. *J. Lipid Res.* 6, 397–410.
- Hayami, K., Shino, K., 1964. Digestibility of chlorophyll in *Chlorella* algae in rats. *Rep. Res. Commit. Essent. Amino Acids* 24, 49–50 (in Japanese).
- Hsu, S.T., Ma, C.I., Hsu, S.K., Wu, S.S., Hsu, N.H., Yeh, C.C., Wu, S.B., 1985. Discovery and epidemiology of PCB poisoning in Taiwan: a four-year followup. *Environ. Health Perspect.* 59, 5–10.
- Jacobson, J.L., Jacobson, S.W., 1996. Intellectual impairment in children exposed to polychlorinated biphenyls in utero. *N. Engl. J. Med.* 335, 783–789.
- Jensen, E., Bolger, P.M., 2001. Exposure assessment of dioxins/furans consumed in dairy foods and fish. *Food Addit. Contam.* 18, 395–403.
- Jödicke, B., Ende, M., Helge, H., Neubert, D., 1992. Fecal excretion of PCDDs/PCDFs in a 3-month-old breast-fed infant. *Chemosphere* 25, 1061–1065.
- Komiyama, K., Hirokawa, Y., Morota, T., Umezawa, I., 1986. An acidic polysaccharide chlon A, from *Chlorella pyrenoidosa*. 2. Antitumor activity and immunological response. *Chemotherapy* 34, 302–307.
- Konishi, F., Tanaka, K., Himeno, K., Taniguchi, K., Nomoto, K., 1985. Antitumor effect induced by a hot water extract of *Chlorella vulgaris* (CE): Resistance to Meth-A tumor growth mediated by CE-induced polymorphonuclear leukocytes. *Cancer Immunol. Immunother.* 19, 73–78.
- Landers, J.P., Bunce, N.J., 1991. The Ah receptor and mechanism of dioxin toxicity. *Biochem. J.* 276, 273–287.
- Leung, H.W., Wendling, J.M., Orth, R., Hilcman, F., Paustenschach, D.J., 1990. Relative distribution of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in human hepatic and adipose tissues. *Toxicol. Lett.* 50, 275–282.
- Matsubara, Y., Sawabe, A., Iizuka, Y., 1990. Structures of new limonoid glycosides in lemon (*Citrus limon* BURM.f.) peelings. *Agric. Biol. Chem.* 54, 1143–1148.
- Masuda, Y., Kuroki, H., Haraguchi, K., Nagayama, J., 1985. PCB and PCDF congeners in the blood and tissues of Yusho and Yu-Cheng patients. *Environ. Health Perspect.* 59, 53–58.
- Merchant, R.E., Rice, C.D., Young, H.F., 1990. Dietary *Chlorella pyrenoidosa* for patients with malignant glioma tumor: Effects on immunocompetence, quality of life, and survival. *Phytotherapy Res.* 4, 220–231.
- Mitsuda, H., Nishikawa, Y., Higuchi, M., Nakajima, K., Kawai, F., 1977. Effect of the breaking of *Chlorella* cells on