Estimation of PBDD/DF Toxicity Equivalency Factors from Ah receptor binding affinity and clearance rate in rat

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Introduction

Polybrominated dibenzo-*p*-dioxins and dibenzofurans (PBDD/DFs) 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]. Additionally, human adipose tissue concentration levels of 2,3,7,8-TeBDD of the general Japanese were 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[2].

The toxic properties of individual PBDD/DFs congeners strongly depend on the substitution numbers and position of bromine similarly to those of chlorinated analogues. Recently, a few data on the general toxicity of 2,3,7,8-TeBDD for experimental animals have been published as well as the results of studies 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[3,4].

However, PBDD/DFs 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 Toxicity Equivalency Factor of PBDD/DFs estimated from Ah receptor binding affinity and clearance rate in rat.

Materials and Methods

CALUX® assay

The mouse hepatoma H1L6.1c2 cells were cultured in 96-well culture plates, and the initial solvent of chemical standards was changed to dimethylsulfoxide (DMSO). The standard solutions were made plural dilution series with DMSO and mixed with the culture medium (RPMI1640). The prepared solutions were added to the cells. The plates were incubated at 35℃ in 5% carbon dioxide atmosphere for 24h. After exposure for 20-24h, the culture medium was removed, and the cells were washed with phosphate-buffered saline (PBS). Adding luciferin as the substrate, the luciferase activity was determined under a luminometer（Centro LB 960, BERTHOLD TECHNOLOGIES, Germany) and reported as relative light units (RLU). For expression of AhR ligand activity, the EC50 of each chemical is generally used. The EC50 estimation is defined as the concentration that induces 50% of the maximum induction by the compounds, however EC50 is dependent on the maximal response Therefore in this study, EC_{TCDD50} was calculated in addition to EC50. The EC_{TCDD50} is concentrations producing luciferase activity equal to 50% of the maximal response of TCDD. Response of the compound was compared to the response of the TCDD standard curve as REP values. REP_{EC50} value was calculated as the ratio of EC50 for target compound to the EC50 derived from TCDD. REP_{TCDD50} values were calculated as the ratio of EC_{TCDD50} for target compound to the EC50 derived from samples.

Clearance of PBDD/DFs in rats

Animal maintenance

Male Wister rats 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 temperature of 23±1 °C.

PBDD/DFs and TCDD treatment

TCDD and PBDD/DFs were added to corn oil. TCDD and PBDD/DFs were interperitoneal administered to 7 groups of 15 rats each at dose of Table.1. The control group were received only corn oil. The total volume administrated to rats was 0.5mL/rat. The animals underwent complete necropsy without pain on days 3, 7, 14, 28, and 56 after treatment.

Table1: 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 PBDD/DFs tissue concentrations

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 200 ml using dichloromethane three times. The extracts were spiked with $^{13}C_{12}$-labeled internal standards and were treated with concentrated sulfuric acid. Then the extracts were purified by a two-layer column chromatography) (44% sulfuric acid silicagel: 2g and Florisil:3g) and by an active carbon column chromatography(silicagel with active carbon:0.3g). The effluents of column chromatography were spiked with $^{13}C_{12}$-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.

Results and Discussion

CALUX® assay

Dose-response curves and Relative potencies of PBDD/DFs and PXDD/DFs

REP values were indicated in Table 2. The REPs of PCDD/DFs are the results of Nakamura et al[5]. The values of REP_{EC50} were similar to the values of REP_{TCDD50} for most of the congeners investigated, or REP_{TCDD50} showed slightly higher than REP_{EC50}. And REPs obtained in this study are similar to REP_{EC50} values reported in other studies of PBDD/F and PXDD/F congeners by Behnisch et al[6] and Nakamura et al[v].

As for PBDF and PXDF congeners, it seems that the potencies of 2,3,7,8-substituted tetra-PXDF congeners are significantly higher than their chlorinated analogues. It should be noted that 2,3,7,8-TeBDF was ten times more potent than 2,3,7,8-TeCDF, the values of REP_{EC50} 2,3,7,8-TeBDF and 2,3,7,8-TeCDF being 0.83 and 0.021 respectively. 1,2,3,7,8-substituted penta-PBDF and PXDF congeners showed similar

PCDD/Fs		REP_{EC50}
PCDDs	2,3,7,8-TeCDD	1
	1,2,3,7,8-PeCDD	0.73
	1,2,3,4,7,8-HxCDD	0.17
	1,2,3,6,7,8-HxCDD	0.085
	1,2,3,7,8,9-HxCDD	0.086
	1,2,3,4,6,7,8-HpCDD	0.047
	OCDD	0.0011
PCDFs	2,3,7,8-TCDF	0.021
	1,2,3,7,8-PeCDF	0.092
	2,3,4,7,8-PeCDF	0.64
	1,2,3,4,7,8-HpCDF	0.11
	1,2,3,6,7,8-HxCDF	0.11
	1,2,3,7,8,9-HxCDF	0.07
	2,3,4,6,7,8-HxCDF	0.15
	1,2,3,4,6,7,8-HpCDF	0.014
	1,2,3,4,7,8,9-HpCDF	0.067
	OCDF	0.0025

