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Determination of *in vitro* relative potency (REP) values for mono-*ortho* polychlorinated biphenyls after purification with active charcoal

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Abstract

The TEF system for dioxin-like compounds has included assignment of TEF values for mono-*ortho* polychlorinated biphenyls (MO-PCBs). Small traces of aryl hydrocarbon receptor (AhR)-active impurities could result in artifactually higher relative potency (REP) values. MO-PCBs -105, -118, -156, and -167 were purified on an active charcoal column to remove AhR agonists that could be present as impurities. Activation or inhibition of AhR-dependent gene expression by purified MO-PCBs was studied in stably transfected cell lines (H1G1.1c3 mouse, H4G1.1c2 rat hepatoma), containing an AhR-responsive (AhR-EGFP) reporter gene. In addition, EROD activity was used as marker for CYP1A1 activity in these cell lines. MO-PCBs -105, -118, -156 induced AhR-EGFP expression in both rodent cell lines, with PCB-156 (10 μM) being most effectively; inducing gene expression to ~27% of TCDD (mouse cells) and 62.5 ± 3.4% (rat cells) of TCDD. This concurred with increased EROD activity in both cell lines to maxima of 20.5 ± 1.5% and 68 ± 3.2% of TCDD, respectively. No induction was observed for PCB-167. In the H1G1.1c3 mouse cells, PCB-105, -118 and -156 (10 μM) significantly reduced TCDD-induced AhR-EGFP expression to 50.9 ± 2.9%, 58.3 ± 2.2% and 70.8 ± 1.3% of TCDD. Reduced EROD activity was also observed, of 39.3 ± 2.8%, 67 ± 5% and 48.3 ± 4% compared to TCDD. PCB-167 did not result in significant reduction. In rat cells, only PCB-156 resulted in significant decrease in TCDD-induced AhR-EGFP expression of 35%, suggesting species differences play a role. Our results suggest that purification of MO-PCBs is an essential step in determining accurate REP values, and could very likely lead to lower TEF values than those presently assigned by the WHO.

Keywords

MO-PCB; TEF; AhR; EROD; CYP1A1; AhR-EGFP

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1. Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and biphenyls (PCBs) are important classes of persistent organic compounds found in the global environment and many individual congeners are highly toxic (Safe, 1994). Because of their insulating and flame retardancy properties, PCBs were extensively used in industrial applications, and have been commercially produced since the 1920s. Releases of PCBs into the environment have resulted in a widespread global distribution into all environmental and biological matrices (Hong et al., 1998). These compounds are lipophilic and accumulate in lipid rich body tissues and fluids from man and wildlife species and can biomagnify up the food chain. PCBs were banned for open applications in many countries in the Western world since the late 1970s due to their persistent nature and toxic effects (Ballschmiter and Zell, 1980). PCB exposure, especially in occupational settings and wildlife, has been associated with e.g. chloracne, carcinogenesis, teratogenesis, impaired immune responses, endocrine disruption, as well as the induction of gene expression (Ahlborg et al., 1994; Safe, 1994).

Environmental contamination by PCDDs and PCDFs has occurred trough emission from waste incinerators and general combustion reactions, their presence as impurities in industrial products such as herbicides and generation during the bleaching of wood pulp for paper production (Ahlborg et al., 1994; Safe, 1994; Van den Berg et al., 1998). Human exposure to PCBs and dioxin-like compounds occurs primarily through occupational and dietary exposure, although concentrations of PCBs and dioxins have declined over the last decades due to the decreased use of the compounds (Ahlborg et al., 1992; Baars et al., 2004; Shadel et al., 2001).

There are 209 possible PCBs congeners and their dioxin-like biological and toxic effects and relative potency depend on their chlorination pattern which dictates their ability to bind to and activate the aryl hydrocarbon (Ah) receptor and AhR-dependent toxicity (Ahlborg et al., 1992; Andersson et al., 1999; Giesy and Kannan, 1998; Safe, 1990, 1994). In this regard, PCBs have been structurally divided into three groups; the non-*ortho* PCBs with a planar conformation, ability to activate the AhR and produce dioxin-like effects; the mono-*ortho* (MO-) substituted PCBs (e.g. PCB-105, -118, -123, -156, -157, -167, and -189) with some planar conformation, weak ability to activate the AhR and produce some dioxin-like effects; multiple-*ortho* substituted PCBs with a non-planar configuration, no or very low affinity for the AhR and inability to produce dioxin-like effects. The biological and toxic effects of PCBs have been studied extensively. Research has primarily focused on the non-*ortho* substituted congeners, i.e. PCBs -77, -126 and -169 as these are potent agonists for the AhR that produce dioxin-like toxic effects. As a consequence, these congeners have been assigned toxic equivalency factors (TEF) values by the World Health Organization (WHO) (Ahlborg et al., 1992; Giesy and Kannan, 1998; Van den Berg et al., 1998). While the MO-PCBs have always been thought to have low affinity for the AhR, with TEF values varying from 1×10^{-5} to 1×10^{-4} (Ahlborg et al., 1992; Safe, 1990, 1994; Van den Berg et al., 1998), due to their relatively high concentrations in food and human milk these PCBs contribute significantly to the overall total toxic equivalency (TEQs) of a sample (Ahlborg et al., 1992, 1994). The MO-PCBs, together with the non-*ortho* substituted dioxin-like PCBs typically represent a higher percentage of the total TEQs of a sample, than that from the PCDDs and PCDFs (Ahlborg et al., 1992).

