DR-CALUX®- AND EROD-TEF VALUES FOR DIOXIN-LIKE COMPOUNDS (PXBs/PXDDs/Fs; X=Br, Cl) AND OTHERS (e.g. PAHs)

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1. Introduction

The aim of this study is to compare the activity of PBDD/F-congeners and their chlorinated homologues in *in vitro* CYP1A1- (Micro-EROD-bioassay)[1,2] and luciferase induction (DR-CALUX®-bioassay)[3,4]. DR-CALUX®- and EROD-TEF values for several PXDD/PXDFs/PBBs-congeners and mixtures were measured. In addition, the dioxin-like potency of several other Ah receptor agonists and mixtures such as polyaromatic hydrocarbons (PAHs), polybrominated diphenylethers (PBDEs) and brominated flame retardants (Firemaster BP6/Dow FR 250) were determined.

2. Methods and Materials

All standards were commercially available from Cambridge Isotope Laboratories (all PXDD/Fs, PCBs), AccuStandards (PBBs, Firemaster BP6, Dow FR250), Supelco (all PAHs), or Wako Pure Chemical Industries (TBBPA, p-BP, 2,4-di-BP). (a) Studies with Micro-EROD-bioassay (with H4IIE cells) were according to earlier publications[1,2]. (b) DR-CALUX®-bioassay: The validation samples were analyzed according to the guidelines from BDS (www.biodetectionsystems.com) and recently published studies[3,4]. The luciferase activity was measured using LucLite™ (Packard) and the TopCount NXT® Microplate Scintillation & Luminescence Counter (Packard). No protein amount adjustment have been used for the TEQ calculation for both bioassays. TEQ values were obtained from the dilution which had its response closest to the limit of quantitation (DR-CALUX®-bioassay: 1.4 pM of TCDD; $TCDD_{EC50}$=14.9 pM).

3. Results and Discussion

Several dioxin-like compounds and other Ah receptor agonists were analyzed by DR-CALUX® and Micro-EROD bioassay. The resulted relative equivalents potencies (REP) are listed in Table 1 and 2 and were compared to already published data (for review see literature 5). Additionally, dose-response curves of several standards are shown in Graph 1. The following list of compounds did not show activity in the DR-CALUX® in the applied concentration ranges and the resulting DR-CALUX® -TEQ values are listed in brackets (molar based): 2,2',4,5',6-PBB [only maximum concentration active ≤ 6.4E-5]; p-bromo-phenol [only maximum concentration active: ≤ 1.8E-4]; 2,4-Bromo-phenol [< 3.0E-3]; TBBPA [< 0.034]; 2,2,4,4'-T4BDE [< 5.4E-4]; 2,2,4,4',5-P5BDE [< 7.4E-4]; 2,2,4,4',5,5'-H6BDE [< 9.6E-4]; 2,3,3'4,4'5,6-H7BDE [< 1.2E-3]. In case of the PBDE-congeners REP values analyzed by DR-CALUX® have been already reported, indicating

that the in the present study used concentrations were not high enough: 1) 2,2',4,4'-TBDE (7.1 x 10^{-7}); 2) 2,2',4,4',5-PBDE (5.9 x 10^{-6}); 3) 2,2',4,4',5,5'-HBDE (4.3 x 10^{-6}); and 4) Bromkal 70-5-DE (4.8 x 10^{-6})[6].

In case of PXDDs similar REPs could be analyzed, whilst for the PXDFs significant differences could be measured (molar based): 2,3,7,8-TBDF (EROD: 0.71/DR-CALUX®: 0.74 compared to WHO-TEF_{TCDF} 0.1), 1,2,3,7,8-PBDF (EROD: 0.52/DR-CALUX®: 0.72; compared to WHO-$TEF_{12378\text{-}PCDF}$ 0.05) and 2,3,4,7,8-PBDF (EROD: 0.087/ DR-CALUX®: 0.12; compared to WHO-$TEF_{23478\text{-}PCDF}$ 0.5). The decrease of the dioxin-like-potency with increase of halogenation was confirmed (see WHO, 1998)[7]. Further research is necessary to understand the impact of these brominated dioxin-like compounds to the biopotency in samples of waste management or in environmental samples.

