CLEARANCE OF BROMINATED DIBENZO-P-DIOXINS AND DIBENZOFURANS AFTER A SINGLE INTERPERITONEAL ADMINISTRATION IN COMPARISON WITH 2,3,7,8－TETRACHLORODIBENZO-P-DIOXIN IN MALE WISTAR RAT-A PRELIMINARY RESULT

Okimoto M, Takechi Y, Nose K*, Matsuda M, Kawano M, Morita M

Department of Environment Conservation, Ehime University,
Tarumi 3-5-7, Matsuyama, Ehime, 790-8566, Japan
Iagnostic Division, Otsuka Pharmaceuticals co,.Ltd.,
224-18 Ebisuno, Hiraoshi, Kawauchi-cho, Tokushima, 771-0195 Japan

Introduction

Polybrominated dibenzo-p-dioxins and dibenzofurans (PBDD/F congeners) are unintentionally produced during various combustion processes of plastics, textiles and other materials containing brominated flame retardants (BFRs) or present as contaminants in technical mixtures of BFRs[1]. Workplace air concentrations of tetra-BDDs and tetra-BDFs measured during the extruder production and injection molding of polybutyleneterephthalate polymer blended with the grass fiber, Sb_2O_3 and decabromodiphenylether (DBDPE) were reported to be 2 and 34 ng/m^3, respectively[4]. And adipose tissue levels of 2,3,7,8-TeBDD in humans from the general Japanese pollution was reported to be a median value of 1.7 pg/g with a range of 0.8-4.2 pg/g in 1970 and a median value of 0.5 pg/g with a range of 0.1-2.0 pg/g in 2008[5].

The toxic properties of individual PBDD/F congeners strongly depend on the substitution numbers and position of bromine similarly to those of chlorinated analogues. Recently, a few data the general toxicity of 2,3,7,8-TeBDD in experimental animals have been published as well as result of studys on reproductive toxicity. Single oral and interperitoneal administrations of 2,3,7,8-TeBDD were reported to induce teratogenic effects in mice and thymic atrophy, body weight loss and induction of hepatic microsomal enzymes in immature male rats[2,3].

But PBDD/Fs are much less studied than the chlorinated congeners partly due to the lack of available standards and the difficulties in sensitive detection. Still it seems important to know the toxic effects of those chemicals as they are persistent and toxic.

This study focused on experiment of dose and time-dependencies of hepatic levels of PBDD/F congeners. We designed to examine clearance of tetra-octa-BDD/F congeners after a single interperitoneal administration in comparison with 2,3,7,8-TeCDD in male wistar rats.

Materials and methods

Chemicals

Twelve unlabeled 2,3,7,8-substituted isomers (2,3,7,8-TeBDD, 1,2,3,7,8-PeBDD, 1,2,3,4,7,8/1,2,3,6,7,8-HxBDD, 1,2,3,7,8,9-HxBDD, OBDD, 2,3,7,8-TeBDF, 1,2,3,7,8-PeBDF, 2,3,4,7,8-PeBDF, 1,2,3,4,7,8-HxBDF, 1,2,3,4,6,7,8-HpBDF and OBDF) and twelve $^{13}C_{12}$-labeled (2,3,7,8-TeBDD, 1,2,3,7,8-PeBDD, 1,2,3,4,7,8/1,2,3,6,7,8-HxBDD, 1,2,3,7,8,9-HxBDD, OBDD, 2,3,7,8-TeBDF, 1,2,3,7,8-PeBDF, 2,3,4,7,8-PeBDF, 1,2,3,4,7,8-HxBDF, 1,2,3,4,6,7,8-HpBDF and OBDF) isomers were purchased from Cambridge Isotope Laboratories, Inc (USA). Corn oil of biochemistry grade was used for the interperitoneal administration of 2,3,7,8-TeCDD and PBDD/Fs.

Animal maintenance

Female Wister rats (Slc: WisterHannover / RCC) were purchased at the age of 5 weeks (weighing 110 to 130 g) from SLC Inc (Japan). The animals were quarantined and acclimated for 7 days, before the start of the experiment. The animals were kept under conventional conditions at constant day/night cycle (7 a.m. to 7 p.m.) and at a tempature of 23±1 °C. All rats were given sterilizes commercial pellet diet purchased from PMI nutrition international, Inc (USA) and water. Body weights were measured everyday.

TBDD and TCDD treatment

2,3,7,8-TeCDD and PBDD/Fs were added to corn oil. 2,3,7,8-TeCDD and tetra-penta-PBDD/Fs were interperitoneal administered to 7 groups of 15 rats each at dose of table.1. The control group received corn oil. The total volume administrated to rats was 0.5mL/Rat.

Animals underwent complete necropsy on days 3, 7, 14, 28, and 56 after treatment. Wet organ weight was measured at the time of necropsy. And 2,3,7,8-TeCDD and PBDD/F concentrations in the liver were measured on days (n = 3 rats for each time).

