# EFFECTS OF CARBONACEOUS ADSORBENT ON ACCUMULATION AND EXCRETION OF DIOXINS IN RAT

Iwakiri R<sup>1</sup>, Asano R<sup>2</sup>, Honda K<sup>2</sup> <sup>1</sup>Mura Institute of Environmental Science, Miura Co., Ltd., Japan <sup>2</sup>Faculty of Agriculture, Ehime University, Japan

## Abstract

Effects of carbonaceous adsorbent on accumulation and excretion of dioxins in rats were investigated. The rats that experimented in the feed with dioxins for 2 weeks, accumulated 88% of the amounts of dioxins dosed, while those in the feed with dioxins and carbonaceous adsorbent accumulated only 2.8% of them. The excretions of dioxins in rats were also accelerated approximately double the rate by the feeding of feed with carbonaceous adsorbent, and it was remarkable for the relatively high toxic isomers of dioxins. These observations indicate that an administration of carbonaceous adsorbent strongly controls the accumulation and excretion of dioxin in rat.

## Introduction

The main intake sources of Polychlorinated dibenzo-*p*-dioxin and Polychlorinated dibenzofuran (PCDD/DFs; Dioxins) for humans are originated from foodstuffs such as fish, meat and dairy products <sup>1,2,3</sup>. Hence, it is a very important to control and reduce the dioxins in foodstuff for reducing the dioxins related to human health risk. We have been reported that an administration of carbonaceous absorbent could effectively suppress the dioxins accumulation of rat, aquaculture fish and chicken<sup>4,5,6</sup>. The purpose of this study is to assess the effects of carbonaceous adsorbent feeding on accumulation and excretion of dioxins in rat.

## Materials and Methods

# Animals and treatment of feeds

## (Experiment 1)

8 male rats of the Wister/STD strain (five-weeks old, Japan SLC, Inc.) were randomly divided into 2 groups, and were housed individually. Carbonaceous adsorbent (Kanto Chemical CO. INC.), and rat feed of AIN93G (Oriental Yeast Co., Ltd.) were used. Dioxins administrated to feeds were adjusted by PCDD/DFs standard solution (Wellington Laboratories Inc.). The two types of feed were AIN93G with dioxins (Group 1-A), and AIN93G with dioxin and carbonaceous adsorbent (0.5% weight/feed weight) (Group 1-B). The final amounts of dioxins administration were 4400 pg (720 pg-TEQ) of PCDDs, and 5000 pg (380 pg-TEQ) of PCDFs. All the rats were fed for 2 weeks, and the body weight and ratio of the feed intake were measured everyday during the period of experiments.

#### (Experiment 2)

Similar to experiment 1, 15 male rats were divided into 3 groups, and housed individually. All the rats were administrated the feed containing dioxins adjusted from PCDD/DFs standard solution for 5 days. Total amounts of the dioxins administrated were 4400 pg (680 pg-TEQ) of PCDDs, and 5000 pg (380 pg-TEQ) of PCDFs. After the administration of dioxins, a group of the rats was euthanized (Group 2-A). Another 2 groups were given two types of the feeds with AIN93G (Grope 2-B), and with AIN93G and 0.5 % carbonaceous adsorbent (Group 2-C) for 2 weeks further. Body weight and ratio of the feed intake were measured everyday during the period of experiments.

#### **Chemical Analysis**

At the end of the experiments, all the rats were euthanized, and the liver and carcass (whole body without fur and digestive tract) samples were excised for dioxins analysis. All the samples were stored at  $-20 \text{ C}^{\circ}$  until instrumental analysis. The method for dioxins analysis was mentioned elsewhere<sup>7</sup>. Briefly, tissue samples were extracted with hexane after alkaline digestion. Then, the extraction were purified through a multi-layer silica-gel

column, and fractionated by an active carbon dispersion silica-gel column. The quantity of dioxins was carried out by a HRGC/HRMS.

## **Results and Discussion**

## Biological effects of carbonaceous adsorbent

Body weights, fat content of carcass, and food intake ratio are shown in Table 1. In experiment 1, body and liver weights showed significant differences between group1-A rats and group 1-B rats. In experiment 2, no significant difference of body and liver weights and carcass fat content were observed between group 2-B and 2-C. In all the rats, abnormalities and obstruction in the digestive tracts were not observed visually. These results indicated that in this additive condition feed with carbonaceous adsorbent did not have any pernicious biological effects on rats.

