TOXICITY EVALUATION OF BROMINATED AND BROMINATED/CHLORINATED DIOXINS USING THE CALUX BIOASSAY

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Introduction

Polybrominated and mixed brominated/chlorinated dibenzofurans (PBDD/F and PXDD/F congeners ; X=Br or Cl) 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].

The toxic properties of individual PBDD/F and PXDD/F congeners strongly depend on the substitution numbers and position of bromine or chlorine similarly to those of chlorinated analogues. But PBDD/Fs and PXDD/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.

Dioxin-like compounds elicit their toxicities mainly via the aryl hydrocarbon receptor (AhR) pathway. The CALUX® bioassay is based on the mechanism of binding action of dioxin-like compounds and activation of the AhR which gives necessary information for risk assessment. Earlier studies by Behnisch et al [2] and Nakamura et al [3] compared relative potencies (REPs) for commercially available standards of PBDD/Fs and PXDD/Fs in CALUX bioassay. The aim of this study was to evaluate AhR binding potency of more PBDD/Fs and PXDD/Fs (X=Br or Cl) isomers including synthesized isomers in order to understand toxicity structure relationship.

Materials and methods

Chemicals

2-MoBDD, 2-MoBDF, 2-Br-1,3,7,8-TeCDD, 2‐Br‐1,3,4,7,8-PeCDD, 2,3-DiBr-6,7,8,9-TeCDD, 2,3,7-TrBr-6,7,8-TrCDD and 2,4,7,8-TeBr-3-MoCDF were synthesized by bromination of corresponding PCDD/Fs in our laboratory. Other PBDD/Fs and PXDD/Fs were purchased from Cambridge Isotope Laboratories, Inc (USA).

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) by evaporation under nitrogen gas flow. 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). The cells in each well of the plate were checked under the microscope to confirm that the extracts did not produce cytotoxicities, and the cells were lysed. 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). Dose – response curve for the CALUX bioassay was fitted to a sigmoidal curve analyzed with the software Graph Pad Prism 4.0 (GraphPad Software, USA).

For expression of AhR ligand activity, the EC_{50} of each chemical is generally used. The EC_{50} estimation is defined as the concentration that induces 50% of the maximum induction by the compounds, however EC_{50} is dependent on the maximal response[4]. Therefore in this study, EC_{TCDD50} was calculated in addition to EC_{50}. 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 EC_{50} for target compound to the EC_{50} derived from TCDD. And REP_{TCDD50} values were calculated as the ratio of EC_{TCDD50} for target compound to the EC_{50} derived from samples.

Results and Discussion

Dose-response curves

The dose-response curves obtained are presented in Fig.1. As shown in Fig.1, many PBDD/F and PXDD/F congeners tend to induce the maximal response than the maximal response induced by TCDD. OBDD and 2,8-DiBDF were which less active and did not show up the plateau level in the concentration range used in this experiment. The reason of higher maximum response of PXDD/F isomers compared to PCDD/F is not clear but it is important to understand the reason behind it.

