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Brominated dioxin-like compounds: in vitro assessment in comparison to classical dioxin-like compounds and other polyaromatic compounds

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Abstract

Recently, several countries agreed to adopt the Stockholm convention on persistent organic pollutants (POPs). One future obligation will be to add other POPs as new evidence becomes available. In vitro cell-based bioassays offer a rapid, sensitive, and relatively inexpensive solution to screen possible POP candidates. In the present study, we investigated the aryl hydrocarbon (Ah)-receptor activity of several dioxin-like POPs by using the Micro-EROD (Ethoxy-Resorufin-*O*-Deethylase) and DR-CALUX (Dioxin-Responsive-Chemical Activated Luciferase gene eXpression) bioassays, which are two state-of-the-art methods. The Micro-EROD system used in our study utilizes a wild-type rat liver cell line (rat liver H4IIEC3/T cells), while the DR-CALUX bioassay consists of a genetically modified rat hepatoma H4IIE cell line that incorporates the firefly luciferase gene coupled to dioxin-responsive elements (DREs) as a reporter gene. In the case of the DR-CALUX bioassay, we used an exposure time of 24 h, whereas we used a 72-h exposure time in the Micro-EROD bioassay. The aim of this study was to compare conventional dioxin-like POPs (such as polychlorinated dibenzodioxins and -furans, PCDD/Fs and coplanar polychlorinated biphenyls, PCBs) with several other classes of possible candidates to be added to the current toxicity equivalent factor (TEF) model in the future. Therefore, this study compares in vitro CYP1A1 (Micro-EROD bioassay) and firefly luciferase induction (DR-CALUX bioassay) in several mixed polyhalogenated dibenzodioxins and -furans (PXDD/Fs; X = Br, Cl, or F), alkyl-substituted polyhalogenated dibenzodioxins and -furans (PMCDD/Fs; M = methyl), polyhalogenated biphenyls (PXBs, X = Br, Cl), polybrominated diphenyl ethers (PBDEs), pentabromophenols (PBPs), and tetrabromobisphenol-A (TBBP-A). We also evaluate congener-specific relative potencies (REPs) and efficacies (% of $TCDD_{max}$) and discuss the dose–response curves of these compounds, as well as the dioxin-like potency of several other Ah-receptor agonists, such as those of the polyaromatic hydrocarbons (PAHs) and polychlorinated naphthalenes (PCNs). The highest REP values were found for several PXDD/F congeners, followed by some coplanar PXBs, trichlorinated PCDD/Fs, PAHs, PBDE-126, 1-6-HxCN, and some brominated flame retardants (TBBP-A). These in vitro investigations indicate that further research is necessary to evaluate more Ah-receptor agonists for dioxin-like potency.

Keywords: Ah receptor; Bioassay; Brominated flame retardant; CALUX; EROD; PAH; PBDE; PCB; PCDD/F; Reporter gene assay

1. Introduction

During the last century, many chemicals have been produced that enter the environment through different pathways. Often, these chemicals have been produced without sufficient knowledge of the possible environmental harm that they may cause. Among other chemicals, several dioxin-like compounds are unintentionally formed (e.g., PCDD/Fs, through thermal processes) or produced in a variety of applications (e.g., PCBs and PCNs, in electronic equipment such as capacitors or transformers). Several other chemicals, such as some brominated flame retardants (e.g., PBDEs, PBPs, and TBBP-A), are still produced in large quantities for use in electric equipment, plastics, and building materials.

It is well known that dioxins, PCBs, and other related compounds constitute a group of lipophilic, persistent, ubiquitous, and bioaccumulative environmental chemicals exhibiting a broad spectrum of biological (e.g., high toxicity) and chemical (e.g., long-range transport) effects.

In the environment, they often occur as industrial byproducts in low concentrations but with a high dioxin-

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like toxicity (e.g., dioxins), or in higher concentrations but with low dioxin-like toxicity (e.g., PCBs). Other chemicals occur in even higher concentrations with other toxicological endpoints (e.g., PAHs, which are carcinogens), but they are not persistent.

In the environment, such compounds often coexist as complex mixtures of various congeners whose relative concentrations/toxicities differ by orders of magnitude.

To determine the dioxin-like activity of these compounds, it is important to know both their concentration and toxicity to evaluate the integrated risk for adverse human health effects and environmental risk assessment. In the past, many studies evaluated the concentrations of several environmental chemicals, but often congener-specific toxicity data were not included in the risk assessment, because such data were unavailable.

The potential effects of chemicals depend on a number of factors, including level and duration of exposure, relative toxic potencies, mechanism of action, and interactions between chemicals in a mixture. Thus, the toxicity equivalent factor (TEF) approach has been established for dioxin-like compounds with the following premises: that they all act through the same biological pathway, they are persistent, that the effects of congeners are essentially additive at submaximal levels of exposure, and that the dose–response curves are parallel and the organotropic manifestations of all congeners are identical over the relevant range of doses (Birnbaum, 1999; Safe, 1998; Van den Berg et al., 1998, 2000).

Seven polychlorinated dibenzo-*p*-dioxins (PCDDs), 10 polychlorinated dibenzofurans (PCDFs), and 12 polychlorinated biphenyls are collectively referred to as dioxin-like compounds (Birnbaum, 1999; Safe, 1998; Van den Berg et al., 1998, 2000).

When considering the addition of more compounds to this list, in vitro bioassay batteries (Behnisch et al., 2001a,b; Bunce and Petrulis, 2000; Hilscherova et al., 2000; Hoogenboom et al., 1999; Safe et al., 1991; Safe, 1993) can help to give a first indication as to whether the unknown compound will bind to the aryl hydrocarbon receptor (AhR) and whether it may have the potential to cause dioxin-like effects. Information from cell-based bioassays can help establish TEFs relative to 2,3,7,8-TCDD (TCDD). TEFs are estimates of relative potency based upon a wide variety of toxic and biological endpoints. The bioassays, however, give information on in vitro AhR-mediated activity in hepatoma cells of only one species (rat). Therefore, the activity relative to TCDD is expressed in relative potency (REP) values which are based in the here presented study from a single set of experiments and do not represent international consensus.

In the past few years, several studies have used the CALUX or, even earlier, the EROD bioassay, to rank Ah-receptor agonists relative to TCDD (for a review, see, for example, Behnisch et al., 2001b), for example, PCDD/Fs (Behnisch et al., 2001a; Bovee et al., 1998; Brown et al., 2001a,b; Garrison et al., 1996; Jeong et al., 2001; Laier et al., 2001; Li et al., 1999; Murk et al., 1996; Safe, 1990; Sanderson et al., 1996; Schmitz et al., 1996; Villeneuve et al., 2000a,b), PCBs (Behnisch et al., 2001b; Bovee et al., 1998; Brown et al., 2001a,b; Garrison et al., 1996; Jeong et al., 2001; Laier et al., 2001; Li et al., 1999; Murk et al., 1996; Safe, 1984, 1990, 1994; Sanderson et al., 1996; Schmitz et al., 1996; Villeneuve et al., 2000a,b), PXDD/Fs (Behnisch et al., 2001b; Blankenburg et al., 1990; Brown et al., 2001a,b; Hornung et al., 1996a,b; Mason et al., 1987; Mennear and Lee, 1994; Nagao et al., 1990; Weber and Greim, 1997; WHO, 1998), PAHs (Delistry, 1997; Jones and Anderson, 1999; Khim et al., 2000; Machala et al., 2001; Pijnenburg et al., 1995; Schramm et al., 2001; Till et al., 1999; Willett et al., 1997), PBDEs (Bunce et al., 2001; Chen et al., 2001; Chen and Bunce, 2001; Darnerud et al., 2001; De Boer et al., 2000; Meerts et al., 1998; Piskorska-Pliszczynska et al., 1986; WHO, 1994a,b), PCNs (Blankenship et al., 2000; Hanberg et al., 1991; Machala et al., 2001; Villeneuve et al., 2000a,b), and other brominated flame retardants (WHO, 1994a,b; Zacharewski et al., 1988).

The objectives of this study is to compare the TCDD-like activity of several PAHs, PCNs, PCBs, brominated and chlorinated dioxin-like compounds in in vitro CYP1A1- (Micro-EROD-bioassay) and luciferase induction (DR-CALUX®-bioassay). DR-CALUX®- and EROD-REP values for several PAHs/PXDD/PXDFs/PXBs/PBDEs/PCNs-congeners determined.

2. Materials and methods

All standards were at the highest purity commercially available and obtained from Cambridge Isotope Laboratories [PCDD/Fs (purity: >97.0–>99.0%), PBDD/Fs (purity: 96.0–>99.0%), PBrCDD/Fs (purity: 97.9–>99.0%), PCBs (purity: >98.0%)], PCNs (purity: 96.0–98.0%), PBDEs (purity: >98.0% or >99.0%), α-HBCD (purity: >98.0%), β-HBCD(purity: >98.0%), γ-HBCD(purity: >98.0%)], AccuStandards [PBBs (purity: >98.0%)], Supelco [PAHs (purity: 97.2–99.7%)], Wellington Laboratories [PXCDD/Fs; X = CH_3, F, I (purity: >98.0%)], Wako Pure Chemical Industries [*p*-bromophenol (purity: 98%)], Tokyo Kasei Kogyo [TBBP-A (purity: >98.0%)], and Dr. Ehrenstorfer [2,4,6-tribromophenol, pentabromophenol (purity: 98.6% and 99.3%, respectively)]. All standards have been transferred from their original solvent (nonane, toluene, methanol, methylenechloride) to DMSO before preparing their dilution series. Possible impurities or concentrations of these standards have not been checked (only the used TCDD has been checked).

