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# *Chlorella vulgaris* May Excrete Dioxin-like PCB-138, -153 *via* Urine of Rats

**Ae-son Om[1], Hye-seoung Shin[2], Jae-young Shim[1], Jae-Gab Han[3] & Jae-hyoun Kim[4]**

[1]Department of Food and Nutrition, College of Human Ecology, Hanyang University, Seoul 133-791, Korea
[2]Hankyung National University, Analysis Center, Gyeonggi 456-749, Korea
[3]DAESANG Corp. Health R & D Center, 125-8, Pyokyo-Ri, Majang-Myun, Icheon City, Gyeonggi 467-813, Korea
[4]Department of Health Science, School of Natural Science, Dongduk Women's University, Seoul 136-714, Korea
Correspondence and requests for materials should be addressed to A. S. Om (aesonom@hanyang.ac.kr) and J. H. Kim (kjhyon@dongduk.ac.kr)

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## Abstract

**The effect of *Chlorella vulgaris* (CV) on the urinary excretion of di-ortho PCB congeners (PCB-138, -153) was investigated. Sprague-Dawley rats (6-weeks-old, n=10 rats/group) were randomly divided into one control (0CV) or 2% CV (2CV) or 5% CV (5CV) or 10% CV (10CV) groups, respectively. Composition of normal and chlorella meal-based diet were made up of 30% casein, 15% cornstarch, 50% sucrose, 5% cellulose, 5% coconut oil, 3.5% mineral mixture, 1% vitamin mixture. All rats had free access to water and diet for 4 weeks. A significant increase in both PCB 138 and 153 in urinary level was detected in CV fed groups, 540% and 167% for 2CV, 155% and 89% for 5CV, 114% and 144% for 10CV group, respectively, when compared with their controls. These findings suggest that CV may have potential to eliminate body burden levels of dioxin-like PCB compounds.**

**Keywords:** *Chlorella*, PCB 138, PCB 153, Dioxin-like PCB

Dioxins and polychlorinated biphenyls (PCBs) are groups of compounds that may be carcinogens at low exposure levels over a relatively long period of time[1,2]. These organochlorine compounds cause cancer in animals and are probablely carcinogenic in humans (group 2A classification, International Agency for Research on Cancer). They may have other types of toxicological effects including thymic involution[3-6], immunosuppression[7], nephrotoxicity[8] and teratogenicity in animals[9]. PCB-138 (22′344′5′) and 153 (22′44′55′-) are non-coplanar, hexachloro di-ortho-substituted, and have chlorines at positions 2, 4 and 5. PCB 138 and PCB 153, highly biopersistent PCBs, are predominantly stored in adipose tissue. They are the predominant congeners found in human tissue worldwide and typically account for around 50-60% of the total PCBs[10,11].

Tolerances for PCBs for feeds and foods in USA can be found in the Food Code (21 CFR 19.30 and 509.30). Also, FDA, in conjunction with the European Union and the USDA, is addressing international and domestic dioxin and PCB concerns. Since there are no tolerances or action levels for dioxins in foods or feeds in Korea, the appearance of these compounds in a food or feed supply is of great concern regarding health and food safety. Hence, many scientists have raised concerns to find functional ingredients in foods to influence excretion of dioxins and dioxin-like compounds to reduce their toxicity[12-15].

There has been increasing interest in finding natural food(s) or biomaterial(s) to alleviate the intoxication of toxic substances in the body. In relation to this fact, several studies demonstrated that a calcium rich diet[16], a protein rich diet[17], a diet containing alginate[18], a green tea containing catechin[19], and an extract of the *Omija*[20] can stimulate the excretion of heavy metals. In addition, the optimum amount of dietary copper and iron with increased dietary calcium and protein[21] and vitamin C[22,23] can be involved in the mechanism of metal detoxification.

*Chlorella vulgaris* (CV) is unicellular green algae containing high concentration of chlorophyll and protein, vitamins, minerals, dietary fiber, and nucleic acid[12]. Due to its nutrients characteristics, CV is most commonly sold as food supplements and functional foods in Korea, Japan and US.