Table.1 Administrated chemicals and the dosage

Group	Isomer	ng/Rat
Group①	2, 3, 7, 8-TCDD	5ng
Group②	2, 3, 7, 8-TeBDD	5ng
	2, 3, 7, 8-TeBDF	5ng
Group③	1, 2, 3, 7, 8-PeBDD	10ng
	1, 2, 3, 7, 8-PeBDF	10ng
	2, 3, 4, 7, 8-PeBDF	10ng
Group④	1, 2, 3, 4, 7, 8-HxBDD	50ng
	1, 2, 3, 6. 7, 8-HxBDD	50ng
	1, 2, 3, 7, 8, 9-HxBDD	50ng
	1, 2, 3, 4, 7, 8-HxBDF	50ng
Group⑤	1, 2, 3, 4, 6, 7, 8-HpBDF	1000ng
Group⑥	OBDD	1000ng
	OBDF	1000ng
Group⑦	Control	Corn oil

Determination of TCDD and TBDD tissue concentration

For determination of the tissue concentration of TBDD, samples of liver (3, 7, 14, 28, and 56 days after treatment) were stored at -30 °C and handled in the dark to avoid photochemical decomposition.

Extraction was carried out by 100 ml using dichloromethane three times. The extracts were spiked with 13C12-labeled internal standards and were treated with concentrated sulfuric acid. Then the extracts were purified by a two-layer column chromatography2） (44% sulfuric acid silicagel: 2g and Florisil:3g) and by an active carbon column chromatography(silicagel with active carbon:0.3g). The column chromatography effluents were spiked with 13C12-labeled standards (syringe spike), and then concentrated to a small volume(50µl), and subjected to the analysis using HRGC (6890 series, Agilent, USA)/HRMS (JMS-800D, JEOL, Japan). HRGC/HRMS was run at high resolution of 12,000 and measured in selected-ion monitoring (SIM) mode. Because 13C12-labeled HpBDD was not available, recovery correction of HpBDD was made using 13C12 -HxBDD.

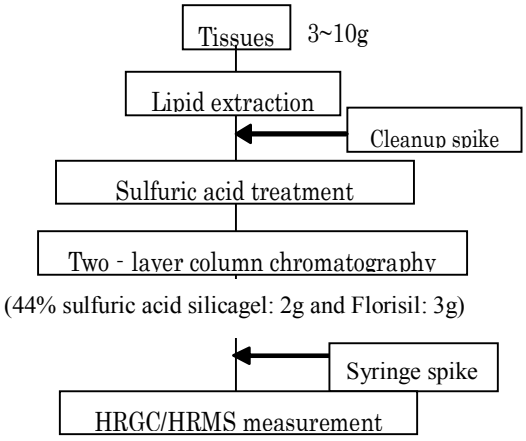

Fig.1 Analytical procedure

Results and Discussion

Time course of body weight and liver weight

During the 56 days of experiment, all rats did not demonstrate any death, decreased locomotor activity and emaciation. Time course of body weight and liver weight from animals treated with a single dose of 2,3,7,8-TeCDD and PBDD/Fs showed no significant differences when compared to control animals. (Fig.2, Fig.3)

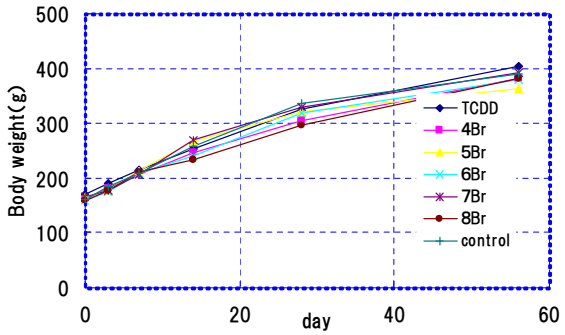

Fig.2 time course change of body weight

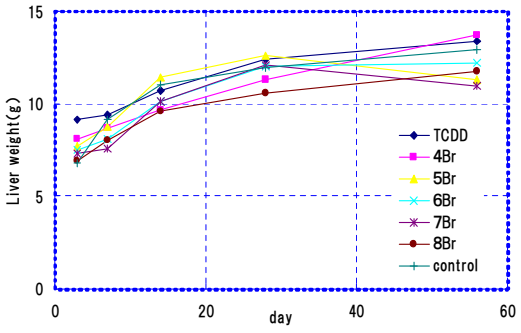

Fig.3 time course change of liver weight

Time course of tissue concentrations of TBDD and TCDD in liver and adipose tissue

Because of the limited number of samples analysed, the result is not conclusive but the following result is obtained by the experiment.

In the liver, the highest tissue concentrations were observed on day 3 in both 2,3,7,8-TeCDD and 2,3,7,8-TeBDD treated animals (Fig. 4). It was calculated that 5~10% of the total dose of 2,3,7,8-TeCDD and 2,3,7,8-TeBDD in the liver three days following treatment. Thereafter, hepatic concentrations of 2,3,7,8-TeCDD and 2,3,7,8-TeBDD gradually decreased. Half-lives ($t_{1/2}$) of the clearance in the liver were virtually identical: 15.5days for 2,3,7,8-TeCDD and 14.8 days for 2,3,7,8-TeBDD. This result is in agreement with results published by Nagao et al[6].