2.1. Micro-EROD bioassay

The Micro-EROD bioassay with rat hepatoma H4IIEC3/T cells was performed as described previously (Behnisch et al., 2002). Briefly, cells were cultured in α-MEM medium (Gibco

Table 1
Comparison of DR-CALUX-REP values (24 h kinetic) based on EC_5 and EC_{50} value for several brominated dioxins and furans

PXDD/Fs	REP EC_{5TCDD} (*A*)	REP EC_{50} (*B*)	Ratio *A*/*B*	REP_{mean}
2-Br-3,7,8-TriCDD	0.23	0.67	0.34	0.45
1-Br-2,3,7,8-TCDD	0.35	0.28	1.25	0.32
2-Br-3,6,7,8,9-PeCDD	0.19	0.19	1.00	0.19
2,3-diBr-7,8-diCDD	1.05	0.86	1.2	0.96
2,3,7,8-TBDD	0.73	0.77	0.95	0.75
1,2,3,7,8,9-HxBDD	0.041	0.017	2.41	0.03
2,3,7,8-TBDF	0.97	0.60	1.62	0.79
1,2,3,7,8-PeBDF	0.13	0.14	0.93	0.52
Mean			*1.21*	

REPs were determined from three independent measurements.

BRL 41061-029) supplemented with 10% v/v fetal calf serum (FCS; JRH12103-78P or TSF907040-500) under standard conditions (37 °C, 5% CO_2). Cells were seeded into 96-well cell culture plates (Iwaki, Japan) at a density of $(0.4–1) \times 10^4$ cells/well. After 3 days of growth (density about 70–90% confluence), TCDD (0.3–300 pM, Cambridge Isotope Laboratories) or the test material was added in 200 μl of FCS-containing medium. All TCDD standards or samples were dissolved in dimethyl sulfoxide (DMSO; Dojin, Wako, Japan) and added to the cell cultures in triplicate to a final concentration of solvent in the medium of 0.4%. The cells were then exposed for another 72 h. TCDD and the sample were simultaneously analyzed in minimal five doses in comparison to a blank sample on each 96 well plate. Then, the medium was removed, and 100 μl of fresh medium containing 16 μM 7-ethoxyresorufin (Sigma E3763) and 10 μM dicumarol (Sigma M1390) was added. After incubation at 37 °C for 60 min, 90 μl of the reaction mixture was transferred to another 96-well plate containing 130 μl of methanol. Resorufin-associated fluorescence was measured at 550-nm excitation and 585-nm emission by using a multi-well fluorescence reader (Corona MTP-32 or MTP-F2).

2.2. DR-CALUX bioassay (with recombinant H4IIE cells)

The DR-CALUX bioassay used in this study was established essentially as in the guidelines from BioDetection Systems, Amsterdam, The Netherlands, and recently published studies (Hamers et al., 2000; Pauwels et al., 2000).

Table 2
DR-CALUX-REP and Micro-EROD-REP values for several dioxin-like compounds (WHO, 1997) relative to 2,3,7,8-TCDD

Standard samples	WHO-TEF	DR-CALUX-REP (pM)[a]			Micro-EROD-REP (pM)[b]			Efficacy % of TCDD B_{max}	
		EC_{5TCDD}	EC_{20}	EC_{50}	EC_{5TCDD}	EC_{20}	EC_{50}	DR-CALUX	Micro-EROD
TCDD	1	1.00	1.00	1.00	1.00	1.00	1.00	100	100
1,2,3,7,8-PeCDD	1	0.75	0.80	0.54	0.72	0.65	0.61	118	120
1,2,3,4,7,8-HxCDD	0.1	0.43	0.42	0.30	0.18	0.21	0.14	110	104
1,2,3,6,7,8-HxCDD	0.1	0.23	0.30	0.14	0.17	0.15	0.15	104	112
1,2,3,7,8,9-HxCDD	0.1	0.14	0.10	0.066	0.059	0.090	0.049	106	102
1,2,3,4,6,7,8-HpCDD	0.01	0.15	0.14	0.046	0.071	0.074	0.034	115	120
OCDD	0.0001	0.0013	0.0013	0.0005	NT[c]	–	–	94	–
2,3,7,8-TCDF	0.1	0.49	0.51	0.32	0.35	0.29	0.24	109	109
1,2,3,7,8-PeCDF	0.05	0.40	0.31	0.21	0.22	0.28	0.21	116	111
2,3,4,7,8-PeCDF	0.5	0.93	0.90	0.50	0.58	0.60	0.39	114	113
1,2,3,4,7,8-HxCDF	0.1	0.22	0.23	0.13	0.13	0.16	0.15	115	93
1,2,3,6,7,8-HxCDF	0.1	0.059	0.055	0.039	0.082	0.065	0.046	104	100
1,2,3,7,8,9-HxCDF	0.1	0.17	0.21	0.11	0.19	0.21	0.12	89	105
2,3,4,6,7,8-HxCDF	0.1	0.33	0.34	0.18	0.092	0.12	0.11	102	99
1,2,3,4,6,7,8-HpCDF	0.01	0.040	0.039	0.029	0.019	0.020	0.018	104	120
1,2,3,4,7,8,9-HpCDF	0.01	0.048	0.060	0.041	0.032	0.032	0.021	95	132
OCDF	0.0001	0.011	0.012	0.0065	NT	–	–	105	–
PCB-77	0.0001	0.0015	0.0016	0.0013	4.7×10^{-4}	5.1×10^{-4}	4.5×10^{-4}	69	80
PCB-81	0.0001	0.0043	0.0046	0.0042	NT	–	–	83	–
PCB-105	0.0001	2.5×10^{-5}	1.7×10^{-5}	1.2×10^{-5}	6.2×10^{-6}	1.1×10^{-5}	9.8×10^{-6}	51	49
PCB-114	0.0005	1.1×10^{-4}	6.8×10^{-5}	4.8×10^{-5}	2.8×10^{-5}	3.2×10^{-5}	3.2×10^{-5}	79	79
PCB-118	0.0001	7.3×10^{-6}	NA[d]	NA	5.2×10^{-6}	9.7×10^{-6}	1.0×10^{-5}	27	48
PCB-123	0.0001	8.6×10^{-5}	3.5×10^{-5}	2.4×10^{-5}	1.2×10^{-5}	2.0×10^{-5}	1.4×10^{-5}	64	51
PCB-126	0.1	0.079	0.073	0.067	0.044	0.051	0.046	98	113
PCB-156	0.0005	1.5×10^{-4}	2.5×10^{-4}	2.1×10^{-4}	7.7×10^{-5}	8.6×10^{-5}	9.9×10^{-5}	60	101
PCB-157	0.0005	7.7×10^{-5}	1.0×10^{-4}	8.0×10^{-5}	3.9×10^{-5}	4.7×10^{-5}	4.2×10^{-5}	66	83
PCB-167	0.00001	5.4×10^{-6}	6.9×10^{-6}	8.2×10^{-6}	NT	–	–	69	–
PCB-169	0.01	0.0025	0.0034	0.0034	0.0016	0.0019	0.0022	69	98
PCB-189	0.0001	3.2×10^{-6}	5.2×10^{-6}	6.7×10^{-6}	NT	–	–	66	–

[a] Mean of three determinations.
[b] Mean of two or three determinations.
[c] Not tested.
[d] Full dose–response curve was not obtained.

In this assay, Ah-receptor agonists are measured by using stable rat H4IIE hepatoma cells transfected with the AhR-controlled luciferase reporter gene construct pGudLuc 1.1. In our test system, the cells were cultured under the same conditions as our wild-type H4IIE cells. After seeding the cells (density, $(7.0-10)\times 10^4$ cells/well) into 96-well view plates (Packard 6005181), they were grown to confluence (90–100%) in 24 h. TCDD calibration standards (0.3–300 pM, Cambridge Isotope Laboratories) were diluted with DMSO (Dojin) and subsequently dissolved in α-MEM (Gibco BRL 41061-029) supplemented with 10% v/v FCS (JRH12103-78P or TSF907040-500). A blank sample with the same FCS medium and DMSO was added separately. Then, the cells were exposed in triplicate to serial dilutions of the TCDD calibration standard and samples for another 20–24 h. Each well contained 200 μl of medium including 0.4% v/v DMSO, which was used as the vehicle. After incubation, the medium was removed, and the cells were washed with 100 μl of Dulbecco's phosphate-buffered saline (PBS, with Ca/Mg, Gibco BRL 14040-141). Afterward, 100 μl of PBS and 100 μl of LucLite/buffer mixture (Packard 6016911) were added at room temperature in the dark. The view plates were sealed with a white cover on the bottom. After 20–30 min in a black box, light production was measured in the dark with a TopCount NXT microplate scintillation and luminescence counter (Packard) using an autosampler. Measurements were started after 1 min for adaptation to the dark in the luminometer and for each plate lasted about 10 min.