The toxic and biological effects of PCDDs, PCDFs and those PCBs with dioxin-like activity are mediated by the AhR, a ligand-dependent transcription factor. While the AhR can be found in the cytoplasm of almost all vertebrate cells, significant species and tissue differences exist in its concentration and distribution (Denison et al., 2002; Denison and Heath-Pagliuso, 1998; Guengerich, 1993). In addition to the planar halogenated

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polyaromatic compounds, recent studies have revealed the ability of very structurally very diverse chemicals to bind and/or activate the AhR and AhR-dependent gene expressions (Denison et al., 2002; Denison and Heath-Pagliuso, 1998; Hahn, 2002). Upon ligand binding, the ligand:AhR complex translocates into the nucleus, wherein it binds to the AhR nuclear translocator protein (ARNT) (Whitlock et al., 1996). Binding of the resulting ligand:AhR:ARNT complex to its specific DNA recognition site, the dioxin response element (DRE), stimulates transcription of a variety of genes, including that of Cytochrome P450 1A1 (*CYP1A1*) (Hankinson, 2005; Kuramoto et al., 2002; Lees and Whitelaw, 1999; Mimura et al., 1999; Nebert and Gonzalez, 1987).

The induction of CYP1A1 has been used both as a model system to study the molecular mechanism of AhR activation and as a bioanalytical method to determine the relative potency of AhR agonists (Denison et al., 2004). Given the role of the AhR in both gene induction and toxicity of these chemicals, it is not surprising that there is a high degree of correlation between the relative potency (REP) of PCDDs, PCDFs and PCBs to induce AhR-dependent gene expression (e.g., CYP1A1) and their relative toxic potency (i.e., their TEF value).

The ability of MO-PCBs to induce CYP1A1 has been previously examined in various human cell lines. While MO-PCBs failed to induce EROD activity in the human hepatoma (HepG2) cell line, or in a human prostate carcinoma (LNCaP) cell line, they were weak inducers of CYP1A1 in the rat hepatoma (H4IIE) cell line (Endo et al., 2003; Zeiger et al., 2001). In addition, although PCB-156 could induce CYP1A1 in fish (PLHC-1) hepatoma cells, two other MO-PCBs, -105 and -118, induced little or no CYP1A1 activity (Hestermann et al., 2000). In primary cultures of rat and cynomolgus monkey hepatocytes, MO-PCBs were found to induce CYP1A1-dependent EROD activity (Chen and Bunce, 2004; Van der Burght et al., 1998, 1999; Zeiger et al., 2001).

In contrast, no CYP1A1 induction was observed with individual MO-PCBs (except PCB-156) in *in vivo* experiments with rats and mice (DeVito et al., 1993; Kuriyama et al., 2003). Thus, when reviewing the relative potencies of MO-PCBs in various *in vitro* and *in vivo* systems there are significant, yet unexplained differences, which are in strong contrast with consistent induction results obtained with PCB-126 (Haws et al., 2004, 2006).

While species, tissue and cell-type differences could contribute to the variability in the results of the above studies, recent questions regarding the presence of impurities in MO-PCB preparations that have relatively potent dioxin-like activities have complicated clear interpretation of prior studies (DeVito et al., 2003; Van den Berg et al., 1998). The presence of low levels of potent AhR-active compounds (such as PCDFs or coplanar PCBs) as impurities in a test compound that is presumed to be pure, could actually be responsible for some or all of the AhR-mediated responses that would otherwise be contributed to the test compound (Brown et al., 2003; Koistinen et al., 1996).

Here we have used several MO-PCBs (PCB-105, -118, -156, and -167; Fig. 1) that have been extensively purified to eliminate the presence of dioxin-like PCDDs, PCDFs and PCB contaminants This was done in order to clearly determine whether these MO-PCBs can bind to and activate AhR-dependent gene expression and/or whether their previously documented ability to activate the AhR was due to the presence of other AhR-active agonist contaminants present in the MO-PCB preparations.

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2. Materials and methods

2.1. Chemicals

The mono-*ortho*-PCBs -105, -118, -156, and -167 were purchased from Cambridge Isotope Laboratories (Woburn, MA, USA); each congener was subjected to purification on activated charcoal and Celite to remove possible contamination with undesired dioxin-like contaminants (Marsh et al., 1999). The compounds were subsequently analyzed by HRGC-MS to determine whether the contaminants were still present in the mixture.