Table 1 Weight, fat contents, and feed intake ratio of rat					
	Body weight	Liver weight	Fat contents in	Feed intake ratio	
	(g)	(g)	carcass (%)	(%)	
Experiment 1					
Group 1-A	208±8.6	13.5±0.56	17.2±0.034	96±2.8	
Group 1-B	192±4.3*	11.1±0.41*	17.5±0.029	99±1.7	
Experiment 2					
Group 2-A	130±4.0	4.8±1.0	9.7±2.5	$99 \pm 0.7^{a}$	
Crown 2 D	$210 \pm 6.0$	$7.0 \pm 1.1$	15 9 2 6	$98{\pm}0.6^{a}$	
Gloup 2-B	210±0.0	) 7.9±1.1 15.6±5.0	13.0±3.0	$94 \pm 5.3^{b}$	
Group 2 C	200 + 4.2	74+00	162120	$99 \pm 0.2^{a}$	
010up 2-C	200±4.3	7.4±0.9	10.3±2.9	$92\pm2.7^{b}$	

\* p < 0.05 (Mann- Whitey U-test vs. group 1-A)

a: dioxin administrated period (5days) b: depuration period (two weeks) after dioxin administration

#### Effects of dioxin accumulation and excretion

Fig 1 shows dioxin accumulation ratio in experiment 1. The dioxin accumulation ratio in rat was calculated from the total burden of dioxin in carcass and liver (TEQ value) and the amount of dioxin intake via feed (TEQ value) at the end of experiment. The accumulation ratio of dioxins in group 1-B (2.6%) was greatly reduced compared to group1-A (88%).

In experiment 2, the time trend of dioxins accumulation in rats are shown in Fig.2. After the administration, the total burdens of dioxins in the carcass and liver of Group 2-A rats were 860 pg-TEQ. After the depuration of two weeks, the burdens of dioxins in group 2-B rats and group 2-C rats were 740pg-TEQ and 620pg-TEQ, respectively.

These results suggest that the feed with carbonaceous adsorbent has effective clearance of dioxins in the rat body. It is considered that suppression-mechanism of





the dioxins accumulation in rat was mainly a mixing of carbonaceous adsorbents and dioxins in the gastrointestinal organs such as stomach and small intestine. While it was expected that the excretion dioxin was accelerated by an adsorption of the carbonaceous adsorbent and the dioxins excreted from small intestine wall<sup>8</sup>.



Fig.2 Body burden of dioxins in rats fed with or without carbonaceous adsorbents after dioxin treatment

## **Biological half-life**

Biological half-lives of dioxin isomers in the rats that fed the feed with carbonaceous adsorbent and without carbonaceous adsorbent were calculated from the results of experiment 1 and 2, and are shown in Table 2. The biological half-lives of the dioxins in rats that fed the feed with carbonaceous adsorbent were shortened compared to the rats for feed without carbonaceous adsorbent. Especially, it was remarkable for the high toxic isomers (2,3,7,8-TeCDD, 1,2,3,7,8-PeCDD, 2,3,4,7,8-PeCDF).

In our studies of another cases, similar results have been also confirmed with animals such as farm animals and aquaculture fish. Therefore, It is concluded that feeds with carbonaceous adsorbent can be used in various applications in aquaculture and animal farming, which facilitates to reduce the dioxin risk in human health.

	Without carbonaceous	With carbonaceous
Isomers	adsorbent (days)	adsorbent (days)
2,3,7,8-TeCDD	54	26
1,2,3,7,8-PeCDD	120	30
1,2,3,4,7,8-HxCDD	72	31
1,2,3,6,7,8-HxCDD	120	33
1,2,3,7,8,9-HxCDD	42	27
1,2,3,4,6,7,8-HpCDD	41	68
OCDD	54	38
2,3,7,8-TeCDF	12	11
1,2,3,7,8-PeCDF	10	9.0
2,3,4,7,8-PeCDF	71	39
1,2,3,4,7,8-HxCDF	69	44
1,2,3,6,7,8-HxCDF	86	50
1,2,3,7,8,9-HxCDF	21	20
2,3,4,6,7,8-HxCDF	69	41
1,2,3,4,6,7,8-HpCDF	38	42
1,2,3,4,7,8,9-HpCDF	30	29
OCDF	10	10

Table 2 Biological half-lives of dioxin isomers in rat

## References

- 1. Ministry of the Environment, Japan, Results of survey on dioxin accumulation and exposure and brominated dioxins in fiscal 2002, 2003
- 2. European Commission DG Environment Compilation of EU Dioxin exposure and health data Task 4: Human exposure, 1999
- 3. Charnley G and Doull J, Food and Chemical Toxicology 2005; 43: 671-679
- 4. Iwakiri R, Honda K, Yamashita H, Nakajima K, Sasaki K, Abstract of 14th Symposium of Environmental Chemistry, 2005: 174-175
- 5. Iwakiri R, Asano R, Sasaki K and Honda K, Abstract of 15th Symposium of Environmental Chemistry, 2006: 26-27
- 6. Iwakiri K, Guruge KS, Asano R, Yamanaka N, Mikami O, Hasegawa J, Honda K, Organohalogen comp., inpress
- 7. Ministry of the Environment, Japan. Manual for surveys of wildlife pollution by dioxins 2002
- 8. Yoshimura H, Yamamoto H, Bull. Environ. Contamin. Toxcol., 1975; 13: 681-688