3. REP calculation

Dose–response curves for the DR-CALUX (luminescence) and Micro-EROD (fluorescence) bioassays were

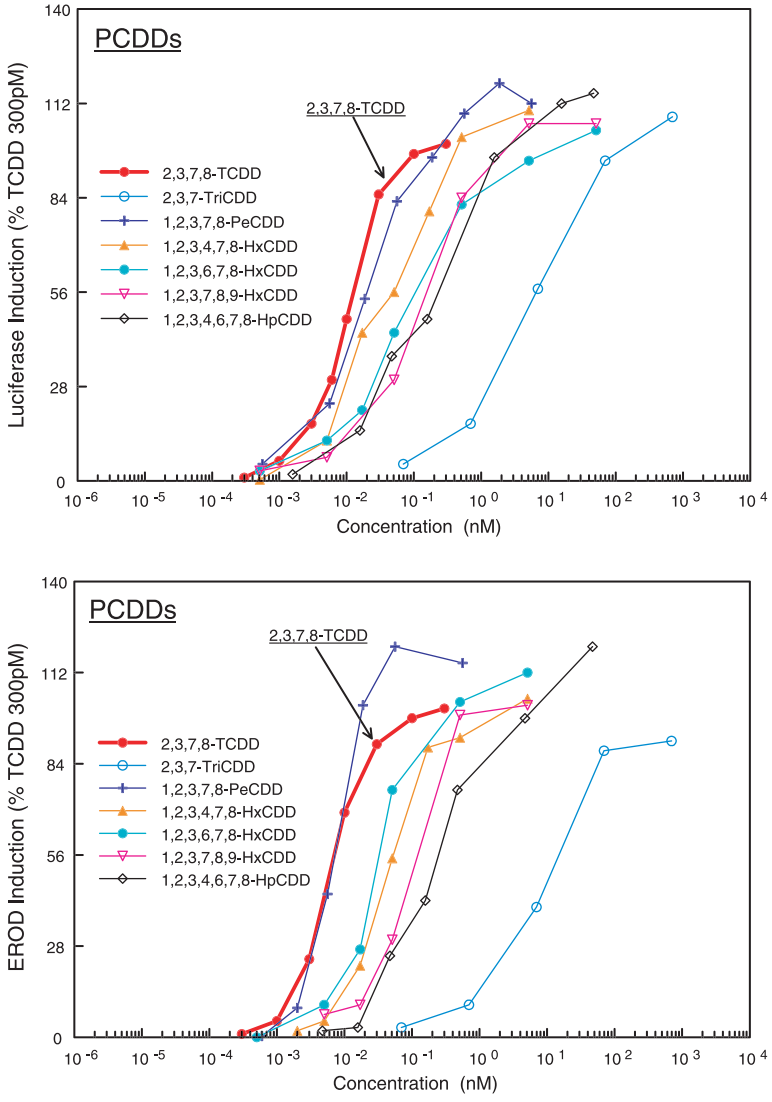

Fig. 1. Dose–response curves of PCDD congeners determined by DR-CALUX (first graph; luciferase induction) and Micro-EROD (second graph; EROD induction) bioassays.

fitted to a sigmoidal curve from which the EC_{20} and EC_{50} values could be calculated (SlideWrite Plus Version 5.0, Advanced Graphics Software, Encinitas, CA). REP values based on EC_{20} (REP EC_{20}) and EC_{50} (REP EC_{50}) were calculated by dividing the EC_x for TCDD by EC_x for the test compound (where $x=20$ or 50). REP values based on EC_5 (REP EC_{5TCDD}) were calculated by interpolation of the response induced by the test compound on the dose–response curve for TCDD. In this case, the diluted solution of the test compound that resulted in a response close to the EC_5 of the TCDD response was used. This is the most linear part of the dose–response curve, and quantifications based on this part of the curve are very reproducible.

We compared REP values for several PBDD/Fs calculated by using traditional EC_{50} values with the REP values based on EC_5 (Table 1). For these compounds, which exhibited TCDD-like slopes, the differences were less than 21%, but, in compounds exhibiting non-TCDD-like slopes, the differences will be certainly larger. The dose–response curves for the TCDD concentration standards were analyzed simultaneously on every 96-well plate. Cells were evaluated microscopically for cellular degeneration and obvious toxicity. Since no direct toxicity was observed, a lack of response in either cell line to a treatment was not due to its being near to a toxic concentration.

3.1. Statistics

Each dilution was analyzed at least three times (DR-CALUX) or two times (Micro-EROD) in at least

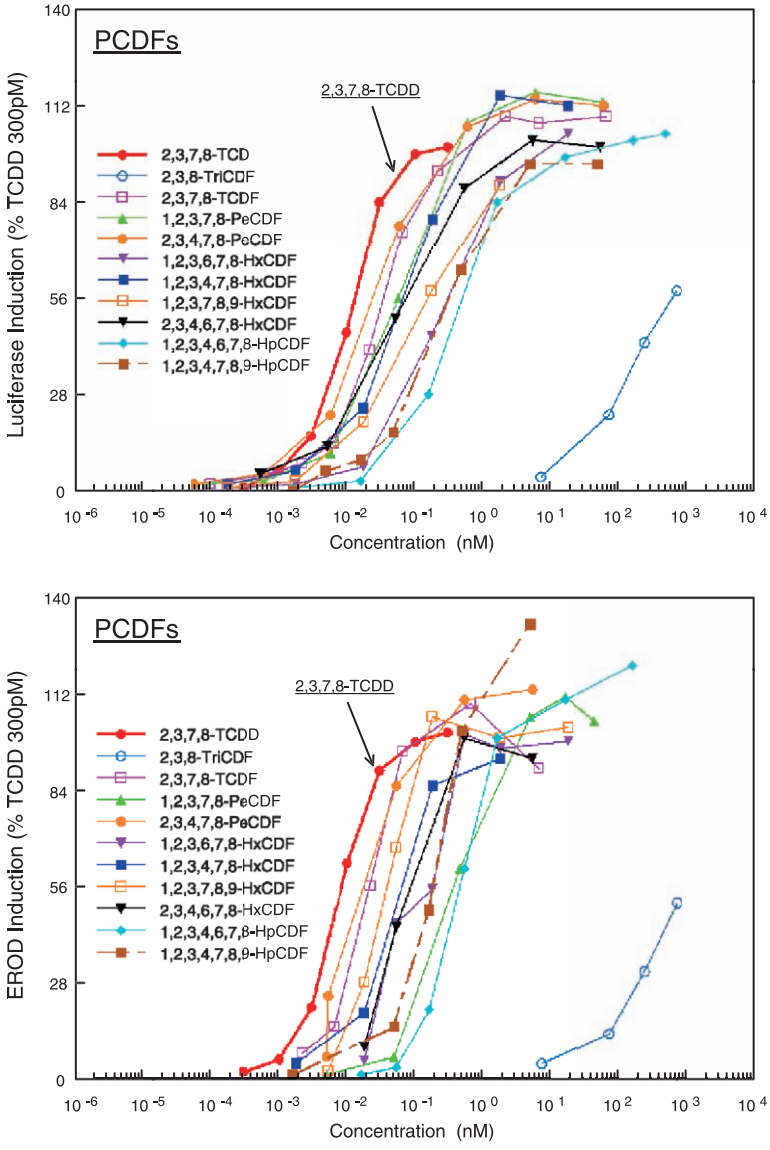

Fig. 2. Dose–response curves of PCDF congeners determined by DR-CALUX (first graph; luciferase induction) and Micro-EROD (second graph; EROD induction) bioassays.

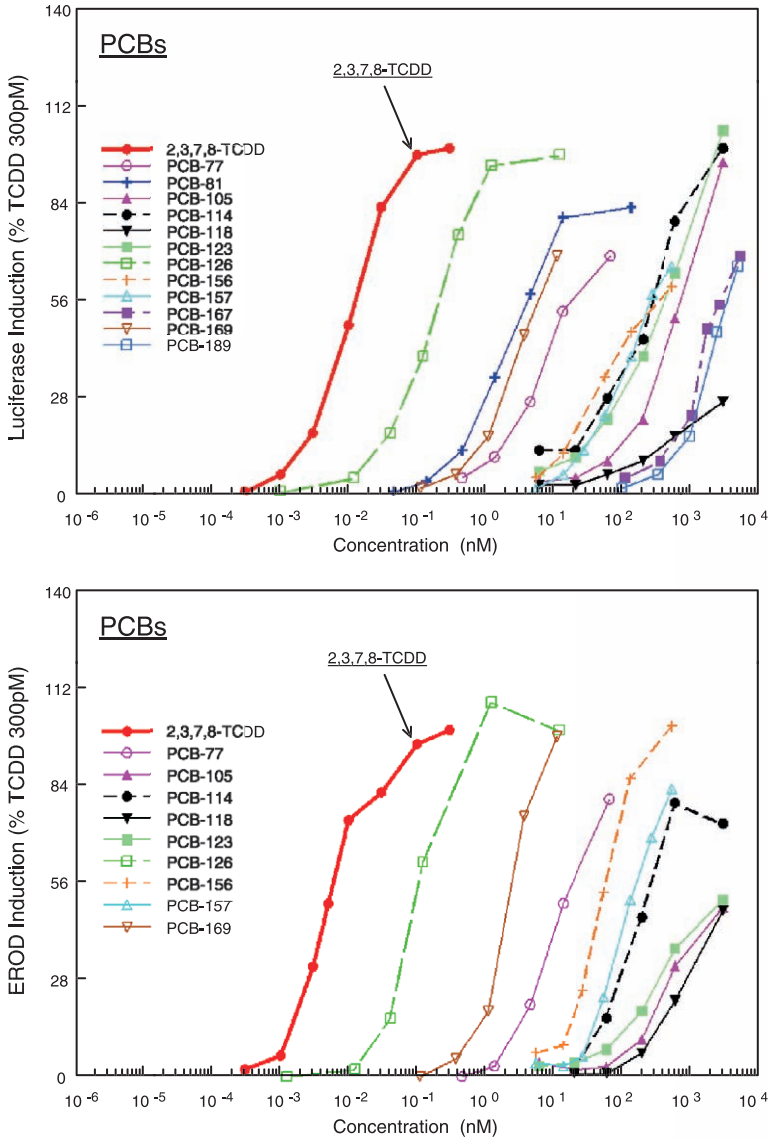

Fig. 3. Dose–response curves of PCB congeners determined by DR-CALUX (first graph; luciferase induction) and Micro-EROD (second graph; EROD induction) bioassays.

three (DR-CALUX) or two (Micro-EROD) independent experiments. No adjustments were made for protein amount in the toxicity equivalents (TEQ) calculation for either bioassay, because it was previously shown that such adjustments had no major influence on the outcome (Bovee et al., 1998). Dose–response curves were fitted using a one-site ligand (Eq. (1)) or user-defined (Eq. (2)) curve fit (SlideWrite Plus Version 5.0):

$$\text{Measured response (luminescence or fluorescence)}\,y = \text{max. response}\ a_0 \times \text{conc. of test compound}\ x / (\mathrm{EC}_{50}a_1 + \text{conc. of test compound}\ x) \quad (1)$$

or

$$\text{Measured response (luminescence or fluorescence)}\,y = \text{max. response}\ a_0/1 + [(\text{conc. of test compound}\ x / \mathrm{EC}_{50}a_1)^{\text{slope of the curve}\ a_2}] \quad (2)$$

Concentrations of the TCDD stock solutions were checked by gas chromatography/mass spectrometry (GC/MS).