Recently, a supplementation of CV has also shown to alleviate the Cd toxicity in rats[24-26]. Morita *et al.*[12] demonstrated that *Chlorella* have the beneficial effects

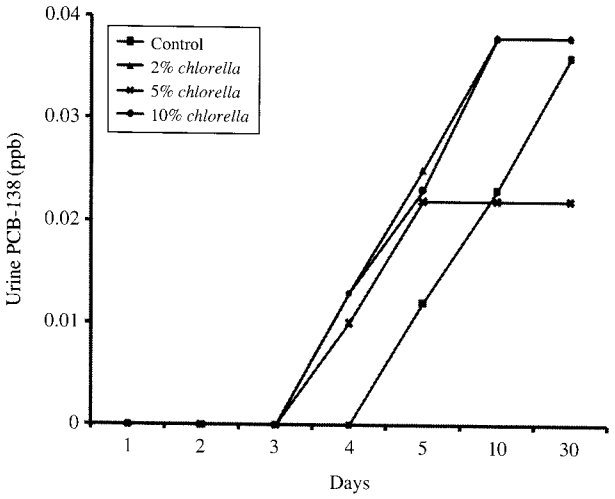


**Figure 1.** Effect of *Chlorella vulgaris* on urinary excretion of PCB138.

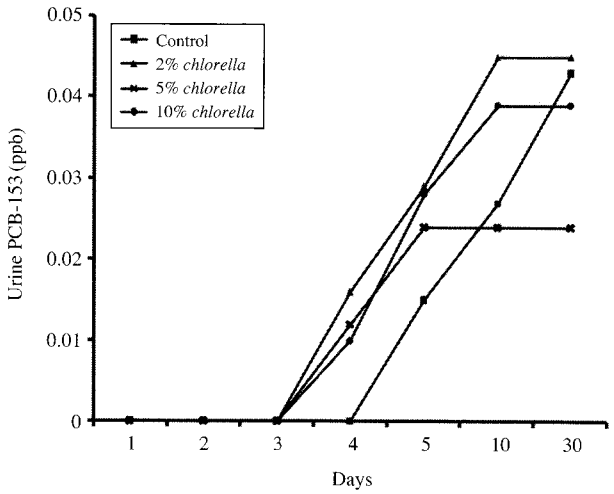


**Figure 2.** Effect of *Chlorella vulgaris* on urinary excretion of PCB153.

on fecal excretion of polychlorinated dibenzo-*p*-dioxin (PCDD) congeners and polychlorinated dibenzofuran (PCDF) congeners in Wistar rats administered the rice oil that caused Yusho disease, as a substitute for purified dioxin. These findings suggest that the administration of *Chlorella* may be useful in preventing gastrointestinal absorption and for promoting the excretion of dioxin already absorbed into the tissues.

There is some research on the relation of CV and dioxin excretion[12-15] however there are only few studies on the excretion of individual PCB congeners, PCB-138 and -152 from both animals and humans. Therefore, this study was performed to examine if CV has influenced excretion of dioxin-like compounds in rats.

### Body Weight Gain

The body weight gain in all the groups increased in a time-dependent manner, ranging from 112 to 127 g in which the result was not statistically significant. Both food intake and total fecal excretions had no statistically significant differences during the experimental period (data is not shown).

### The Urinary Excretion of PCB 138 and PCB 153

Figure 1 and Figure 2 depict the mean of the urinary excretion of PCB 138 and PCB 153 over the 30-day study. The excretion of PCB 138 started on 3$^{rd}$ day in CV-fed groups while that of PCB 138 on 4$^{th}$ day in 0CV group. The maximal concentration of PCB 138 excretion in urine was observed on the 10$^{th}$ day, the 5$^{th}$ day, and the 10$^{th}$ day in 2CV, 5CV, and 10CV group, respectively, while the urinary excretion of PCB 138 had significant decreases by 540%, 155%, and 114% in 2CV, 5CV, and 10CV group, respectively when compared with in 0CV group. Thus, PCB 138 showed a strong association with the excretion by feeding CV in a concentration dependent manner. The urinary excretion of PCB 153 demonstrated a significant enhancement in the CV groups, giving 167% for 2CV, 89% for 5CV, and 144% for 10CV group, compared with in the 0CV group. Similar findings were observed in PCB 153 as was shown with PCB 138, showing that the maximal concentration was shown on the 10$^{th}$ day, the 5$^{th}$ day, and the 10$^{th}$ day in 2CV, 5CV, and 10CV groups, respectively, when compared with 0CV group. It indicated that CV dose-dependently excreted PCBs through urine.