2,3,7,8-TCDD (>99% pure) was purchased from Cambridge Isotope Laboratories (Woburn, MA, USA); cell culture media Dulbecco's Modified Eagle Medium (DMEM), phosphate buffered saline (PBS), and fetal calf serum (FCS) were obtained from Gibco BRL (Breda, The Netherlands). All other chemicals were obtained from Sigma Chemical Company (St. Louis, MO, USA).

2.2. Cell culture

The mouse and rat hepatoma cell lines (H1G1.1c3 and H4G1.1c2) were created by stable transfection of mouse hepatoma (Hepa1c1c7) and rat hepatoma (H4IIE) cells with the AhR-responsive enhanced green fluorescent protein (AhR-EGFP) reporter plasmid pGreen1 as reported earlier (Nagy et al., 2002). Both cell lines were cultured in DMEM supplemented with 10% heat inactivated FCS, 100 U/ml penicillin, and 100 μg/ml streptomycin in an incubator (5% CO_2, 37 °C).

2.3. Enhanced green fluorescent protein (EGFP) assay

The H1G1.1c3 and H4G1.1c2 cell lines were stably transfected with an enhanced green fluorescent protein (EGFP) reporter containing approximately 500 bp of the CYP1A1 promoter including 4 XRE sequences (Nagy et al., 2002). Both cell lines were seeded in normal culture medium in 96 well plates (1×10^4 cells/well) and exposed after 24 h to the solvent control DMSO (0.1%), positive controls TCDD (0.001–1 nM) and PCB-126 (0.01–10 nM), the indicated MO-PCBs (0.01–10 μM), or co-exposed to various concentrations of both TCDD and MO-PCBs. After 72 h the cells were washed twice with phosphate buffered saline (PBS, 37 °C), PBS was added to each well and fluorescence of the intact cells was measured using an excitation wavelength of 485 nm and emission wavelength of 510 nm in a Fluostar fluorescence plate reader (BMG). Induced AhR-EGFP activity was determined by subtracting the background fluorescence in the DMSO sample from the fluorescence treated samples (Galietta et al., 2001).

2.4. 7-Ethoxyresorufin-O-deethylase (EROD) assay

Ethoxyresorufin-*O*-deethylation (EROD) activity was used as a marker for CYP1A1 catalytic activity using a modification of the method described by Burke and Mayer (1974) as we have recently reported (Peters et al., 2004). Directly following the AhR-EGFP assay, the PBS in the well plates was replaced with serum-free medium supplemented with 5 mM $MgCl_2$, 5 mM 7-ethoxyresorufin (7-ER), and 10 mM dicumarol. Metabolic conversion of 7-ER into the fluorescent resorufin product was followed over a 10 min period at 37 °C using an excitation wavelength of 530 nm and emission wavelength of 590 nm.

2.5. Cell viability

After cells were incubated with the designated compounds for 72 h, they were washed and medium was replaced with a 1 mg/ml MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution. The conversion of MTT to formazan in the presence of the mitochondrial enzyme succinate dehydrogenase was allowed for 1 h at 37 °C (Denizot

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and Lang, 1986). After the incubation period, cells were washed and the formazan was extracted by adding 0.1 ml of isopropanol and measured spectrophotometrically (595 nm). The results were expressed as % of cell viability compared to the vehicle control DMSO (100% viable cells).

2.6. Statistical analysis

All experiments were carried out three times, in triplicate. Statistical differences among treatments were determined by a two-tailed Student *t*-test, with a level of statistical significance of 95% ($p < 0.05$).

3. Results

Singular exposure with either TCDD or PCB-126 or the MO-PCBs did not result in any cytotoxicity in either cell lines as measured with the MTT assay. The highest concentration tested for the PCBs was 10 μM since higher concentrations resulted in a visible suspension, likely resulting from the limited solubility of the chemicals. Co-exposure of the cells to TCDD and PCB-167 resulted in a significant decrease in cell viability for TCDD (≥0.1 μM) and PCB-167 at 10 μM and thus, these concentrations were therefore excluded from the experiments.

3.1. Agonistic effects of mono-ortho PCBs on AhR-EGFP expression

Both TCDD and PCB-126 induced strong AhR-EGFP expression in both mouse H1G1.1c3 and rat H4G1.1c2 cells to a maximum for PCB-126 of 50 ± 7.7% and 83 ± 5.6%, respectively, compared to TCDD (100%) (Figs. 2A and 3A and Table 2). EC_{25} and EC_{50} values for TCDD and PCB-126 were comparable between the two cell lines (Tables 1 and 2) with REP values for PCB-126 of 0.05 (EC_{50}) and 0.07 (EC_{25}) in the mouse cell line and 0.04 (EC_{50}) and 0.07 (EC_{25}) in the rat cell line (Table 2).