3.2. Quality criteria

The maximum induction factor was set to be at least 6-fold and no more than 30-fold. The EC_{50} value was

accepted only if it was in the range of 6 to 18 pM TCDD (the most linear part of concentrations). The mean value of the coefficient of determination R^2 of the fitted TCDD curve was greater than 0.98 (DR-CALUX) or 0.95 (Micro-EROD).

4. Results and discussion

4.1. World Health Organization (WHO) criteria for dioxin-like compounds

The WHO criteria for including a compound in the TEF scheme and therefore adding it to the list of dioxin-like compounds are as follows: (a) the compound must share certain structural relationships with the PCDD/Fs; (b) it must bind to the aryl hydrocarbon receptor (AhR); (c) it must elicit AhR-mediated biochemical and toxic responses; and (d) it must be persistent and accumulate in the food chain. In our earlier literature review (Behnisch et al., 2001b), we compared REP values for several Ah-receptor agonists from different studies. Therefore, we will compare here only results from cell-based bioassays similar to the H4IIE-luc and H4IIE-EROD bioassays used in this study.

Different endpoints, such as EC_{20}, EC_{50}, or EC_{80}, have been used to evaluate REP values in the past. Because of the lack of efficacy in the full dose–response curve of several congeners (e.g., some PCBs) and because we expected lower inhibitory effects, we chose to use REP values based on responses close to the EC_5 of the TCDD response. This choice may explain some of the differences between the REP values obtained in our study compared with those from previously published data.

4.2. Dioxin-like compounds

The H4IIE-EROD bioassay has been already extensively used to evaluate REP values for dioxin-like compounds (Behnisch et al., 2001b). Using the same H4IIE wild-type cell line, Li et al. (1999) reported REP values, based on EC_{50}, of several dioxin-like compounds, and we here compare those values with our results (our results↔comparison): 2,3,7,8-TCDF: 0.15↔0.24; PCB-126: 0.05↔0.05; PCB-77: 2.7×10^{-4}↔4.5×10^{-4}.

Several studies that used the CALUX system (H4IIE-pGudLuc 1.1 cells, 24-h kinetic) have previously reported REP values for several dioxin-like compounds (Bovee et al., 1998—first value; Laier et al., 2001—second value; Sanderson et al., 1996—third value), which we have compared to our values (after the symbol →; see Table 2): 1,2,3,7,8-PeCDD: 0.49/0.79/0.79 → 0.54–0.80; 2,3,4,7,8-PCDF: 0.34/0.69/0.51 → 0.50–0.93; 1,2,3,6,7,8-HxCDD: 0.068/not analyzed (n.a.)/0.36 → 0.14–0.30; PCB-126: 0.065/0.017/0.28 → 0.067–0.079; PCB-169:

Table 3
DR-CALUX-REP and Micro-EROD-REP values for several brominated dioxins and furans, and mixed substituted dioxins and furans (PXCDD/Fs) relative to 2,3,7,8-TCDD

Standard samples	DR-CALUX-REP (pM)[a]			Micro-EROD-REP (pM)[b]			Efficacy % of TCDD B_{max}	
	EC_{5TCDD}	EC_{20}	EC_{50}	EC_{5TCDD}	EC_{20}	EC_{50}	DR-CALUX	Micro-EROD
2,3,7-TriCDD	0.0049	0.0034	0.0015	0.0018	0.0013	0.0008	108	91
2,3,8-TriCDF	1.0×10^{-4}	8.8×10^{-5}	6.0×10^{-5}	3.7×10^{-5}	3.8×10^{-5}	2.4×10^{-5}	58	51
2-B-3,7,8-TriCDD	0.23	0.44	0.67	0.19	0.25	0.53	99	108
1-B-2,3,7,8-TCDD	0.35	0.35	0.28	0.28	0.37	0.58	108	93
2-B-1,3,7,8-TCDD	0.52	0.47	0.37	0.41	0.58	0.27	111	98
2-B-3,6,7,8,9-PeCDD	0.19	0.28	0.19	0.19	0.23	0.16	97	92
2,3-diB-7,8-DiCDD	1.05	1.15	0.86	0.35	0.56	0.95	118	110
3-B-2,7,8-TriCDF	1.28	1.09	0.74	0.52	0.72	0.40	98	91
2,3,7-TriBDD	0.081	0.062	0.033	0.031	0.039	0.036	92	103
2,3,7,8-TBDD	0.73	0.87	0.77	0.45	0.34	0.72	110	111
1,2,3,7,8-PeBDD	0.26	0.25	0.21	0.26	0.16	0.30	110	116
1,2,3,6,7,8-HxBDD	0.007	0.017	0.016	0.007	0.014	0.014	85	84
1,2,3,7,8,9-HxBDD	0.041	0.043	0.017	0.017	0.041	0.039	90	87
OBDD	$<5.6 \times 10^{-5}$	–	–	$<4.6 \times 10^{-5}$	–	–	12	19
2,3,7,8-TBDF	0.97	0.86	0.60	0.53	0.78	0.53	108	100
1,2,3,7,8-PeBDF	0.13	0.19	0.14	0.17	0.27	0.22	103	105
2,3,4,7,8-PeBDF	0.12	0.14	0.094	0.094	0.14	0.095	104	102
1,2,3,4,7,8-HxBDF	0.017	0.022	0.017	0.008	0.013	0.010	110	107
1,2,3,4,6,7,8-HepBDF	0.0019	0.0035	0.0027	0.0013	0.0028	0.0022	89	102
8-M-2,3,7-TriCDD	0.081	0.045	0.011	0.0078	0.0068	0.0032	106	103
8-F-2,3,4-TriCDF	4.6×10^{-4}	2.7×10^{-4}	1.7×10^{-4}	2.5×10^{-4}	2.0×10^{-4}	1.3×10^{-4}	89	84
8-I-2,3,4-TriCDF	0.0014	0.0018	0.0011	4.2×10^{-4}	4.9×10^{-4}	4.6×10^{-4}	107	81
7,8-diM-2,3,4-TriCDF	0.019	0.026	0.0063	0.0028	0.0021	0.0012	98	86

[a] Mean of three determinations.
[b] Mean of two or three determinations.

0.0015/0.00055/n.a. → 0.0025–0.0034; PCB-156: 3.8×10^{-5}/n.a./3.0×10^{-4} → $(1.5–2.5) \times 10^{-4}$; PCB-118: 4.9×10^{-6}/$< 1 \times 10^{-6}$/6.8×10^{-6} → 7.3×10^{-6}; PCB-105: 2.1×10^{-6}/1×10^{-6}/n.a. → $(1.2–2.5) \times 10^{-5}$; PCB-77: n.a./7.1×10^{-5}/n.a. → 0.0013–0.0016.

These results all fall within a reasonable range for different REP calculations based on data obtained in different laboratories with standards from different distributors.

4.2.1. Dose–response curves

The DR-CALUX and EROD activities of PCDD/Fs are shown in Figs. 1 and 2. Most of the PCDD/F congeners induced activity up to the maximum level induced by TCDD. Only the concentrations up to the plateau level are shown, because for the calculation of the REP value, we focused on the linear part of the curve, from around EC_5 until EC_{50} (which we measured at least three times).

4.3. Polychlorinated biphenyls (PCBs)

Eight dioxin-like and non-dioxin-like PCBs showed lower REP values based on EC_{50} than expected from the WHO TEF values (the ratios of EC_{50} REP values to WHO TEF values were 0.01–0.5), but the EC_{50} REP values of one Co-PCB (PCB-77) was due to his rapid metabolism in the here used in vitro systems more than one magnitude higher than the WHO TEF value (see Table 2). Our results show excellent agreement between REP values obtained by the Micro-EROD and DR-CALUX bioassays, indicating that these two bioassays may have a similar responsiveness.