Table2: Relative potencies of PBDD/DFs and PXDD/DFs

PBDD/Fs		REP_{EC50}	REP_{TCDD50}
	●2,3,7,8-TeCDD	1	1
PBDDs	2-MoBDD	0.00042	0.00035
	2,3,7-TrBDD	0.00025	0.0013
	2,3,7,8-TeBDD	0.80	1.1
	1,2,3,7,8-PeBDD	0.28	0.47
	1,2,3,4,7,8-HxBDD	0.019	0.026
	1,2,3,6.7,8-HxBDD	0.0086	0.015
	1,2,3,7,8,9-HxBDD	0.020	0.029
	OBDD	0.000038	0.00035
PBDFs	2-MoBDF	0.0020	0.0019
	2,8-DiBDF	0.0015	—
	2,3,7,8-TeBrDF	0.83	0.89
	1,2,3,7,8-PeBrDF	0.12	0.14
	2,3,4,7,8-PeBrDF	0.39	0.36
	1,2,3,4,7,8-HxBDF	0.063	0.056
	1,2,3,4,6,7,8-HpBDF	0.0045	0.0034
PXDDs/DFs	2-Br-3,7,8-TrCDD	0.81	0.94
	1-Br-2,3,7,8-TeCDD	0.52	0.80
	2-Br-1,3,7,8-TeCDD	1.2	2.4
	2-Br-1,3,4,7,8-PeCDD	0.22	0.52
	2-Br-3,6,7,8,9-PeCDD	0.11	0.19
	1-Br-2,3,6,7,8,9-HxCDD	0.069	0.075
	2,3-DiBr-7,8-DiCDD	1.2	1.4
	2,3-DiBr-6,7,8,9-TeCDD	0.12	0.22
	2,3,7-TrBr-6,7,8-TrCDD	0.12	0.26
	3-Br-2,7,8-TrCDF	0.52	0.58
	1-Br-2,3,7,8-TeCDF	0.16	0.20
	2,4,7,8-TeBr-3-MoCDF	0.32	0.31

potency as their chlorinated analogues. Overall, it seems that most of PBDD/DF and PXDD/DF congeners were similarly potent with their individual chlorinated analogues. However in the case of 2,3,7,8-substituted tetra-PXDF had greater potencies compared to PCDF.

Clearance of PBDD/DFs in rats

Time course of tissue body burdens of PBDD and TCDD in liver and adipose tissue

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 in treated animals (Fig.1). 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 on 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: 15.2 days for 2,3,7,8-TeCDD and 18.1 days for 2,3,7,8-TeBDD. 2,3,7,8-TeBDF and almost penta-hexa-BDD/DF congeners showed the highest tissue concentrations on day 7, and HpBDF, OBDD and OBDF were observed on day 14. Therefore, it was considered that HpBDF and OBDD/F were transported more slowly to liver. As for 2,3,4,7,8-PeBDF, it was formed that 25~50% of the total dose was present in liver with high rate compared to other penta-BDD/DF 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 would be repeatedly confirmed. High body burden of TCDD and PBDD/DF congeners were measured in adipose tissue. It was calculated that 30~80% of the total dose. The body burden of liver were also decreased to 1/2 from 28 to 56, but in adipose tissue were constant after 28 day. Half-lives of the clearance in the adipose tissue were much longer than in liver for TCDD and tetra~penta-PBDD/DFs.