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Graph 1: Dose-response curves obtained in the DR-CALUX®-bioassay with a number of different PXDD/Fs (X=Br, Cl), PCBs and PAHs. TCDD-curve fitting was done by using a one-ligand curve-fit. Data points of all standards are means of three independent measurements.

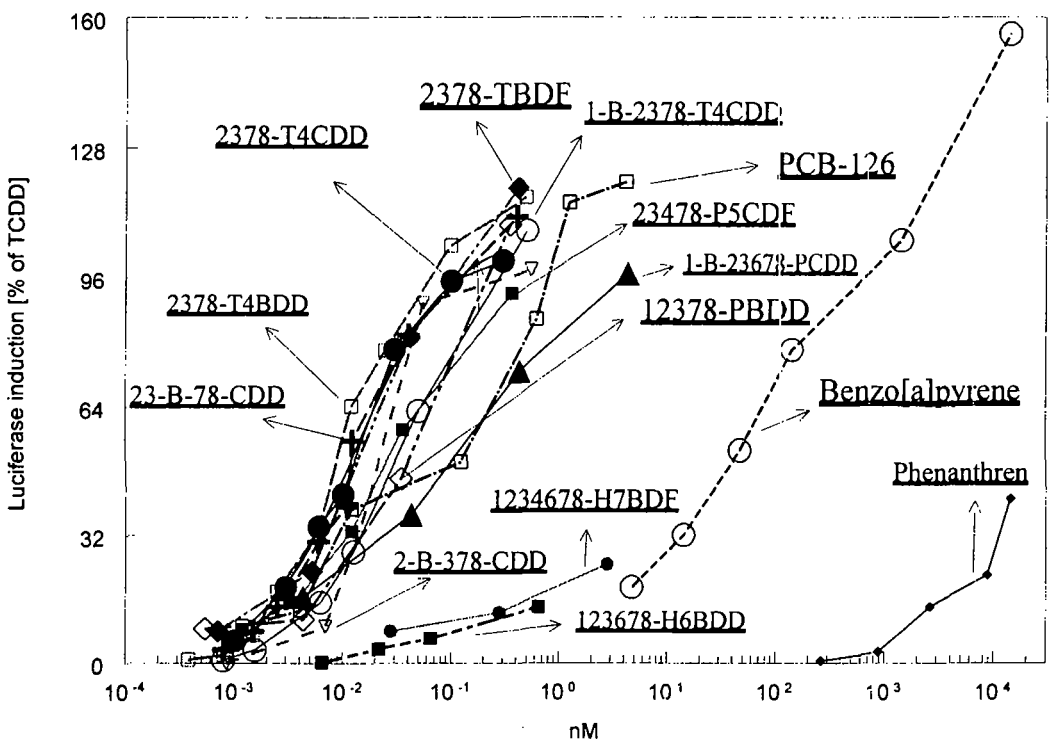

Table 1. DR-CALUX®-TEF (24 h kinetic) and Micro-EROD-TEF (72 h kinetic) values (molar based) for several PXDD/Fs (X=Br, Cl) relative to 2,3,7,8-TCDD [MDL minimal detection limit; published CALUX/EROD data in this table listed are from Sanderson et al. (1996)[12] and Bovee et al. (1998)[3]]