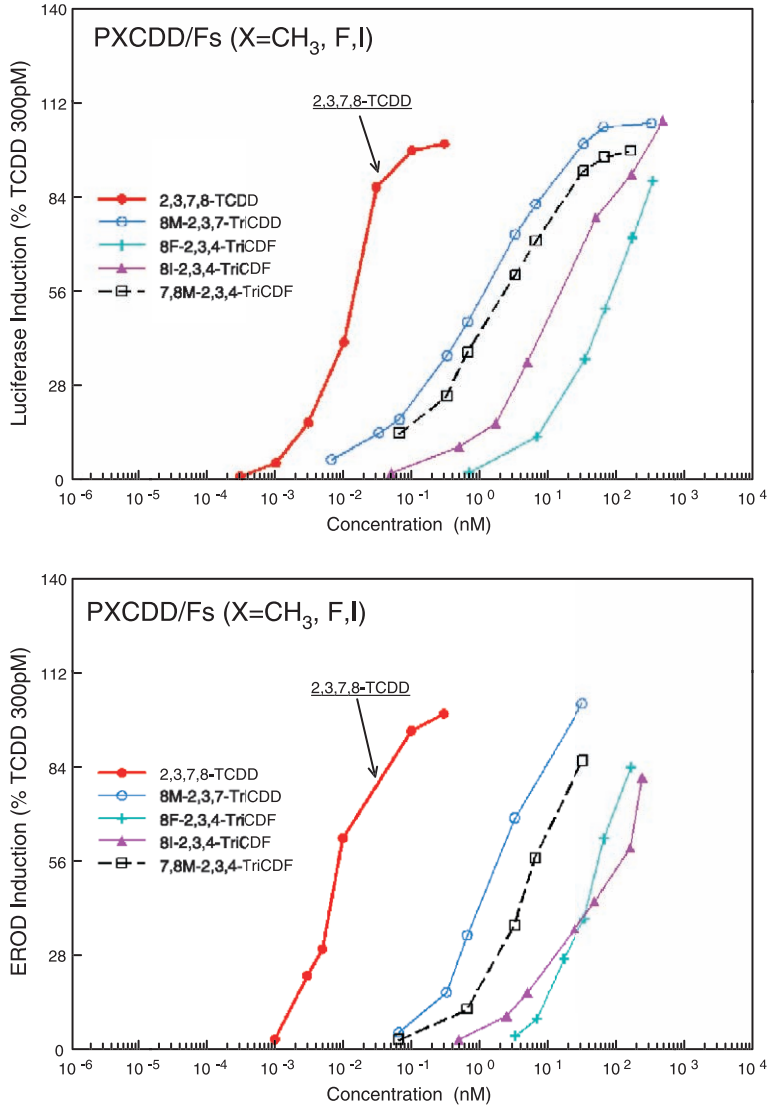

Fig. 4. Dose–response curves of several methylated or polyhalogenated PXDD/Fs (X = F, I, Cl) determined by DR-CALUX (first graph; luciferase induction) and Micro-EROD (second graph; EROD induction) bioassays.

4.3.1. Dose–response curves

The DR-CALUX and EROD activities of dioxin-like PCBs are shown in Fig. 3. Only PCB-126 induced activity in both assays up to the maximum level induced by TCDD. In general, the efficacy obtained using the Micro-EROD bioassay was higher. Only a few PCB congeners (such as PCB-105, PCB-118, or PCB-123) induced activity to a maximum of about 50% in at least one of the bioassays. Induction was less in the mono-ortho-PCBs in comparison with the planar non-ortho-PCBs.

4.4. Polyhalogenated aromatics

A huge number of polyhalogenated, e.g., chlorine- and/or bromine-containing, aromatic hydrocarbons (PHAHs) are suspected to cause health problems: biphenyls (PXBs), diphenyl ethers (PXDEs), benzenes, phenols (PXPs), dibenzo-*p*-dioxins/-furans (PXDD/Fs), etc. Brominated organic compounds are widely used as flame retardants, and their production is still increasing. Most common are PBDEs, PBBs, tetrabromobisphenol-A (TBBP-A), and hexabromocyclododecane (HBCD). In thermal processes, highly toxic PBDD/Fs can be formed from these parent compounds. Several reviews of these classes of mixed halogenated aromatic hydrocarbons are available (Behnisch et al., 2001b; Mason et al., 1987; Mennear and Lee, 1994; Safe, 1984; Weber and Greim, 1997; WHO, 1998).

4.5. Methylated and polyhalogenated (X=F, I) dibenzodioxins and -furans (PXCDD/Fs)

In Table 3, we present REP values for some methylated and polyhalogenated (X = F, I) dibenzodioxins and -furans (PXCDD/Fs) measured by both bioassays. When a methyl

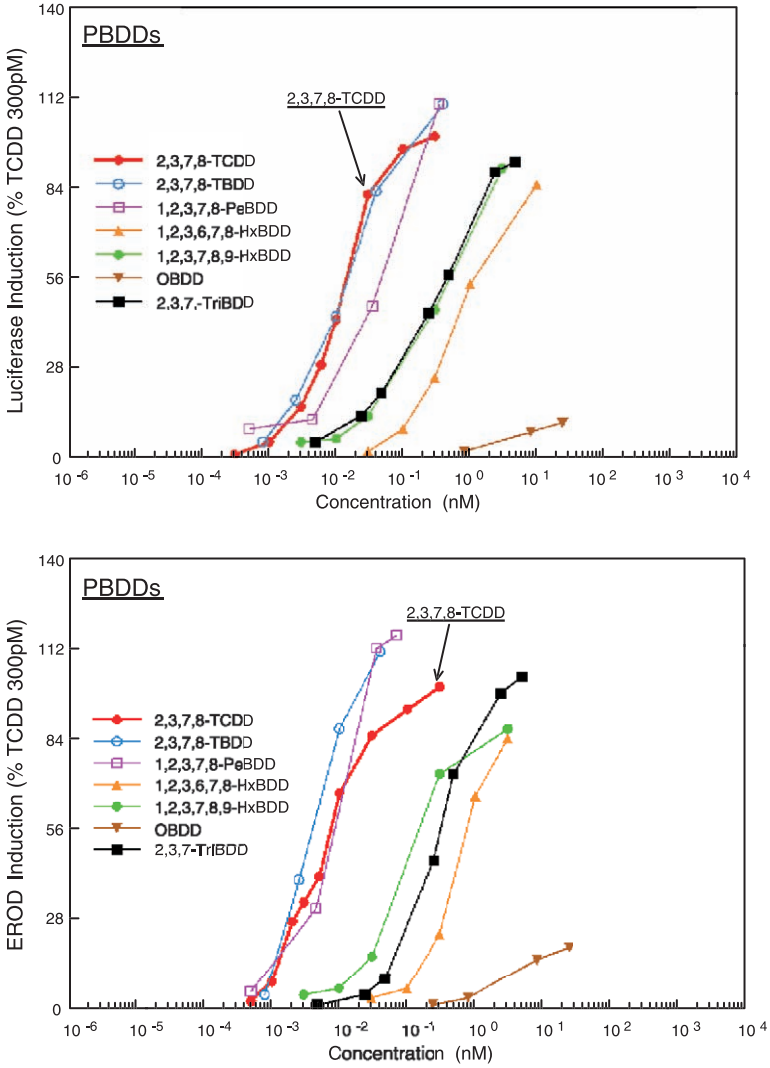

Fig. 5. Dose–response curves of PBDD congeners determined by DR-CALUX (first graph; luciferase induction) and Micro-EROD (second graph; EROD induction) bioassays.

group is substituted for one chlorine atom, the REP value decreases from 1 to 0.081 (DR-CALUX; EC_{5TCDD}) or 0.0078 (Micro-EROD). If two chlorine atoms are replaced with methyl groups in the 7 and 8 positions of 2,3,4,7,8-PeCDF, the DR-CALUX REP value decreases from 0.93 to 0.02 (EC_{5TCDD}), and the Micro-EROD value decreases from 0.58 to 0.003.

Compounds in which fluorine or iodine replaced one chlorine of 2,3,4,8-TCDF also showed significant activity (EC_{5TCDD}) in the DR-CALUX (REP values 4.6×10^{-4} and 0.0014, respectively) and Micro-EROD (2.5×10^{-4} and 4.2×10^{-4}, respectively) bioassays.

4.5.1. Dose–response curves

The DR-CALUX and EROD activities of methylated and polyhalogenated PXCDD/Fs are shown in Fig. 4. Most of these PXCDD/F congeners induced activity up to the maximum level induced by TCDD. We show here only the concentrations up to the plateau level, because for the calculation of the REP value, we focused on the linear part of the curve from about EC_5 to EC_{50}. Both bioassays showed less induction by the more methylated or iodated/fluorinated PXDD/F congeners.

4.6. Mixed polyhalogenated dibenzodioxins and -furans (PXDD/Fs; X=Br, Cl)

There are 4600 potential mixed congeners. The biological effects of PBDD/Fs are similar, if not identical, to those of their chlorinated analogues (PCDD/Fs). Both groups of compounds show similar effects, such as induction of aryl hydrocarbon hydroxylase (AHH)/EROD activity, and toxicity, such as induction of wasting syndrome, thymic atrophy, and liver toxicity, in rhesus monkeys, rats, and guinea pigs (for reviews, see, e.g., Behnisch et al., 2001b). Previously, we reviewed REP values relative to 2,3,7,8-TCDD

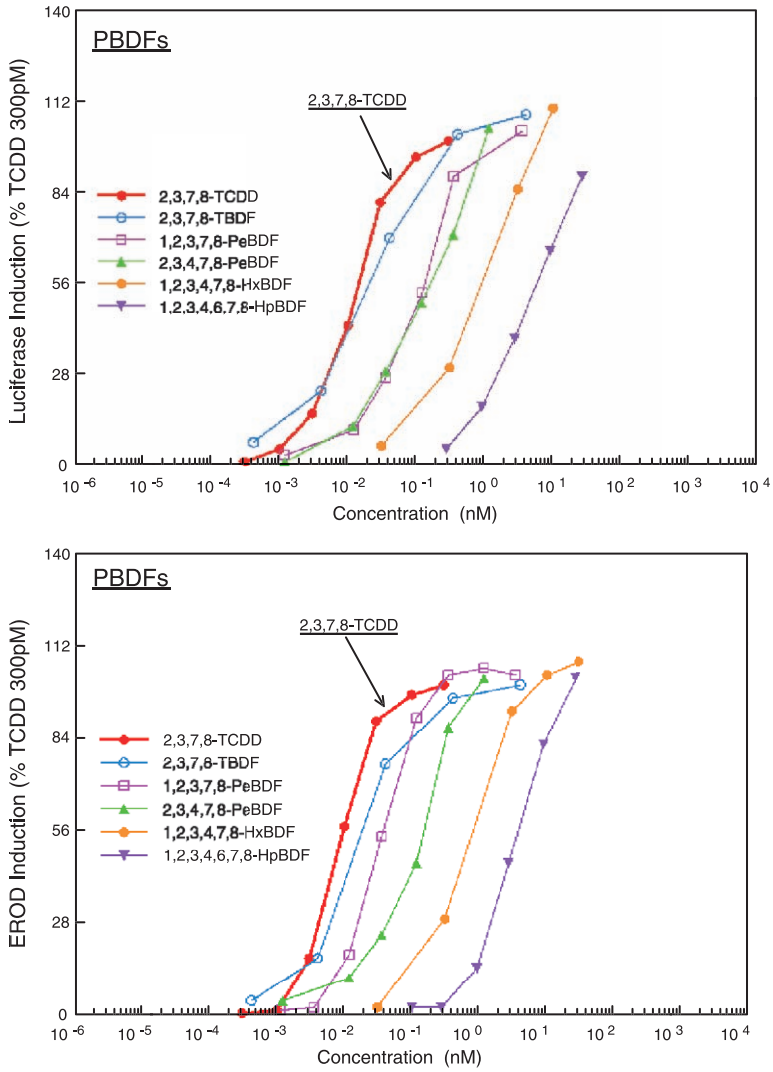

Fig. 6. Dose–response curves of PBDF congeners determined by DR-CALUX (first graph; luciferase induction) and Micro-EROD (second graph; EROD induction) bioassays.