2,3,7,8-TeBDF and almost penta-hexa-BDD/F congeners showed the highest tissue concentrations on day 7, and HpBDF, OBDD and OBDF were observed on day 14. Therefore, it was consider that HpBDF and OBDD/F are transported more slowly to liver.

As for 2,3,4,7,8-PeBDF, It was formed that 25~50% of the total dose is present in liver with high rate compared to other penta-BDD/F congeners. It may be suggested that 2,3,4,7,8-PeBDF have a special affinity to accumulate in liver at higher level compare to other congeners if the experiment is repeatedly confirmed.

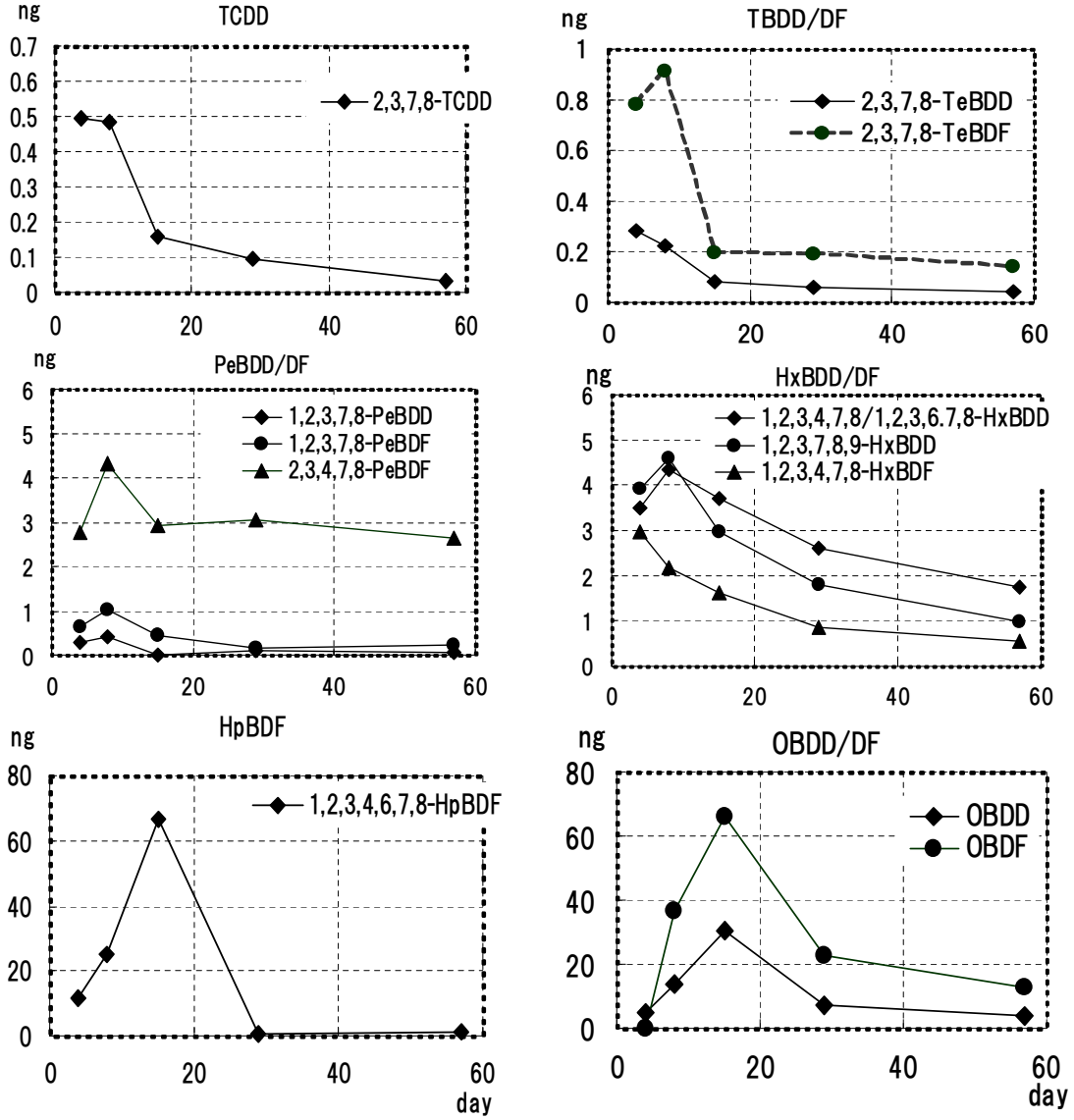

Fig.4 Time course change of TCDD and PBDD/DFs (ng) in liver after single peritoneal administration of TCDD and PBDD/Fs

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