PXDD/Fs	DR-CALUX® MDL based Present study [n/ CV/% $TCDD_{max}$]	DR-CALUX® EC_{50} based Present study [n/ CV/% $TCDD_{max}$]	Micro-EROD MDL based Present study [n/ CV/ % $TCDD_{max}$]	CALUX[12] /CALUX[3] / EROD[12]	Safe (1991)[8] *in vitro* EROD	Safe (1991)[8] *In vivo* AHH	Schramm et al. (2000)[10] EROD *in vitro*	Mason et al. (1987)[11] *in vitro* EROD
1,2,3,7,8-P5CDD	0.49 [3/26/110]		0.56 [6/32/-]	0.79/0.49/ 0.30				
2,3,7,8-T4CDF			0.075 [5/29/100]					
2,3,4,7,8-P5CDF	0.79 [4/8/92]		0.45 [12/32/60]	0.69/0.34/ 0.28				
2-Br-3,7,8-T3CDD	0.43 [3/26/98]	0.51 [3/5.1/98]	0.20 [3/31/86]		0.23	1.6		0.10
1-Br-2,3,7,8-T4CDD	0.30 [3/21/110]	0.29 [3/16/110]	0.42 [3/5/90]					
1-Br-2,3,6,7,8,9-H6CDD	0.29 [3/10/98]		0.42 [2/-/90]					
1-Br-2,3,4,6,7,8,9-H7CDD	0.17 [2/-/52]							
2,3-diBr-7,8-diCDD	0.92 [6/12/110]	0.59 [3/15/110]	0.55 [5/14/100]		3.4	8.2		1.4
(1,3,7,8-T4BDD)					0.0031	0.00062		0.001
2,3,7,8-T4BDD	0.84 [3/16/84]	0.72 [3/12/84]	0.65 [3/29/100]		2.3	5.3	0.74	0.35
1,2,3,7,8-P5BDD	0.88 [3/11/110]		0.29 [3/6/100]		0.27	0.16	0.11	0.12
1,2,4,7,8-P5BDD					0.024	0.02		0.01
1,2,3,4,7,8-H6BDD	0.082 [2/-/88]		0.033 [3/24/89]					
1,2,3,6,7,8-H6BDD	7.8E-3 [3/11/25]		0.022 [3/18/46]				0.055	
1,2,3,7,8,9-H6BDD	0.063 [3/32/91]	0.044 [3/6.3/91]	0.027 [4/30/97]					
O8BDD	1.6E-4 [4/47/24]		3.3E-4 [4/6/27]					
2,3,7,8-T4BDF	0.74 [5/14/124]	0.64 [3/20/120]	0.71 [3/40/101]				0.37	
1,2,3,7,8-P5BDF	0.72 [3/13/91]	0.69 [3/17/91]	0.52 [3/7/102]					
2,3,4,7,8-P5BDF	0.12 [5/32/92]		0.087 [4/40/-]				0.056	
1,2,3,4,7,8-H6BDF	0.046 [2/-/74]		0.017 [2/-/99]					
1,2,3,4,6,7,8-H7BDF	4.7E-3 [3/4/42]		4.5E-3 [2/-/23]					

Table 2 : DR-CALUX®-TEF (24 h kinetic) and Micro-EROD-TEF (72 h kinetic) values (molar based) for several AhR agonists compared to 2,3,7,8-TCDD and in comparison to other studies [n independent measurements/ CV correlation of variation/ % $TCDD_{max}$]

PXDD/Fs	Present study DR-CALUX® H4IIE-luc	Present study Micro-EROD H4IIE	Bovee et al. (1998)[3] H4IIE-luc	Schramm et al. (2001)[9] H4IIE	Khim et al. (2000)[13] H4IIE-luc; REP_{20}	Willett et al. (1997)[14] H4IIE	Sanderson et al. (1996)[12] H4IIE-luc/H4IIE
PCB-77	2.1E-4 [2/-/33]						7.1E-4/3.4E-4
PCB-126	0.073 [6/19/100]	0.050 [6/36/69]	0.065				0.017/0.047
PCB-118	1.6E-5 [4/17/54]		4.9E-6				<1E-6/<1.5E-6
PCB-156	3.4E-5 [3/45/41]		3.8E-5				
PCB-157	1.5E-4 [3/51/42]	2.5E-5 [3/49/65]					
PCB-169	0.049 [2/-/50]	0.013 [3/40/51]	0.0015				5.5E-4/0.0015
PBB-77	0.080 [4/44/90]	0.019 [4/31/80]					
PBB-169	0.021 [3/12/62]]						
Firemaster BP6*	3.4E-4 [3/18/80]	3.8E-5 [3/23/87]					
Dow FR 250*	3.6E-4 [4/15/50]	7.9E-7 [3/29/40]					
* not molar based							
Benzo[a]pyrene	2.7E-4 [5/19/140]	5.5E-5 [2/-/96]		3.0E-4	2.4E-6	3.5E-4	
Phenanthrene	2.7E-6[4/38/43]						
Indeno[c,d]pyrene	2.7E-3 [4/47/92]	1.5E-4 [2/-/89]		8.6E-5	3.4E-5	1.1E-3	
Benz(a)-anthracene		2.6E-5 [2/-/96]		2.7E-5	2.2E-6	2.5E-5	

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