for several PBDD/Fs and PBBs analyzed by in vitro AHH/EROD induction and in vivo AHH induction in the rat and in a rainbow trout early-life-stage mortality bioassay (Behnisch et al., 2001b). Safe et al. (Safe, 1990, 1993; Safe et al., 1991) reported REP values, determined in vivo, for AHH activity in immature male rat for several brominated and mixed brominated–chlorinated dioxins, and found that the toxic responses of these brominated dioxin congeners were similar. In addition, he reported the in vitro EROD activity (in rat hepatoma H4IIE cells, see first number) of several polybrominated dioxins, which are here compared to some of our results (REP values based on EC_{50}; see second number and Table 3): 2,3,7,8-TBDD: 2.3↔0.77; 2,3-diB-7,8-diCDD: 3.4↔0.86; and 2-B-3,7,8-triCDD: 0.23↔0.67.

In our study, Micro-EROD/DR-CALUX REP values for PXDD congeners were similar to earlier reported values from the WHO (1), while PXDF congeners showed significant differences (REP values based on EC_{5TCDD}; see Table 3): 2,3,7,8-TBDF (EROD: 0.53/DR-CALUX: 0.97 compared with earlier reported values from the WHO (1) for TCDF: 0.1), 1,2,3,7,8-PeBDF (EROD: 0.17/DR-CALUX: 0.13; compared with earlier reported values from the WHO (1) for 1,2,3,7,8-PCDF: 0.05), and 2,3,4,7,8-PeBDF (EROD: 0.094/ DR-CALUX: 0.12 compared with earlier reported values from the WHO (1) for 2,3,4,7,8-PCDF: 0.5).

The Cl/Br ratio, for example, of two PXDD/F congeners analyzed by DR-CALUX | EROD bioassay is for 1,2,3,7,8-PeXDD, 2.9 | 2.8, and for 2,3,4,7,8-PeXDF, 7.8 | 6.2. This shows for these two congeners a pattern whereby the REP value of the chlorinated congener is significantly higher in both bioassays.

The DR-CALUX/Micro-EROD TEF ratio for 14 PCDD/F congeners ranged from 0.7 to 3.7 (mean 1.8) and for 18 PBCDD/Fs from 0.8 to 3.0 (mean 1.8).

Thus, a decrease in the dioxin-like potency with an increase in number of halogens was confirmed (WHO, 1998).

4.6.1. Dose–response curves

The DR-CALUX and EROD activities of PBDD/Fs are shown in Figs. 5 and 6. Most of the PBDD/F congeners induced activity up to the maximum level induced by TCDD. Only OBDD did not show significant induction in either bioassay. We show here only the concentrations up to the plateau level, because for the calculation of the REP values, we focused on the linear part of the curve from about EC_5 until EC_{50}.

4.7. Polybrominated biphenyls (PBBs)

In our studies, PBB-77 showed the highest activity (REP EC_{5TCDD}) in both bioassays (DR-CALUX/Micro-

Table 4
DR-CALUX-REP and Micro-EROD-REP values for several polybrominated compounds relative to 2,3,7,8-TCDD

Standard samples	DR-CALUX-REP (pM)[a]			Micro-EROD-REP (pM)[b]			Efficacy % of TCDD B_{max}	
	EC_{5TCDD}	EC_{20}	EC_{50}	EC_{5TCDD}	EC_{20}	EC_{50}	DR-CALUX	Micro-EROD
3,3′,4,4′-TBB (PBB-77)	0.083	0.13	0.080	0.058	0.084	0.043	107	103
2,2′,4,5′,6-PeBB (PBB-103)	0.0015	0.0041	0.0028	4.6×10^{-4}	0.0013	0.0012	57	48
3,3′,4,4′,5-PeBB (PBB-126)	0.12	0.21	0.16	NT[c]	–	–	90	–
3,3′,4,4′,5,5′-HxBB (PBB-169)	0.0031	0.0056	0.0047	0.0026	0.0048	0.0041	102	96
TBBP-A	2.5×10^{-6}	3.4×10^{-6}	2.6×10^{-6}	$<7.3 \times 10^{-7}$	–	–	46	17
PBDE-47	$<1.1 \times 10^{-6}$	–	–	NT	–	–	0.2	–
PBDE-66	$<2.0 \times 10^{-6}$	–	–	NT	–	–	7	–
PBDE-77	$<1.1 \times 10^{-5}$	–	–	$<2.8 \times 10^{-6}$	–	–	25	9
PBDE-85	$<2.1 \times 10^{-6}$	–	–	NT	–	–	7	–
PBDE-99	$<5.6 \times 10^{-7}$	–	–	NT	–	–	0.1	–
PBDE-100	$<1.2 \times 10^{-6}$	–	–	NT	–	–	3	–
PBDE-105	$<3.8 \times 10^{-6}$	–	–	NT	–	–	14	–
PBDE-119	1.1×10^{-5}	NA[d]	NA	$<3.3 \times 10^{-6}$	–	–	31	10
PBDE-153	$<2.3 \times 10^{-6}$	–	–	NT	–	–	9	–
PBDE-183	$<1.5 \times 10^{-6}$	–	–	NT	–	–	5	–
PBDE-190	$<4.6 \times 10^{-5}$	–	–	NT	–	–	14	–
PBDE-209	1.6×10^{-5}	NA	NA	$<4.0 \times 10^{-6}$	–	–	32	17
PBDE-126	9.3×10^{-5}	1.8×10^{-4}	1.3×10^{-4}	4.3×10^{-5}	1.7×10^{-4}	1.3×10^{-4}	83	43
α-HBCD	$<2.1 \times 10^{-5}$	–	–	NT	–	–	11	–
β-HBCD	$<1.7 \times 10^{-5}$	–	–	NT	–	–	9	–
γ-HBCD	$<2.4 \times 10^{-5}$	–	–	NT	–	–	13	–
p-BP	$<2.4 \times 10^{-8}$	–	–	NT	–	–	4	–
2,4,6-TriBP	$<2.5 \times 10^{-8}$	–	–	NT	–	–	1	–
PeBP	$<4.6 \times 10^{-7}$	–	–	NT	–	–	8	–

[a] Mean of three determinations.
[b] Mean of two or three determinations.
[c] Not tested.
[d] Full dose–response curve was not obtained.

EROD: 0.08/0.06), whereas PBB-103 and PBB-169 activity was lower by at least one order of magnitude (see Table 4). Comparison of the chlorinated and brominated biphenyl congeners tested showed that PBB-77 (DR-CALUX/Micro-EROD: 0.08/0.06) had a higher REP value in both bioassays than its chlorinated analogue (Table 2; 0.0015/0.0005), whereas the higher hexabrominated PBB-169 (0.0031/0.0026) and its chlorinated analogue showed similar activity (Table 2; 0.0025/0.0016). The statement of Safe (1994) that PBB-77 appears to be more active than the chlorinated analogue due to "inherent properties of the chloro and bromo substituents which facilitate increased receptor-binding affinities and increased receptor-mediated biological and toxic effects for the brominated analogs" is thus confirmed, but the PBB-169 congeners may be too large for strong AhR binding because of the larger bromine substituents.

4.8. Polybrominated diphenyl ethers (PBDEs)

Previously, we reviewed current REP value data from bioassays for PBDEs (Behnisch et al., 2001b). REP values so far analyzed by the H4IIE-CALUX bioassay (Jeong et al., 2001), often incubated for 24 h, are several orders of magnitude lower than that of 2,3,7,8-TCDD: (a) TBDE-47 (7.1×10^{-7}; % $TCDD_{max}$: 51); (b) PBDE-99 (5.9×10^{-6}; % $TCDD_{max}$: 32); (c) HBDE-153 (4.3×10^{-6}; % $TCDD_{max}$: 64); and (d) Bromkal 70-5-DE (4.8×10^{-6}; % $TCDD_{max}$: 49).

In another study for which the H4IIE-CALUX bioassay was used, 17 PBDE congeners were able to activate the AhR in an agonistic (e.g., BDE-166 and BDE-190), partly agonistic and partly antagonistic (BDE-85, -99, and -119), or fully antagonistic way (BDE-47, -77, and -138) way. EC_{50} values of BDE-166 (2,3,4,4′,5,5-HBDE; 1400 nM) and BDE-190 (2,3,3′,4,4′,5,6-HpBDE; 800 nM) were in the same range as those of PCB-105 and PCB-118 (Meerts et al., 1998).