The MO-PCBs PCB-105, -118, and -156 produced a significant induction of AhR-EGFP expression in the mouse cell line to a maximum of 11.4 ± 3.5%, 7.5 ± 1.0%, and 26.8 ± 4.7%, respectively. A higher induction was observed in the rat cell line of 29.6 ± 2.6%, 18.2 ± 0.6%, and 62.5 ± 3.5% that of TCDD, respectively (Tables 1 and 2). Since the MO-PCBs did not reach a maximal induction compared to TCDD, calculation of the EC_{50} or EC_{25} was merely theoretical and considered not a realistic comparison with TCDD. Therefore, $EC_{5\ TCDD}$ values were also calculated, as previously proposed by Behnisch et al. (2003). PCB-156 was the most efficacious inducer, resulting in REP values of 0.000004 ($EC_{5\ TCDD}$) in the mouse cells (Table 1), and 0.000025 ($EC_{5\ TCDD}$) in the rat cell line (Table 2). This is respectively 125–20 times lower than the current WHO value of 0.0005 (Van den Berg et al., 1998). PCB-105 resulted in 100 (mouse cell line, Table 1) to 50 (rat cell line, Table 2) times lower REP ($EC_{5\ TCDD}$) values than the current WHO value of 0.0001. PCB-118 also resulted in 50–100 times lower REP ($EC_{5\ TCDD}$) values than the WHO value of 0.0001, in the mouse and rat cell line, respectively (Tables 1 and 2).

3.2. Agonistic affects of mono-ortho PCBs on EROD activity

As observed in the AhR-EGFP assay, both TCDD and PCB-126 induced EROD activity in mouse and rat cell lines to a maximum for PCB-126 of 58 ± 5.6% and 79 ± 4.1%, respectively, compared to TCDD (100%) (Figs. 2A and 3A and Tables 1 and 2). EC_{25} and EC_{50} values for TCDD were 6.3×10^{-12} M and 1.9×10^{-11} M, respectively in the mouse cells, and 8.9×10^{-12} M and 1.8×10^{-11} M, respectively in the rat cells. For PCB-126, these values were 7.9×10^{-11} M and 2.2×10^{-10} (EC_{25} and EC_{50} values, respectively) in the mouse cells, and 1.3×10^{-10} M and 2.1×10^{-10} M (EC_{25} and EC_{50} values, respectively) in

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the rat cells (Table 2). This indicates a relative potency for PCB-126 of approximately 0.08 based on both EC_{50} and EC_{25} values.

Similar to results obtained in the AhR-EGFP assay, PCB-167 failed to induce EROD activity. PCB-156 was also the most efficacious inducer of EROD activity, reaching a maximum 20.5 ± 1.5% of TCDD (100%) at a concentration of 5 μM in the mouse cells, and 68.0 ± 3.2% of TCDD (100 ± 21%) at a concentration of 10 μM (Tables 1 and 2) in the rat cells. As the MO-PCBs did not reach a maximal EROD induction compared to TCDD, calculation of the EC_{50} or EC_{25} was merely theoretical and considered not a realistic comparison with TCDD. Therefore, $EC_{5\ TCDD}$ values were again calculated, as previously proposed by Behnisch et al. (2003). Corresponding REP values for PCB-156 were 0.000019 ($EC_{5\ TCDD}$) in the mouse cell line (Table 1) and 0.000045 ($EC_{5\ TCDD}$) in the rat cell line (Table 2), 26–11 times lower than the WHO value of 0.0005, respectively. REP values for PCB-105 were <0.000001 (mouse cell line, Table 1) to 0.000002 (rat cell line, Table 2) based on the $EC_{5\ TCDD}$ values, which is 100–50 times lower than the WHO values, respectively. PCB-118 resulted in REP values of <0.000001–0.000001 based on $EC_{5\ TCDD}$ values in mouse and rat cells, respectively, both 100 times lower than the WHO value of 0.0001 (Tables 1 and 2).

3.3. Antagonistic effects of mono-ortho PCBs on AhR-EGFP expression

In contrast to their agonist effects on AhR-mediated gene expression, were more efficacious inducers in rat cells, antagonistic effects of MO-PCBs were more pronounced in the mouse cell line. Co-exposure of TCDD (0.001–1 nM) and MO-PCBs (0.1–10 μM) resulted in repression of TCDD-induced AhR-EGFP expression in the mouse cell line, PCB-167 (1–5 μM) and PCB-156 (1–10 μM) had minimal effects on TCDD-induced expression, while PCB-105 (1–10 μM) and PCB-118 (1–10 μM) reduced the TCDD-induced expression significantly in the mouse cell line (Fig. 4A) with 10 μM inhibiting to a maximum of 50.9 ± 2.9% and 58.3 ± 2.2%, respectively (Table 3). In the rat cell line, no significant dose-dependent antagonistic effects on AhR-EGFP expression were observed at the highest PCB concentrations (Fig. 5A and Table 3). The EC_{25} and EC_{50} values were essentially unaffected by co-incubation with TCDD/PCB (Table 3); the main inhibitory effect was observed on the maximal level of inducible AhR-EGFP expression.

