Ah-receptor activity induced by brominated and mixed brominated/chlorinated dibenzodioxins in DR-CALUX and RTL-W1 cell lines

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Introduction

The aim of this study was to obtain relative potency (REP) values for ten brominated and mixed brominated/chlorinated compounds (PBDDs and PXDDs), for the dioxin-specific bioassays DR-CALUX and RTL-W1. DR-CALUX is a mammalian cell line based on luciferase induction in recombinant H4IIE rat hepatoma cells,[1] whereas RTL-W1 uses the endogenous induction of EROD in rainbow trout liver cells.[2]

Polyhalogenateddibenzo-p-dioxins are present as complex mixtures in the environment. To facilitate the risk assessment of these compounds, a WHO working group introduced the toxic equivalency factor (TEF) concept.[3] However, it was concluded that consensus TEF values could only be established for certain chlorinated congeners (PCDDs), for which a large amount of background data were available. Brominated and mixed brominated/chlorinated dibenzo-*p*-dioxins (PBDDs and PXDDs) are much less studied than the chlorinated congeners, due to analytical obstacles and the lack of available standards. The relatively few studies addressing the biological activity of PXDDs and PBDDs report a toxicology similar to that of PCDDs, mediated by the Ah-receptor (AhR) pathway.[4, 5]

Although TEFs are consensus values based on *in vivo* studies if available, *in vitro* determination of AhR activation potency may be helpful in risk assessment and establishment of future TEFs. Also, bioassay-specific relative potency values (REPs) are necessary for mass balance calculations when comparing bioassay TCDD-equivalents (bio-TEQ) with GC-MS-analysis based WHO-TEQ.

Method and materials

Experimental design: Chemical standards of ten PBDDs and PXDDs were obtained from Wellington laboratories (Guelph, Ontario, Canada), purity 95% - >98%. The initial solvent of the standards (toluene) was changed to dimethylsulfoxide (DMSO). Aliquots of each standard were distributed to the two labs performing DR-CALUX (Biodetection Systems, Amsterdam, NL) or RTL-W1 assays, respectively. Standard solutions were tested at least twice in a dose-response manner in the respective bioassay.

REP calculation: Dose-response curves were analysed with the software GraphPadPrism®, using the Hill equation. The bottom value was set to the response of the solvent controls. All compounds tested in sufficient doses reached a maximum similar to that of TCDD, and therefore, this was assumption was made also for compounds not tested in sufficient doses, to allow REP estimation. REPs were determined based on both the EC_{25} and EC_{50} of each standard compound to give a measurement of uncertainty. This was done on a weight basis (pg/ml) [3, 6].

DR-CALUX assay: Confluent cells in 96-well plates were exposed to ten or twelve concentrations of each standard compound in 3x dilutions. The total volume was 200 ul and the final DMSO concentration was 0.8% in all wells. As internal standard, dilution series of TCDD (0-300 pM) was run on the same plate. After 24h exposure, cells were washed and lysed. Luciferase induction was measured using the Luclite assay kit (Perkin Elmer) and a 96-well plate reader for luminescence (Victor^2, Wallac 1420).

RTL-W2 EROD assay: Confluent cells in 96-well plates were exposed to eight doses of each standard compound in 3x dilution. As internal standard, dilution series of TCDD (0-300 pM) was run on the same plate (3.15-200 pM). Cells were exposed for 72h and EROD induction and protein content was measured using ethoxyresorufin and

fluorescamine reactions, using a fluorescence plate reader (GENios, Tecan, Cralsheim, Germany).

Results and discussion

REP-values based on EC_{25} and EC_{50} are presented in table 1. Brominated and mixed brominated/chlorinated induced AhR-mediated activity in the dioxin-specific bioassays DR-CALUX and RTL-W2. All ten compounds tested induced luciferase activity in the DR-CALUX, as did all but one, 1,2,3,4-BDD, in the RTL-W1, even if the response was very low for some congeners. The mixed brominated/chlorinated were the most potent inducers. Overall, the tested compounds were 10- to 100-fold more potent in the DR-CALUX than in the RTL-W1, compared to TCDD. The reason for this is unknown. However, ranking the REP-values shows that the relative potencies between congeners were similar between the two assays, as follows:

2,3,7,8-tetra/pentaXDD > 2,3,7,8-tetra/pentaBDD > triBDD/-XDD > non-2,3,7,8-tetraBDD

The dose-response curves obtained are presented in figure 1 and 2. All tested congeners tested in sufficient doses reached a similar induction maximum to that of TCDD. The EC_{25}REPs were generally higher compared to EC_{50}REPs, due to a larger slope deviation between the tested standard compound and TCDD in the lower part of dose-response curves. This was most prominent for the PXDDs. Thus, it may be of great significance from which part of the curve the chosen REP has been obtained, when performing mass-balance calculations in order to explain activity of samples in bioassays. Also, it is important to choose bioassay-specific REPs. DR-CALUX REP values for PBDDs were very similar to earlier reported values for different CALUX-assays[6, 7]. PXDDs gave slightly higher REPs than reported by Behnisch et al.[7] The most potent PXDD, the penta-halogenated 2-Br-1,3,7,8-Cl-dioxin had a REP of 0.7-1.9 (at EC_{50}). In the EROD-based RTL-W1, bell-shaped dose-response curves indicated substrate inhibition.[8] The assays were equally sensitive for TCDD, with mean EC_{50} 15.7 ± 4.0 pM (DR-CALUX) and 12.2 ± 4.0 pM (RTL-W1).

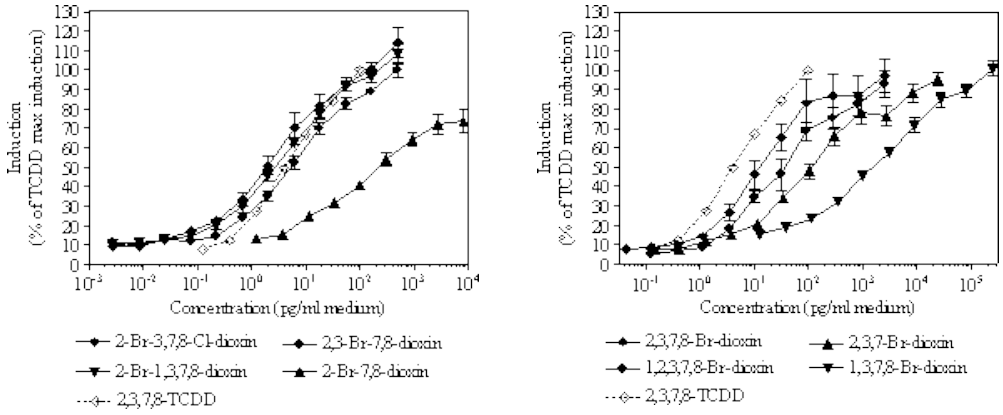

Figure 1. Dose-response curves for several brominated and mixed brominated/chlorinated dibenzo-*p*-dioxins determined by the DR-CALUX bioassay. (n=1-2 separate assays for tested compounds, n=6 separate assays for TCDD).

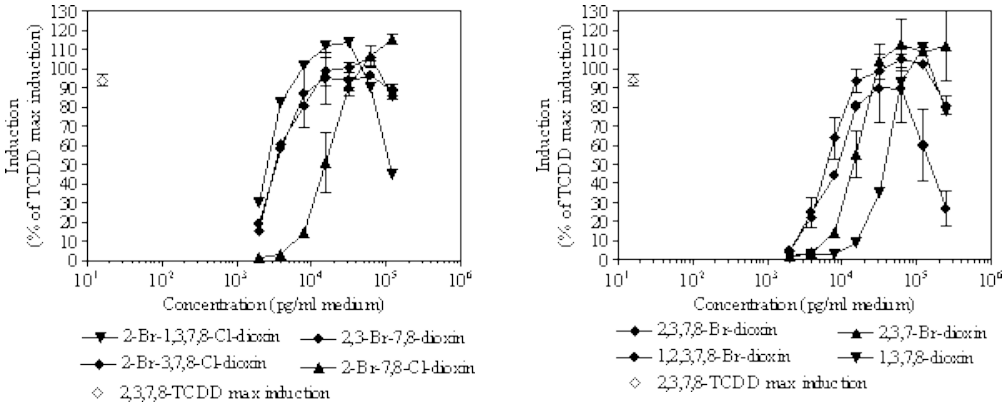

Figure 2. Dose-response curves for several brominated and mixed brominated/chlorinated dibenzo-*p*-dioxins determined by the RTL-W1 bioassay. (n=1-2 separate assays for tested compounds, n=6 separate assays for TCDD).