## Discussion

We have previously reported that oral administration of CV enhances excretion of dioxin in rats treated with dioxin[15]. In this study, we investigated if CV would be able to excrete dioxin-like PCB compounds such as PCB 138 and PCB 153 *via* urine.

The body weight gain was measured to check if animals were damaged, which is known as a reliable indicator for health. As we expected, all rats gained weight over the study period and there was no significant effect of CV treatment. Levels of feed intake exerted no significant differences during the experimental period (data is not shown). The values of body weight gain and feed intake were time dependent. Hence, it can be assumed that all rats in this study were in normal status by the end of experiment in 4

weeks.

Although all rats used in this study did not get any chemical treatments, PCBs were detected in their urine samples. Congeners that do not have adjacent un-substituted carbon atoms may be metabolized very slowly and are therefore cleared very slowly according to a study[27]. Then it may be assumed that mothers exposed to PCBs during pregnancy transfer its PCBs to babies through the placental cord while giving birth. Otherwise, all rats might be contaminated by PCBs *via* contamination during the experimental period, since PCBs or polychlorinated biphenyls were used extensively in a wide range of industrial applications. In particular, PCBs were widely used as coolants and lubricants in electrical transformers, capacitors and lamp ballasts.

The excretion of PCB 138 started one day earlier in CV-fed groups than that in 0CV group. The PCB 138 in CV-fed rats was excreted on the 5$^{th}$ day and the 10$^{th}$ day with the maximal concentration unlike that in 0CV-fed rats on the 30$^{th}$ day. The urinary excretion of PCB 138 significantly decreased by feeding chlorella, however, it did not show in concentration dependant manner. Thus, PCB 138 was found to be strongly associated with possible excretion by feeding CV.

Likewise, the urinary excretion of PCB 153 was significantly increased in the CV groups resulting in 167% for 2CV, 89% for 5CV, and 144% for 10CV group, compared with the 0CV group. PCB 153 was excreted in the similar manner to PCB 138. These findings indicated that CV dose-dependently excreted two PCBs *via* urine. 0CV group seems to be excreted slower and is presumed to be associated with direct target organ damage, compared with CV fed groups.

The urinary excretion patterns of two types of PCBs with time following the oral intake of CV are similar among CV fed groups. In particular, rats in 5CV group showed maximal concentration on day 5 of which are half of maximal concentration of rats in 2 and 10CV groups on day 10. This can be considered that animals in 5CV group might be less exposed to PCBs from mothers than those in other groups.

PCBs are resistant to chemical degradation by oxidation or hydrolysis due to lipophilicity. However, biodegradation, especially of lower chlorinated PCBs, can occur. The major route of PCB excretion is in the urine and feces[28]. The net absorption varied widely with the dietary intake for those compounds which bio-accumulate in humans; high dietary intake of chemical resulted in absorption approaching 100% of intake, while low dietary intake resulted in a net excretion several times greater than the dietary intake[29]. Furthermore, excretion rates of the most persistent dioxin-like PCBs such as PCB 138 and PCB 153 were statistically significantly greater for the older subjects, implying that excretion rate is dependent on body burden, and that the majority of dioxin-like PCBs in human feces arise from endogenous excretion[14].

*Chlorella* is well known to have functions to eliminate heavy metals[25,26] and hazard substances[12-15] due to its nutritional characteristics. Morita *et al.*[12,13] has reported that chlorophyll and dietary fiber derived from chlorella inhibit dioxin absorption from gastrointestinal tract and accelerates dioxin excretion in rats. They may function as a chelate which binds to dioxin-like PCBs and then excreted out of body. The present results in our study were in agreement with above evidence that chlorella was effective for inhibiting dioxin absorption and accumulation *via* foods or environmental pathways.