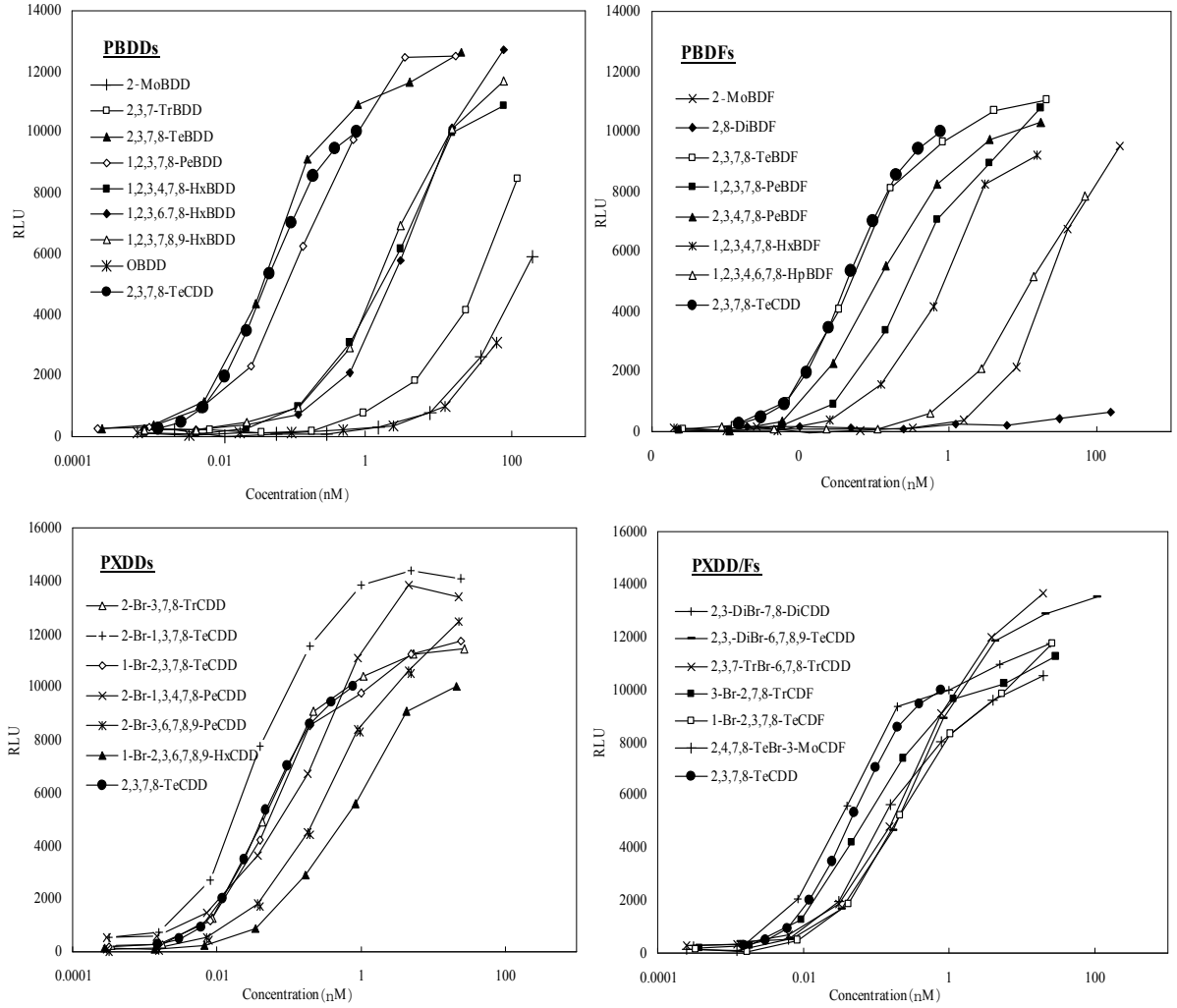


Fig.1 : Dose-response curves of PBDD/F and PXDD/F congeners

Relative potencies of PBDD/Fs and PXDD/Fs

REP values of PBDD/Fs, PXDD/Fs and PCDD/Fs were indicated in Table 1. The comparisons of PBDD/F and PXDD/F congeners REP_{EC50} with their chlorinated analogues were indicated in Figure2. The REPs of PCDD/Fs are the results of Nakamura et al. 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 [2]and Nakamura et al [3].

As shown in Fig.2, in general, PBDDs and PXDDs were similar to their individual chlorinated analogues. Among PBDD and PXDD congeners, relatively high REP potencies were obtained from 2,3,7,8-TeBDD and 2,3,7,8-substituted tetra-PXDD, namely REP $_{EC50}$ values of 2,3,7,8-TeBDD, 2-Br-3,7,8-TrCDD and 2,3-DiBr-7,8-DiCDD were 0.80, 0.81 and 1.2, respectively. The high REP values of those isomers has been reported in other studies [2,3,5]. REPs of 2-Br-1,3,7,8-TeCDD which is synthesized in our lab showed relatively high potencies.