3.4. Antagonistic effects of mono-ortho PCBs on EROD activity

As observed after the AhR-EFGP assay, co-incubation of TCDD (0.001–1 nM) with MO-PCB-105, -118 and -156 resulted in concentration-dependent antagonistic effects on TCDD-induced EROD-activity in the mouse cell line (Fig. 4B) with maximal inhibition of 26.7 ± 9.3% for PCB-105 (5 μM), 46.5 ± 10.4% for PCB-118 (5 μM) and 48.3 ± 4% for PCB-156 (10 μM) compared to TCDD (100%) (Table 3). In contrast, no significant antagonistic effects on EROD activity were observed in the rat cell line.

4. Discussion and conclusion

In our current study the MO-PCBs -105, -118, -156 induced both low levels of AhR-EGFP expression as well as low EROD activity in both rat and mouse cell lines at concentrations approximately ≥0.1 μM (Figs. 2 and 3). This indicates and confirms that these MO-PCBs can cause AhR-mediated *in vitro* effects in both rodent species, even after an extensive clean-up procedure to remove more efficacious AhR agonist, though with very low potency and at high concentrations. The lack of AhR-mediated effects of PCB-167 could be caused by the chlorine substitution pattern. Other authors have found the chlorination pattern to be of importance with respect to the AhR mediated responses of PCBs (Bandiera et al., 1982;Safe, 1994;Suh et al., 2003). Furthermore, high affinity ligand binding appears to be

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dependent upon key electronic and thermodynamic characteristics of the ligand (Mhin et al., 2002;Tuppurainen and Ruuskanen, 2000;Waller and McKinney, 1995).

The *in vitro* and *in vivo* experimental data that have been obtained concerning the toxicity of MO-PCBs, has resulted in their inclusion by the World Health Organization (WHO) in the dioxin-TEQ approach (Ahlborg et al., 1994; Van den Berg et al., 1998). The TEF values that are published by the WHO for risk assessment in humans and animals are consensus values derived from numerous REPs derived from different *in vivo* and *in vitro* studies (Schmitz et al., 1995).

The WHO TEF values for MO-PCBs vary from 1×10^{-5} for PCB-167 to 1×10^{-4} (PCB-105 and PCB-118) (Van den Berg et al., 1998). When comparing the REP values obtained from our experiments (Tables 1 and 2) with the TEF values assigned by the WHO (Van den Berg et al., 1998), we observed a 11–125-fold difference (based on $EC_{5\ TCDD}$ values as observed for AhR-EGFP expression of PCB-156 in the rat H4G1.1c2 cell line and for EROD activity after exposure to PCB-156 in the mouse H1G1.1c3 cell line). If these *in vitro* endpoints in both rodent species are assumed to correlate with dioxin-like toxicity of MO-PCBs, the suggested WHO TEFs (Ahlborg et al., 1994; Van den Berg et al., 1998) could be an overestimation of approximately one to two orders of magnitude.

In a recent study, Haws et al. (2006) reported a range of REP values obtained from various *in vitro* and *in vivo* experiments for PCBs. The reported *in vitro* (REP_{2004} database) range for PCB-105 was 7.5×10^{-6} to 0.015 with the WHO TEF value in the 36th percentile. Our observed REP values are within the 10–25th percentile of these values. For PCB-118, the reported *in vitro* range was 6×10^{-6} to 0.0011, with the WHO TEF value in the 74th percentile and our REP values below the 50th percentile. For PCB-156, the reported *in vitro* range was 3×10^{-5} to 0.0014; the WHO TEF value was in the 43rd percentile and our observed REP values are also below the 50th percentile (Haws et al., 2006). Clearly, the large variability between the reported REPs is not only due to the differences between *in vitro* and *in vivo* data and species differences, but also due to the differences in endpoints. The endpoints included in this extensive database can vary from biochemical changes to carcinogenicity studies (Haws et al., 2006).

Antagonistic effects of AhR-active PCBs have been reported previously from both *in vivo* as well as *in vitro* studies (Aarts et al., 1995; Bandiera et al., 1982; Besselink et al., 1998; Dean et al., 2002; Petrulis and Bunce, 1999; Safe, 1994; Sanderson et al., 1996). In our *in vitro* mixture experiments antagonistic effects of MO-PCBs (1–10 μM) were observed in the mouse H1G1.1c3 cells (Fig. 4). Surprisingly, in the rat H4G1.1c2 cells, these MO-PCBs had only agonistic potencies (Table 1 and Fig. 3), no antagonistic effects were observed after exposure in combination with TCDD (Table 3).

Aarts et al. (1995) previously reported a similar finding with mouse cells being more sensitive than rat and human-derived hepatocyte cell lines in respect to AhR-mediated antagonistic effects of di-*ortho* PCBs.

With respect to human relevance of the observed AhR-mediated effects of MO-PCBs, it should be noted that medium concentrations in our *in vitro* experiments causing minimal effect were found around 0.1–1 μM. In human blood, concentrations of MO-PCBs are observed in the nM-range (Kimbrough, 1987; Rogan et al., 1986; Schecter and Piskac, 2001), Assuming *in vitro* medium concentrations can be used as surrogate for blood concentrations, a difference of two to three orders of magnitude is still present. This indicates AhR mediated effects of MO-PCBs might not easily be achieved in humans.