In conclusion, CV played an important role in preventing PCBs accumulation by stimulating urinary excretion of dioxin-like PCBs, such as PCB 138 and 153. Consequently, CV may have potential functions to eliminate toxic substances by endogenous and by exogenous contamination.

Thus, further work is needed to examine these effects in a long term study and elucidate the molecular mechanism by which CV inhibits PCB accumulation and excretion in body. Even though a large amount of unchanged PCB 138 and 153 excreted in feces, the polar metabolites may make up a relatively same proportion of the total output[30]. Thus, additional studies are also required to clarify the exact role of CV on removal of polar or nonpolar PCB derivatives.

## Materials & Methods

### Animals and Diet

Five-week-old male Sprague-Dawley (SD) rats, weighing 90-110 g were allowed to acclimatize for one week prior to commencement of the test. Fifty rats (10 rats/group) were randomly divided into one control group and four groups which included a CV free (0CV) or a 2% CV (2CV) or a 5% CV (5CV) or a 10% CV (10CV) group. All rats received diet with free access to water for 4 weeks. Composition of normal and chlorella meal-based diet were made up 30% casein, 15% cornstarch, 50% sucrose, 5% cellulose, 5% coconut oil, 3.5% mineral mixture, 1% vitamin mixture. These diets had nearly the same composition except that chlorella meal-based diet contains 2%, 5% or 10% chlorella. The animals' treatments and procedures were conducted in accordance with Hanyang University Lab Animal Care Committee (HALACC) animal use protocols. CV powder was obtained from Daesang Wellife Co. (Seoul, Korea).

**Table 1.** GC/MS/MS[1] conditions.

| | | | | | |
|---|---|---|---|---|---|
| Column | Ultra-2 (Cross-linked 5% Phenylmethylsilicon, 25 m × 0.2 mm I.D × 0.33 μm, film thickness) | | | | |
| Carrier Gas | He at 0.8 mL/min | | | | |
| Splitless mode | Purge on time : 0.7 min | | | | |
| Injection port temp. : | 250°C | | | | |
| Transfer line temp. : | 280°C | | | | |
| Oven temp. program : | initial temp. (°C) | initial time (min) | rate (°C/min) | final temp. (°C) | hold time (min) |
| | 50 | 1 | 20 | 200 | 0 |
| | | 10 | 310 | 5 | |
| Run Time : | 24.5 min | | | | |
| (solvent oven delay) | 3 min | | | | |
| SIM mode (m/z) (Selected ion monitoring) | 188, 94, 360, 362, 358 | | | | |

[1]Gas chromatography-mass spectrometry-mass spectrometry

### Analysis of PCB 138 and 153

Urine samples from each rat were homogenized and extracted HS-SPME (head space solid phase micro extraction). The extract of each sample was concentrated to approximately 1 mL. After each adding 10 μL of a phenanthrene-d10 and pyrene-d10 internal standards (Sigma-Aldrich, Louis, MO, USA), 0.02 g of $K_2CO_3$ was added to each sample, to adjust pH 11. The commercial SPME holder for manual use and fobers coated with 85 μm polyacrylate were purchased from Supelco Inc. (57347-U, PA, USA). The fibers were conditioned by heating in the injection port of the GC according to the manufacture. All analyses were performed in 4 mL amber glass vials and the solutions were stirred with a magnetic stir bar (Supelco 27006, PA, USA) at 1,300 rpm using PTFE-coated magnetic stir bars. The GC/MS system consists of a Trace GC and Polaris-Q ion-trap MS direct inlet (Thermo Finnigan, NY, USA). Table 1 shows the GC/MS/MS conditions.

### Statistical Analysis

All data are presented as mean ± standard error (SE). Statistical analysis was performed by ANOVA. When significance was established, differences among the fifth groups of data were tested for significance using Duncan's multiple range test. All statistical procedures were performed using SPSS (SPSS Inc. Chicago, IL, USA). The differences were considered significant at $P<0.05$.

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