Using the wild-type H4IIE-EROD bioassay (24-h kinetic, rat hepatocytes), PBDE congeners had the following REP values: PBDE-126 (4.1×10^{-4}), PBDE-77 (2.3×10^{-4}), PBDE-119 (1.0×10^{-4}), PBDE-100 (1.3×10^{-5}), PBDE-183 (3.9×10^{-6}), PBDE-153 (3.4×10^{-5}), PBDE-85 (1.0×10^{-4}), and PBDE-66 (3.2×10^{-5}), whereas PBDE-154, -99, -47, and -28 were inactive (Chen et al., 2001; Bunce et al., 2001).

For comparison, in our studies we calculated for the DR-CALUX bioassay the following REP values: PBDE #77

Table 5
DR-CALUX-REP (24 h kinetic) and Micro-EROD-REP (72 h kinetic) values for several polychlorimated naphthalenes (PCNs) relative to 2,3,7,8-TCDD

Standard samples	DR-CALUX-REP (pM)[a]			Micro-EROD-REP (pM)[b]			Efficacy % of TCDD B_{max}	
	EC_{5TCDD}	EC_{20}	EC_{50}	EC_{5TCDD}	EC_{20}	EC_{50}	DR-CALUX	Micro-EROD
1-MoCN	5.0×10^{-5}	3.0×10^{-5}	1.7×10^{-5}	$<6.4 \times 10^{-6}$	–	–	54	17
2-MoCN	2.7×10^{-5}	2.6×10^{-5}	1.8×10^{-5}	$<1.5 \times 10^{-6}$	–	–	42	10
1,2-DiCN	$<2.9 \times 10^{-7}$	–	–	NT[c]	–	–	2	–
1,4-DiCN	3.0×10^{-5}	5.0×10^{-5}	3.5×10^{-5}	$<1.6 \times 10^{-6}$	–	–	37	10
1,5-DiCN	$<1.2 \times 10^{-6}$	–	–	$<6.6 \times 10^{-7}$	–	–	8	2
1,8-DiCN	1.5×10^{-5}	NA[d]	NA	$<1.7 \times 10^{-6}$	–	–	28	9
2,3-DiCN	3.7×10^{-5}	4.1×10^{-5}	2.7×10^{-5}	$<5.9 \times 10^{-6}$	–	–	45	12
1,2,3-TriCN	$<4.4 \times 10^{-6}$	–	–	$<2.0 \times 10^{-6}$	–	–	22	10
1,2,3,4-TCN	$<2.3 \times 10^{-6}$	–	–	$<1.6 \times 10^{-6}$	–	–	14	7
1,2,5,6-TCN	$<4.1 \times 10^{-7}$	–	–	NT	–	–	2	–
1,3,5,7-TCN	7.5×10^{-6}	NA	NA	$<1.9 \times 10^{-6}$	–	–	28	10
2,3,6,7-TCN	4.2×10^{-5}	4.4×10^{-5}	4.1×10^{-5}	NT	–	–	70	–
1,2,3,4,6-PeCN	5.0×10^{-5}	1.0×10^{-4}	6.8×10^{-5}	2.5×10^{-5}	5.3×10^{-5}	4.3×10^{-5}	46	48
1,2,3,5,7-PeCN	$<3.4 \times 10^{-6}$	–	–	$<1.8 \times 10^{-6}$	–	–	20	9
1,2,3,6,7-PeCN	0.0018	9.6×10^{-4}	5.8×10^{-4}	NT	–	–	89	–
1,2,3,5,8-PeCN	$<1.8 \times 10^{-6}$	–	–	$<1.2 \times 10^{-6}$	–	–	11	5
1,2,3,4,6,7-HxCN	0.0014	0.0017	0.0012	0.00058	0.00053	0.00054	89	127
1,2,3,5,6,7-HxCN	3.8×10^{-4}	6.1×10^{-4}	4.8×10^{-4}	NT	–	–	65	–
1,2,3,5,6,8-HxCN	2.8×10^{-4}	4.8×10^{-4}	4.9×10^{-4}	NT	–	–	59	–
1,2,3,5,7,8-HxCN	7.2×10^{-5}	1.6×10^{-4}	1.1×10^{-4}	2.2×10^{-5}	1.4×10^{-5}	6.4×10^{-6}	41	48
1,2,3,6,7,8-HxCN	0.0097	0.0095	0.0028	NT	–	–	98	–
1,2,4,5,6,8-HxCN	$<1.1 \times 10^{-6}$	–	–	NT	–	–	7	–
1,2,4,5,7,8-HxCN	4.5×10^{-5}	9.0×10^{-5}	6.0×10^{-5}	7.1×10^{-6}	NA	NA	38	32
1,2,3,4,5,6,7-HpCN	3.7×10^{-4}	5.9×10^{-4}	5.2×10^{-4}	NT	–	–	70	–
1,2,3,4,5,6,8-HpCN	4.1×10^{-6}	NA	NA	NT	–	–	23	–
OCN	1.0×10^{-5}	NA	NA	$<4.3 \times 10^{-6}$	–	–	35	21

[a] Mean of three or four determinations.
[b] Mean of two or three determinations.
[c] Not tested.
[d] Full dose–response curve was not obtained.

($<1.1\times10^{-5}$), PBDE #105 ($<3.8\times10^{-6}$), PBDE #119 (1.1×10^{-5}), PBDE #190 ($<4.6\times10^{-5}$), PBDE #209 (1.6×10^{-5}). PBDE #126 (DR-CALUX/Micro-EROD: $9.3\times10^{-5}/4.3\times10^{-5}$) showed similar weak activity in both assays (see Table 4, REP EC_{5TCDD}).

4.9. Brominated flame retardants

Earlier studies with in vitro/in vivo systems indicated that measured TCDD equivalents for several brominated aromatic flame retardant pyrolysates (FireMaster 300 BA and BP-6, Bromkal 70-5-DE, 70-DE, and GI) ranged from 170 to 8960 ppm (in comparison with 0.1 ppm for fly ash) (Zacharewski et al., 1988).

In our study, TBBP-A, α-HBCD, β-HBCD, γ-HBCD, *p*-bromophenol, 2,4,6-tribromophenol, and pentabromophenol did not show any activity (see Table 4).

4.10. Polychlorinated naphthalenes (PCNs)

Blankenship et al. (2000), Villeneuve et al. (2000a,b, 2001), and Sanderson et al. (1996) found that lower chlorinated Halowaxes (1000, 1001, 1099) are inactive in the EROD and DR-CALUX bioassays, while higher tetra- to octachlorinated naphthalenes containing Halowaxes 1013, 1014 (REP: 5.4×10^{-5} to 6.8×10^{-5}), and 1051 are active. Mainly, the penta- to heptachlorinated naphthalenes show measurable activity in comparison with TCDD, with the largest REP value being 0.0035 for hexa-CN-73, followed by hepta-CN-66/67 (0.0023), hexa-CN-63/69 (0.002), hexa-CN-70 (0.0006), penta-CN-54 (0.0002), and tetra-CN-40 (1.65×10^{-5}). Furthermore, Hanberg et al. (1991) reported REP values from 7×10^{-6} to 0.002 for hexachlorinated naphthalenes analyzed by the EROD bioassay.

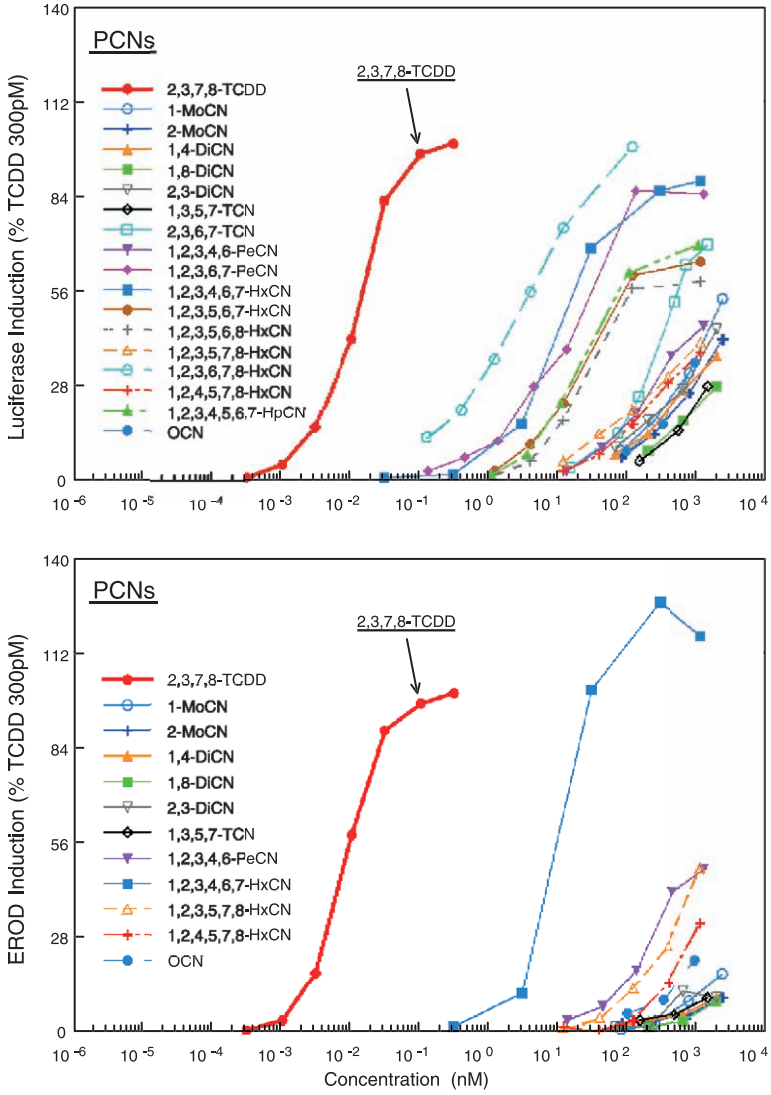

Fig. 7. Dose–response curves of PCN congeners determined by DR-CALUX (first graph; luciferase induction) and Micro-EROD (second graph; EROD induction) bioassays.

