

## PROMOTING FECAL EXCRETION OF ACCUMULATED DIOXINS BY PORPHYRIN-DIETARY FIBER

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### Introduction

This study developed a new evacuation substance to promote more efficient excretion dioxin accumulated in the human. Enhancing the excretion by intake of an evacuation substance has been considered an available means of reducing the of dioxin burden in the body. The evacuation substance needs to be capable of obstructing the absorption of dioxins to which our bodies are continuously exposed as well as promote the excretion of the accumulated form. It has already been reported that the intake of water-insoluble high molecular weight dietary fibers showed a high ability to reduce absorption of dioxins compared to water-soluble low molecular weight fibers<sup>1)</sup>. In a previous study, copper-chlorophyllin is known to stimulate elimination of dioxin from the body in experimental animals and is water-soluble<sup>2)</sup>. From these findings, it considered that the binding of copper-chlorophyllin and water-insoluble high molecular weight chitosan enhances their potency in decreasing dioxin absorption. Therefore, the water-insoluble high molecular weight chitosan bearing linked copper- chlorophyllin (chlorophyllin-chitosan) was prepared, and it was appeared that the intake of the prepared chitosan enhanced fecal excretion of dioxin<sup>1)</sup>.

In this study, the ability of chlorophyllin-chitosan to enhance the excretion of dioxin accumulated in body was assessed.

### Methods and Materials

Three groups (4 or 5 mice) of 10-week-old C57BL/6 female mice were used in the experiment. All three groups were given the basal fiber-free diet for 4 days, then treated with a single oral administration of dioxin (1,2,3,4,7,8-HxCDD) dissolved in ethanol : Tween80 : saline (1:10:80) at a dose of 10\_g/kg body weight. The basal fiber-free diet was continuously given to all groups for 3 days and, feces containing HxCDD, which is not absorbed in the intestine, were collected. Thereafter, two groups were maintained on diets containing (0.1% and 1.0% respectively) chlorophyllin-chitosan as shown in Fig. 1 for experimental period of 21day. Another group (control) was further bred on the basal fiber-free diet. Feces were collected every 3 days for 21 days and analyzed for excreted HxCDD using a gas chromatograph-mass spectrometer. Feces excreted from beginning 3 day after administration of HxCDD is considered to contain the excreted form of HxCDD, which is absorbed by the body. In addition, the amount of HxCDD remaining in adipose tissues, which is a main target tissue for accumulating dioxin isomers, was determined after 21 days. The analytical conditions were previously described<sup>3)</sup>.