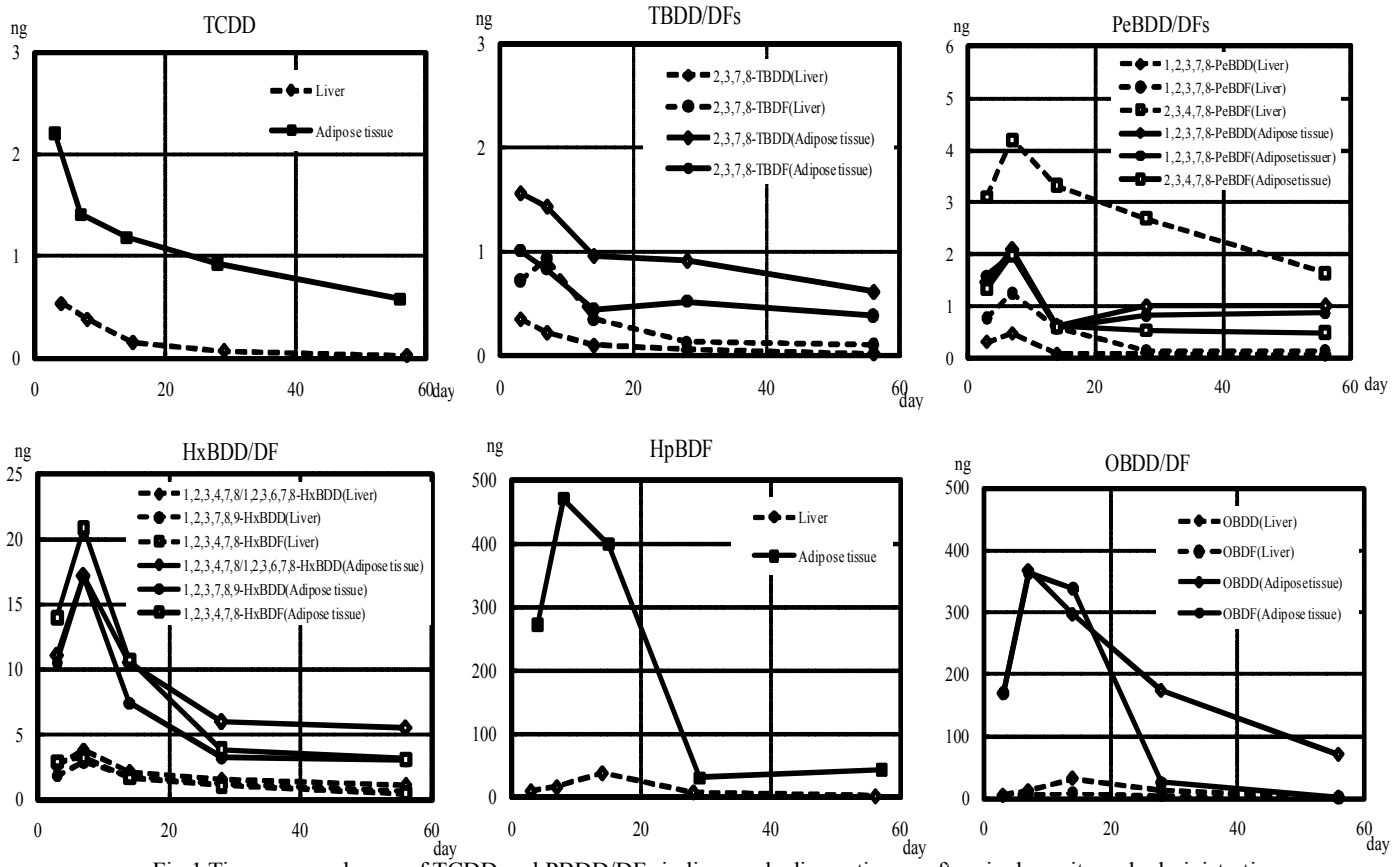

Fig.1 Time course change of TCDD and PBDD/DFs in liver and adipose tissues after single peritoneal administration

Estimation of PBDD/DF TEFs

PBDD/DF TEFs were estimated from the data on CALUX® assay REP and clearance experiment half-lives of hepatic and adipose tissue on PBDD/DFs for rats. Many PBDD congeners of TEFs showed similar to WHO-TEF, On the other hand, PBDF TEFs showed higher values than WHO-TEFs. In particular 2,3,7,8-TBDF and 2,3,4,7,8-PeBDF were suggested that they have more higher TEF values than TCDD. It is not necessarily to using PCDD/DFs WHO-TEF values when we evaluate the contamination of PBDD/DFs in environmental samples. On the basis of our studies we propose to evaluate the toxicities of PBDD/DFs in details.

Table.3 The results of CALUX® assay and half lives of PBDD/DFs in rat tissues and their proposed TEFs

		$t_{1/2}$		Relative-$t_{1/2}$		PCDD/DFs	
	REP_{TCDD50}	Liver	Adipose tissue	Liver	Adipose tissue	Proposed-TEF	WHO-TEF(2006)
●2,3,7,8-TeCDD	1	15.2	34.5	1	1	1	1
2,3,7,8-TeBDD	1.1	18.1	46.3	1.2	1.3	1.3	1
1,2,3,7,8-PeBDD	0.47	28.0	84.0	1.8	2.4	0.9	1
1,2,3,4,7,8/1,2,3,6.7,8-HxBDD	0.026	56.5	43.5	3.7	1.3	0.1	0.1
1,2,3,7,8,9-HxBDD	0.029	26.3	30.1	1.7	0.9	0.05	0.1
OBDD	0.00035	54.3	34.7	3.6	1.0	0.001	0.0003
2,3,7,8-TeBrDF	0.89	20.3	49.2	1.3	1.4	1.2	0.1
1,2,3,7,8-PeBDF	0.14	24.8	70.0	1.6	2.0	0.2	0.03
2,3,4,7,8-PeBDF	0.36	53.2	38.5	3.5	1.1	1.3	0.3
1,2,3,4,7,8-HxBDF	0.056	33.7	26.8	2.2	0.8	0.1	0.1
1,2,3,4,6,7,8-HpBDF	0.0034	31.8	20.7	2.1	0.6	0.01	0.01
OBDF	–	64.8	14.2	4.3	0.4	–	0.0003

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