Table 1. Relative potencies (REPs) of brominated and mixed brominated/chlorinated dioxins, relative to 2,3,7,8-TCDD, in the RTL-W1 and the DR-CALUX cell lines. REPs were determined from EC_{25} and EC_{50} (weight basis), for 1 or 2 separate dose-response curves. nd, no induction detected by the assay. [a]mixture containing 69% of 1,3,6,8-Br- and 31% of 1,3,7,9-Br-dioxin. [b]low induction, REP estimated from the highest dose ~EC_{12}

	RTL-W1 REP		DR-CALUX REP	
Congener	EC25	EC50	EC25	EC50
2,3,7-Br-	$1.7x10^{-04}$, $1.4x10^{-04}$	$2.1x10^{-04}$, $2.0x10^{-04}$	0.072, 0.061	0.064, 0.030
2,3,7,8-Br-	$4.6x10^{-04}$, $3.7x10^{-04}$	$4.9x10^{-04}$, $3.7x10^{-04}$	0.57	0.62
1,3,7,8-Br-	$2.9x10^{-04}$, $5.4x10^{-05}$	$2.9x10^{-04}$, $9.1x10^{-05}$	$4.3x10^{-03}$, $3.6x10^{-03}$	$1.5x10^{-03}$, $8.9x10^{-04}$
1,2,3,4-Br-	nd	nd	$7.0x10^{-05}$, $1.2x10^{-04}$	$5.5x10^{-05}$, $7.8x10^{-05}$
1,3,6,8-Br- & 1,3,7,9-Br- [a]	$4.8x10^{-05}$ [b]	-	$9.1x10^{-05}$, $4.1x10^{-04}$	$8.7x10^{-05}$, $2.0x10^{-05}$
1,2,3,7,8-Br-	$4.3x10^{-03}$, $6.5x10^{-05}$	$4.0x10^{-03}$, $4.5x10^{-05}$	0.24	0.49
2-Br-7,8-Cl-	0.0015, 0.00096	$1.5x10^{-03}$, $9.3x10^{-03}$	0.097, 0.074	0.027, 0.026
2-Br-3,7,8-Cl-	$8.0x10^{-04}$	$9.3x10^{-04}$	3.2, 1.0	2.0, 0.88
2-Br-1,3,7,8-Cl-	$5.8 x10^{-04}$, -	$4.5 x10^{-04}$, 0.0014	0.9, 2.8	0.7, 1.9
2,3,-Br-7,8-Cl-	0.013, 0.016	0.013, 0.042	1.6	0.88

References

1. Murk, AJ., Legler, J., Denison, MS., Giesy, JP., Van De Guchte C., and Brouwer, A. (1996) *Fundam and ApplToxicol* 33:149-160

2. Lee, LE., Clemons, JH., Bechtel, DG., Caldwell, SJ., Han, KB., Pasitschniak-Arts, M., Mosser, D., Bols, NC. (1993) *Cell Biol and Toxicol* 9:279-294

3. van den Berg, M., Birnbaum, L., Bosveld, AT., Brunstrom, B., Cook, P. Feeley, M. et al. (1998) *Environ Health Perspect* 106:775-792

4. Weber LWD and Greim H (1997). *J Toxicol Environ Health* 50:195-215

5. Birnbaum L, Staskal D and Diliberto J (2003). *Environ Int* 29:855-860

6. Brown, D., van Overmeire, I., Goeyens, L., Denison, MS., De Vito, MJ., and Clark, G. (2004). *Chemosphere* 55:1509-1518

7. Behnisch, P.A., Hosoe, K., and Sakai, S. (2004). *Environ Int* 29:861-877

8. Petrulis, JR. and Bunce, NJ. (1999). *Toxicol Letters* 105:251-260