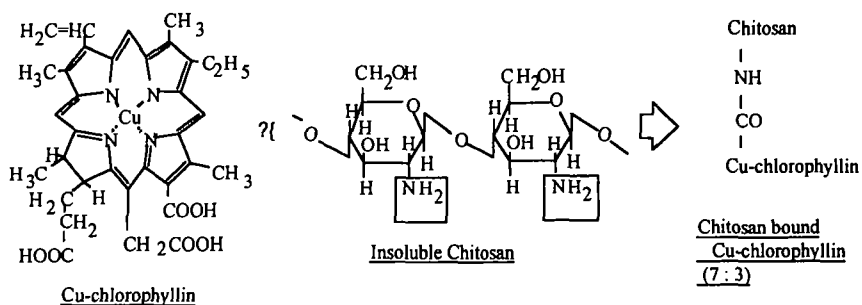


Fig. 1 Structure of Cu-chlorophyllin-chitosan

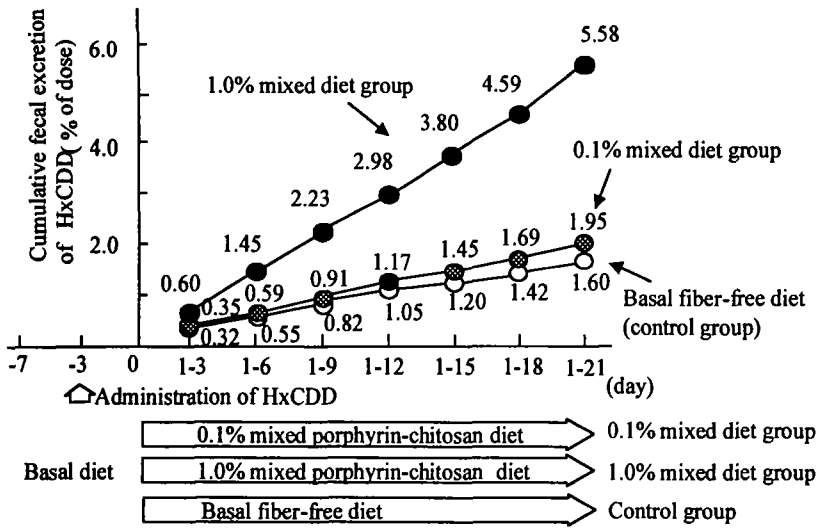
## Results and Discussion

### 1. Promoting fecal excretion of the accumulated HxCDD

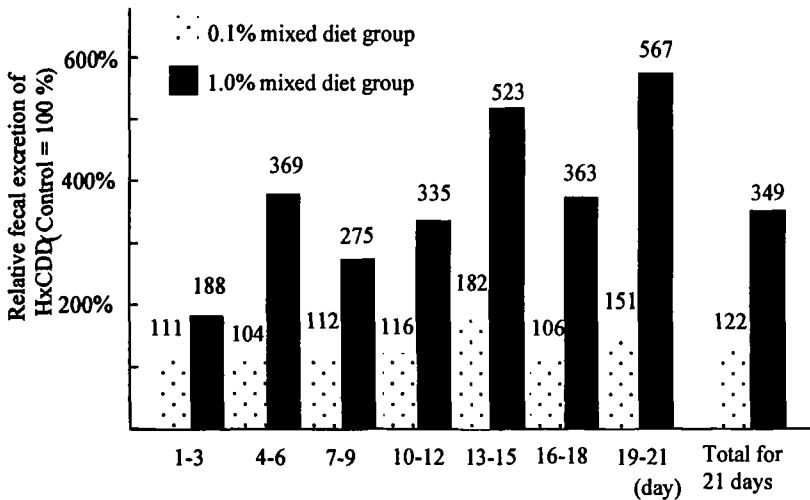
Fig. 2 shows the cumulative amounts of HxCDD excreted in feces every 3 days in the 21-day experimental period when chlorophyllin-chitosan was given to mice beginning 3 days after oral administration of HxCDD. Chlorophyllin-chitosan was mixed with the basal fiber-free diet at a 0.1% and 1.0% ratio, respectively. In the control group given the basal fiber-free diet, HxCDD was excreted in feces at a rate of 0.32% of the dose for first 3 days of the experimental. The cumulative amounts was slightly increased throughout the experimental period, and was increased up to 1.6%. In contrast, the 0.1% mixed chlorophyllin-chitosan diet caused a slight increase in fecal excretion over 21 days to 1.95% relative to that in the control group (1.6%). However, in the mice of the group given the 1.0% mixed diets, a significant increase in HxCDD excretion was observed, showing 5.58% of the HxCDD dose excreted in feces.

Fig. 3 presents the relative amounts of HxCDD excreted in feces every 3 days in the experimental period of 21 days. The fecal excretion of HxCDD increased by intake of a 0.1% mixed diet ranged from 104% to 182% compared to that in mice eating basal fiber-free diet (100%). In the group fed the 1.0% mixed diets, fecal excretion was clearly increased from 188% to 567%. These results indicate that the chlorophyllin-chitosan containing in the diets at a 1.0% ratio remarkably stimulated the excretion of the accumulated HxCDD.

# RISK EVALUATION - POSTERS



**Fig. 2** Effects of porphyrin-chitosan diet on fecal excretion of HxCDD



**Fig. 3** Effects of the porphyrin-chitosan diet on relative fecal excretion of HxCDD every 3 days

## 2. Elimination from adipose tissues

Table 1 shows the fecal excretion for 21 days and the residual amounts of HxCDD in adipose tissues of treated mice. The amount HxCDD remaining in adipose tissue of mice in the 0.1% mixed diet group was approximately 99% of the level in the control group (100%), even though fecal excretion was slightly more than that in the control mice. However, in the 1.0% mixed diet group, HxCDD accumulated at a clearly lower level of 67% compared to that in the control group due to the increased fecal excretion of HxCDD (347% of control group).

## ORGANOHALOGEN COMPOUNDS

From these results, chlorophyllin-chitosan intake could cause rapid elimination of accumulated HxCDD from adipose tissue by stimulating fecal excretion.

### 3. Adsorption of three dioxin isomers in an *in vitro* experiment

The ability of an evacuation substance to enhance fecal excretion of dioxins depends on the potency of dioxin adsorption. To assess the potency of chlorophyllin-chitosan in adsorbing other dioxin isomers, we performed an *in vitro* experiment. Three dioxin isomers (TCDD, HxCDD and OCDD) were respectively incubated for 30 min at 37\_ in the presence of 30mg the chlorophyllin-chitosan. In addition, cellulose and chitin, which are typical dietary fibers, were also assessed by similar procedures. Table 2 shows the adsorption rates for three dioxin isomers by chlorophyllin-chitosan, cellulose and chitin. Chlorophyllin-chitosan absorbed all three dioxin isomers at rates above 80%. The adsorption rates were significant higher than those of cellulose (8.0% to 52%) and chitin (13% to 55%). From these results, it is estimated that chlorophyllin-chitosan can potentially promote the excretion of TCDD and OCDD similar to that observed for HxCDD.

**Table 1 Fecal excretion over 21 days and residual amounts of HxCDD in adipose tissues of treated mice**

	Fecal excretion (Control=100/ mouse/21 days)	Amount in adipose tissue (Control=100)
Control (Non-fiber)	100	100
0.1% mixed diet	122	99
1.0% mixed diet	349	67

**Table 2 Adsorption rates (%) of TCDD, HxCDD and OCDD by cellulose, chitin and chlorophyllin-chitosan**

Isomer	Adsorption rate (%)		
	Cellulose	Chitin	Chlorophyllin- chitosan
TCDD	8.0	13	82
HxCDD	10	21	90
OCDD	52	55	95

### References

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