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With respect to the observed antagonism between TCDD and some of these MO-PCBs it should be noted that these were found at ratios 1×10^4 and 1×10^5 that are also found in human samples like blood and milk (Kimbrough, 1987; Rogan et al., 1986; Sjodin et al., 2004). Thus, the question remains to which extent the observed antagonism could have relevance for the human situation. Here we have to keep in mind that our *in vitro* experiments were performed at lower concentrations MO-PCBs, and in addition, we observed strong differences between mouse and rat cells.

There are several criteria for a compound that should be met for inclusion in the TEF concept: (1) the compound must bind the Ah receptor; (2) it should elicit dioxin-specific biochemical and toxic responses; (3) be persistent and accumulate within the food chain (Ahlborg et al., 1994; Van den Berg et al., 1998). Toxic equivalencies (TEQs) can be derived from complicated mixtures of dioxin-like compounds by multiplying the congener specific concentration with their individual TEF values. When used in this way for risk assessment, additivity is a prerequisite and this is supported by numerous *in vivo* and *in vitro* studies. Although, as in our study, antagonism or synergism have been reported for mixtures of dioxin-like compounds and PCBs, the magnitude of these non-additive effects is considered not to comprise this TEF concept significantly when e.g. compared to differences between species. Results from our present study show that MO-PCBs PCB-105, -118 and -156 should indeed be considered as weak AhR agonists and inclusion in the TEF concept is valid, but their present WHO TEF values might be too high. Based on our experiments, PCB-167 should not be considered as AhR agonist. Nevertheless we question if MO-PCBs used in earlier studies have been sufficiently checked for impurities with potent AhR agonists like 2,3,7,8-substituted PCDDs, PCDFs, or planar PCBs such as PCB-126. It should be noted that earlier studies with PCBs or polybrominated diphenyl ethers have also indicated that these type of impurities could have significant influence on the outcome of a study when using weak AhR agonists (Giesy and Kannan, 1998; Sanders et al., 2005).

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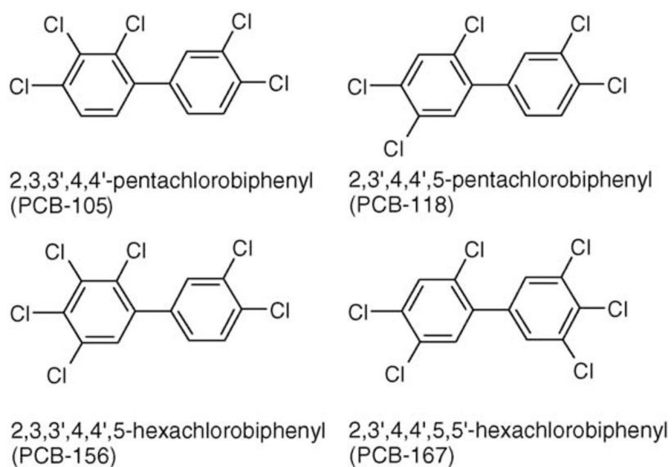

Fig. 1.
Structure of the compounds used in this study.

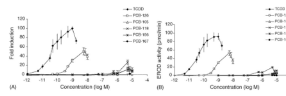

Fig. 2.

Induction of AhR-dependent gene expression by TCDD, PCB-126 and MO-PCBs in recombinant mouse hepatoma cells. H1G1.1c3 cells were incubated with DMSO, TCDD (0.001–1 nM), PCB-126 (0.001–10 nM) or mono-*ortho* PCBs (0–10 μM) for 72 h and AhR-EGFP expression (A) or EROD activity (B) measured as described in Section 2. The data are expressed as mean ± S.E.M. ($n = 3$).

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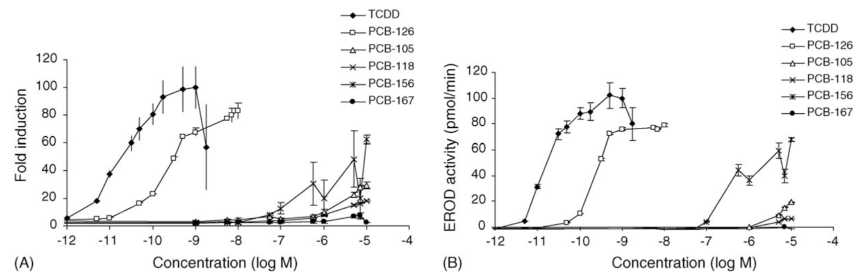

Fig. 3.

Induction of AhR-dependent gene expression by TCDD, PCB-126 and MO-PCBs in recombinant rat hepatoma cells. H4G1.1c2 cells were incubated with DMSO, TCDD (0.001–1 nM), PCB-126 (0.001–10 nM) or mono-*ortho* PCBs (0–10 μM) for 72 h and AhR-EGFP expression (A) or EROD activity (B) measured as described in Section 2. The data are expressed as mean ± S.E.M. ($n = 3$).