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 isomers showed similar potency as their chlorinated analogues. Overall, it seems that most of PBDD/F and PXDD/F 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. If we consider that PBDFs are present as contaminants in technical mixtures of BFRs, special attention should be paid to evaluate the risk assessment for BFRs which could contain 2,3,7,8-TeBDF as a contaminant. Further studies of other need other PBDD/F and PXDD/F isomers present in BFRs would be needed.

Table1: Relative potencies (REP $_{EC50}$ and REP $_{TCDD50}$) of PBDD/Fs and PXDD/Fs

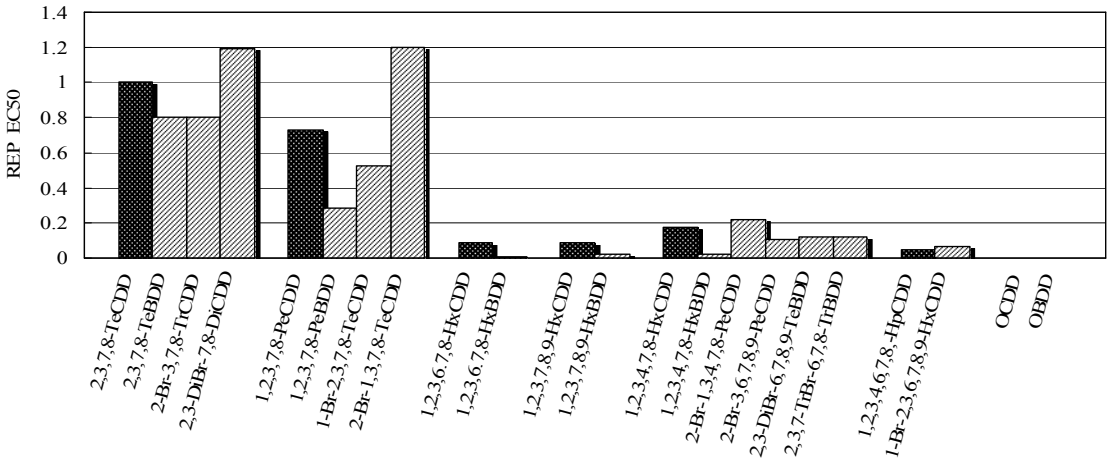
PBDD/Fs

		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

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

• **PBDDs and PXDDs**



• **PBDFs and PXDFs**

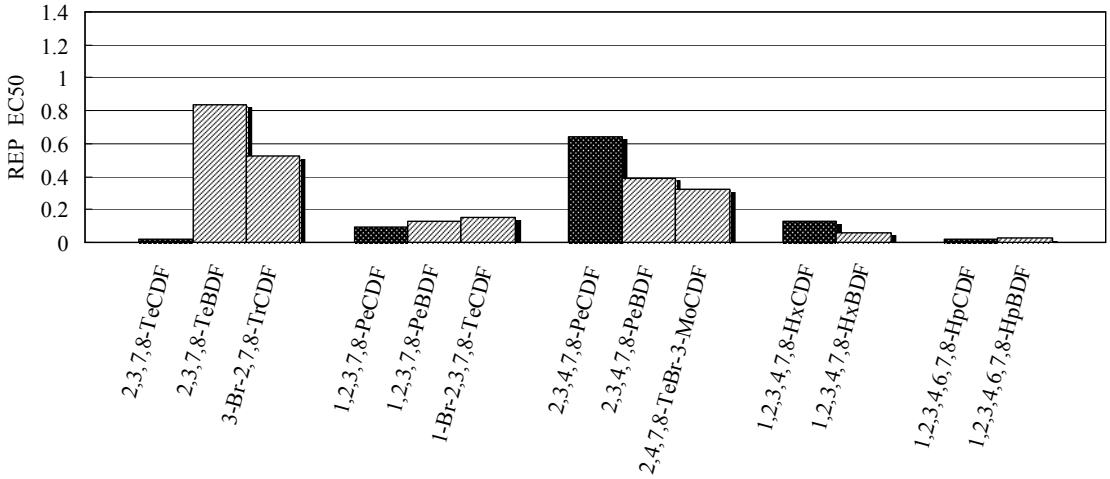


Fig.2: The comparisons of PBDD/F and PXDD/F congeners REP_{EC50} with their chlorinated analogues

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