Table 6
DR-CALUX-REP (24 h kinetic) and Micro-EROD-REP (72 h kinetic) values for several polyaromatic hydrocarbons (PAHs) relative to 2,3,7,8-TCDD

Standard samples	DR-CALUX-REP (pM)[a]			Micro-EROD-REP (pM)[b]			Efficacy % of TCDD B_{max}	
	EC_{5TCDD}	EC_{20}	EC_{50}	EC_{5TCDD}	EC_{20}	EC_{50}	DR-CALUX	Micro-EROD
Dibenzo(*a*,*h*)anthracene	0.0049	0.0039	0.0011	7.0×10^{-4}	0.0013	6.2×10^{-4}	117	90
Benzo(*k*)fluoranthene	0.0041	0.0045	5.4×10^{-4}	0.0013	0.0012	3.2×10^{-4}	127	102
Benzo(*b*)fluoranthene	0.0042	0.0038	9.2×10^{-4}	3.8×10^{-4}	6.5×10^{-4}	2.6×10^{-4}	96	86
Indeno[1,2,3]pyrene	0.0041	0.0026	7.6×10^{-4}	2.8×10^{-4}	2.7×10^{-4}	1.6×10^{-4}	109	104
Benzo(*a*)pyrene	5.8×10^{-4}	5.2×10^{-4}	2.5×10^{-4}	4.1×10^{-5}	4.0×10^{-5}	2.4×10^{-5}	113	89
Benzo(*a*)anthracene	1.7×10^{-4}	1.5×10^{-4}	1.1×10^{-4}	1.8×10^{-5}	6.4×10^{-6}	3.8×10^{-6}	96	79
Pyrene	1.4×10^{-5}	–	–	$< 7.0 \times 10^{-7}$			37	17
Phenanthrene	1.3×10^{-6}	–	–	NT[c]			18	–

[a] Mean of three determinations.
[b] Mean of two or three determinations.
[c] Not tested.

The results from our study with H4IIE-DR-CALUX and -wild-type cells are in good agreement with previous studies showing low activity for most PCN congeners (see Table 5). However, in our study, 1,2,3,4,6,7-HxCN showed REP values ranging from 0.0012 to 0.0017 (DR-CALUX; EC_{5TCDD}) and 0.0005 (Micro-EROD). Furthermore, our

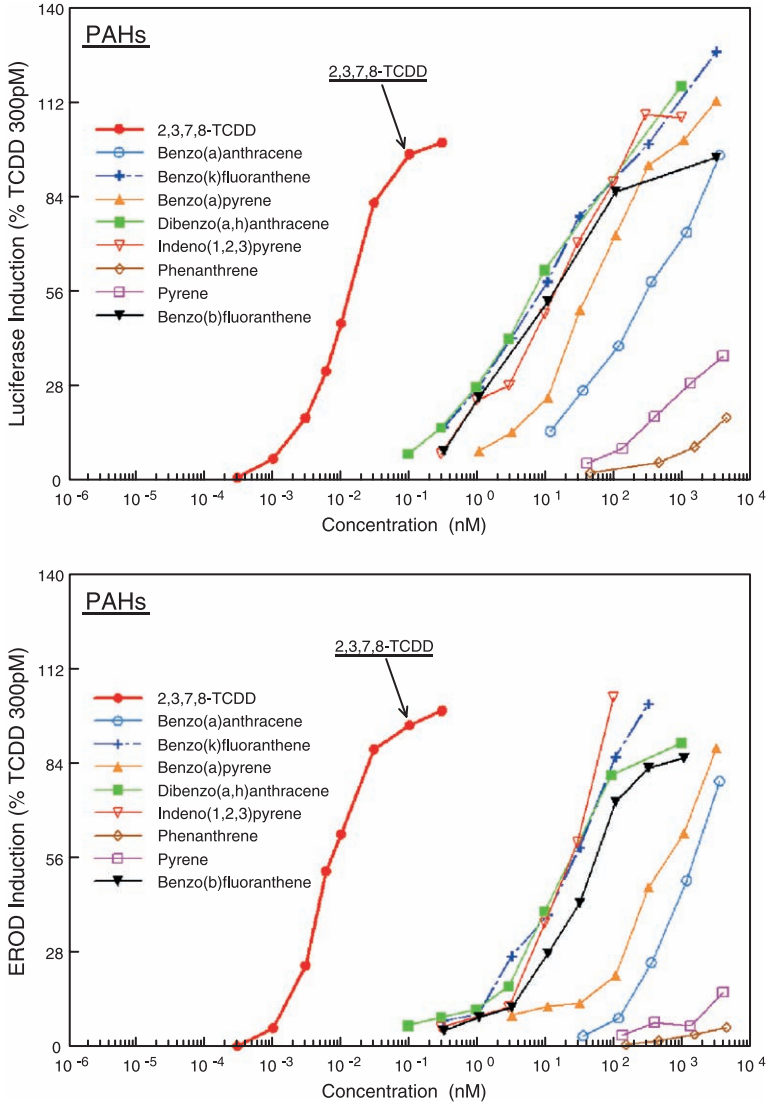

Fig. 8. Dose–response curves of PAH congeners determined by DR-CALUX (first graph; luciferase induction) and Micro-EROD (second graph; EROD induction) bioassays.

study found weak activity (DR-CALUX, REP EC_{5TCDD}) by 1,2,3,4,6-penta-CN-50 (0.00005) and 1,2,3,5,7,8-hexa-CN-69 (0.00007).

4.10.1. Dose–response curves

The DR-CALUX and EROD activities of PCNs are shown in Fig. 7. Most of the PCN congeners could not induce activity to the maximum level inducible by TCDD (in the range of 30–50%). Only 1,2,3,4,6,7-hexa-CN showed TCDD-like induction in both bioassays. For most of the weak agonists, the EROD bioassay (because of the longer incubation time) showed lower induction than the DR-CALUX bioassay.

4.11. Polyaromatic hydrocarbons (PAHs)

These compounds do not meet the criteria for the TEF approach, but in terms of binding to the Ah receptor, they have a high potential toxicity. Recently, several studies have examined REP values of PAHs relative to 2,3,7,8-TCDD by using wild-type and recombinant H4IIE cell-based bioassays (Delistry, 1997; Jones and Anderson, 1999; Khim et al., 2000; Machala et al., 2001; Pijnenburg et al., 1995; Schramm et al., 2001; Till et al., 1999; Willett et al., 1997; for review, see Behnisch et al., 2001b). However, for a comparison of these easily metabolized compounds, the kinetics play an important role. Machala et al. (2001) used the DR-CALUX bioassay, i.e., the same cell system, with the same incubation time as in our test, whereas Khim et al. (2000) used a longer incubation time, which resulted in lower REP values (see Table 6). Our results, when compared with those of Machala et al. (2001), showed significant differences only for benzo[*b*]fluoranthene, while all the other PAHs were different by at most one order of magnitude (see Table 6).

Several studies used H4IIE cells to evaluate the AhR activity of PAHs with a shorter, 24-h kinetic (Willett et al., 1997; Khim et al., 1999; Schramm et al., 2001). In our study, we wanted to know the dioxin-like activity with a longer incubation time (72 h) to understand the possible influence of this compound class on our screening assay, in which we always use a 72-h kinetic because of the higher induction factor for 2,3,7,8-TCDD with the longer incubation time.

Therefore, this study is the first that uses H4IIE cells to measure TCDD-like activity with a 72-h kinetic. Using the same cells and measuring TCDD-like activity after 24 h of incubation, Schramm et al. (2001) reported EROD REP values that differed from our results by only one to two orders of magnitude (see Table 6).

4.11.1. Dose–response curves

The DR-CALUX and EROD activities of PAHs are shown in Fig. 8. Most of the PAHs could induce activity up to the maximum level induced by TCDD. Several of the higher aromatic compounds such as benzo[*a*]pyrene, benzo[*k*]fluoranthene, dibenzo[*a*,*h*]anthracene, and indeno[1,2,3-*cd*]pyrene showed some superinduction effects. Only lower aromatic hydrocarbons such as pyrene or phenanthrene showed induction below 40%. For most of the PAHs, the CALUX bioassay (due to the shorter incubation time) showed higher induction than the Micro-EROD bioassay.

5. Conclusion

Numerous studies have already demonstrated the utility of Ah receptor-based cell bioassays in the assessment of relative potencies of individual chemicals. However, this is the first study that has tested such a wide range of different compound classes in a congener-specific manner with two different rat liver cell based bioassay systems.

By comparing the wild-type (EROD) and recombinant (genetically modified; DR-CALUX) rat liver cell lines tested, we can also confirm, as stated previously (Hoogenboom et al., 1999; Sanderson et al., 1996; Seidel et al., 2000), that the DR-CALUX bioassay system certainly shows significant improvements over the EROD bioassay, because of its better quality criteria (e.g., repeatability, reproducibility), as well as its easier handling and data calculations, achieved in our study. Overall, we have shown that the bioassay approach is an efficient (fast/cost effective) screening system to identify compound classes and evaluate congener-specific toxicity. It can be a useful tool for monitoring possible AhR agonists and can therefore help evaluate them as possible dioxin-like POPs.

The results of the present in vitro study do not, of course, reflect the pharmacokinetics, tissue distribution, biotransformation, or non-receptor-mediated responses that may occur in vivo. However, this approach using newly developed REP values can certainly be used to screen many chemicals and to evaluate on short notice the potential ecotoxicological relevance of the multiplication of the concentrations of these compounds.

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