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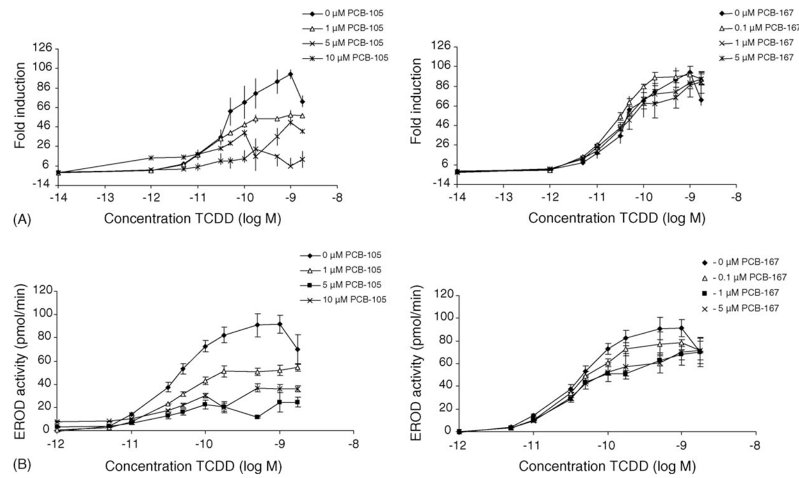

Fig. 4.

Effects of MO-PCB -105 and -167 on the induction of AhR-dependent gene expression by TCDD in recombinant mouse hepatoma cells. H1G1.1c3 cells were incubated with DMSO, TCDD (0.001–1 nM), TCDD and PCB-105 (0–10 μM) or TCDD and PCB-167 (0–10 μM) for 72 h and AhR-EGFP expression (A) or EROD activity (B) measured as described in Section 2. The data are expressed as mean ± S.E.M. ($n = 3$).

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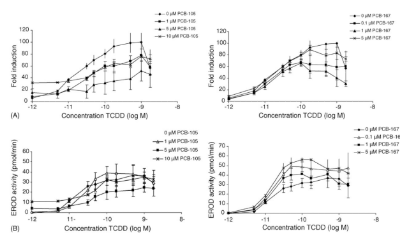

Fig. 5.

Effects of MO-PCB -105 and -167 on the induction of AhR-dependent gene expression by TCDD in recombinant rat hepatoma cells. H4G1.1c2 cells were incubated with DMSO, TCDD (0.001–1 nM), TCDD and PCB-105 (0–10 μM) or TCDD and PCB-167 (0–10 μM) for 72 h and AhR-EGFP expression (A) or EROD activity (B) measured as described in Section 2. The data are expressed as mean ± S.E.M. ($n = 3$).

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Table 1

Effects of mono-*ortho* PCBs, TCDD, and PCB-126 on AhR-EGFP expression and EROD activity in stably transfected mouse hepatoma (H1G1.1c3) cells

Compound	**$EC_{5\,TCDD}$ (nM)**	**EC_{25} (nM)**	**EC_{50} (nM)**	**Concentration**	**AhR-EGFP**	**REP value ($EC_{5\,TCDD}$)**	**REP value (EC_{25})**	**REP value (EC_{50})**
Effects of mono-*ortho* PCBs on AhR-EGFP expression in mouse H1G1.1c3 cells								
TCDD	2.51×10^{-12}	1.58×10^{-11}	3.98×10^{-11}	1 nM	$100 \pm 4.7^{*}$	1	1	1
PCB-126	2×10^{-10}	3.98×10^{-10}	7.50×10^{-10}	5 μM	$50 \pm 7.7^{*}$	0.01	0.08	0.05
PCB-105	1.78×10^{-6}			10 μM	$11.4 \pm 3.5^{*}$	0.000001		
PCB-118	1.58×10^{-6}			10 μM	$7.5 \pm 1.0^{*}$	0.000002		
PCB-156	5.60×10^{-7}	1.3×10^{-6}		5 μM	26.8 ± 4.7	0.000004	0.000023	
PCB-167					nd			
Compound	$EC_{5\,TCDD}$ (nM)	EC_{25} (nM)	EC_{50} (nM)	Concentration	EROD	REP value ($EC_{5\,TCDD}$)	REP value (EC_{25})	REP value (EC_{50})
Effects of mono-*ortho* PCBs on EROD activity in mouse H1G1.1c3 cells								
TCDD	5.60×10^{-12}	6.3×10^{-12}	1.9×10^{-11}	1 nM	$100 \pm 8.4^{*}$	1	1	1
PCB-126	3.20×10^{-10}	7.9×10^{-11}	2.2×10^{-10}	5 μM	$58.2 \pm 5.8^{*}$	0.02	0.04	0.1
PCB-105	$<1 \times 10^{-5}$			10 μM	$5.6 \pm 0.63^{*}$	<0.000001		
PCB-118	$<1 \times 10^{-5}$			10 μM	$5.2 \pm 0.67^{*}$	<0.000001		
PCB-156	2.99×10^{-7}			5 μM	$20.5 \pm 1.5^{*}$	0.000019		
PCB-167					nd			

The data are presented as one representative experiment (±S.E.M.), conducted in triplicate.

* Statistically significant compared to the vehicle control (DMSO 0.1%) ($p < 0.05$).

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Table 2

Effects of mono-*ortho* PCBs, TCDD, and PCB-126 on AhR-EGFP expression and EROD activity in stably transfected rat hepatoma (H4G1.1c2) cells

Compound	**$EC_{5\,TCDD}$ (nM)**	**EC_{25} (nM)**	**EC_{50} (nM)**	**Concentration**	**AhR-EGFP**	**REP value ($EC_{5\,TCDD}$)**	**REP value (EC_{25})**	**REP value (EC_{50})**
Effects of mono-*ortho* PCBs on AhR-EGFP expression in rat H4G1.c1 cells								
TCDD	1.78×10^{-12}	1.50×10^{-11}	4.0×10^{-11}	1 nM	$100 \pm 14.7^{*}$	1	1	1
PCB-126	2×10^{-10}	4.5×10^{-10}	8.9×10^{-10}	5 µM	$83 \pm 5.6^{*}$	0.01	0.07	0.04
PCB-105	1×10^{-6}	5.6×10^{-7}		10 µM	$29.6 \pm 2.6^{*}$	0.000002	0.000004	
PCB-118	1.58×10^{-6}			10 µM	$18.2 \pm 0.6^{*}$	0.000001		
PCB-156	7.08×10^{-8}	1×10^{-7}	2.8×10^{-7}	5 µM	$62.5 \pm 3.4^{*}$	0.000025	0.000032	0.000140
PCB-167					nd			
Compound	$EC_{5\,TCDD}$ (nM)	EC_{25} (nM)	EC_{50} (nM)	Concentration	EROD	REP value ($EC_{5\,TCDD}$)	REP value (EC_{25})	REP value (EC_{50})
Effects of mono-*ortho* PCBs on EROD activity in rat H4G1.c1 cells								
TCDD	5×10^{-12}	8.9×10^{-12}	1.78×10^{-11}	1 nM	$100 \pm 21^{*}$	1	1	1
PCB-126	5.6×10^{-11}	1.26×10^{-10}	2.11×10^{-10}	5 µM	$79 \pm 4.1^{*}$	0.1	0.03	0.08
PCB-105	2.50×10^{-6}			10 µM	$19 \pm 0.8^{*}$	0.000002		
PCB-118	5.60×10^{-6}			10 µM	$7 \pm 0.3^{*}$	0.000001		
PCB-156	1.10×10^{-7}	2.8×10^{-7}	3.2×10^{-7}	5 µM	$68 \pm 3.2^{*}$	0.000045	0.000150	0.000056
PCB-167					nd			

The data are presented as one representative experiment (±S.E.M.), conducted in triplicate.

* Statistically significant compared to the vehicle control (DMSO 0.1%) ($p < 0.05$).

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Table 3

Effects of mono-*ortho* PCBs on TCDD-induced AhR-EGFP expression and TCDD-induced EROD activity in stably transfected mouse hepatoma (H1G1.1c3) and rat hepatoma (H4G1.1c2) cells

Compound	Concentration (μM)	EC_{25} (nM)	EC_{50} (nM)	AhR-EGFP	Compound	Concentration (μM)	EC_{25} (nM)	EC_{50} (nM)	EROD
Effects of mono-*ortho*-PCBs in TCDD-induced AhR-expression and EROD activity in mouse H1G1.1c3 cells									
PCB-105	1	8.51×10^{-12}	2.24×10^{-11}	$58 \pm 4.5^{*}$	PCB-105	1	1.6×10^{-11}	4.0×10^{-11}	$57 \pm 4.8^{*}$
	5	1.12×10^{-11}	5.6×10^{-11}	$56 \pm 8.4^{*}$		5	1×10^{-11}	3.2×10^{-11}	$26.7 \pm 9.3^{*}$
	10	6.31×10^{-13}	3.55×10^{-11}	$50.9 \pm 2.9^{*}$		10	6.7×10^{-12}	3.5×10^{-11}	$39.3 \pm 2.8^{*}$
PCB-118	1	3.5×10^{-11}	4.5×10^{-11}	$73.7 \pm 2.2^{*}$	PCB-118	1	1.9×10^{-11}	4.2×10^{-11}	82.8 ± 3.9
	5	8.9×10^{-12}	3.55×10^{-11}	$42.3 \pm 11^{*}$		5	1.3×10^{-11}	3.8×10^{-11}	$46.5 \pm 10.4^{*}$
	10	5×10^{-12}	5×10^{-11}	$58.3 \pm 2.2^{*}$		10	1.1×10^{-11}	6.3×10^{-11}	$51.1 \pm 1.3^{*}$
PCB-156	1	1.3×10^{-11}	3.3×10^{-11}	90.9 ± 14.9	PCB-156	1	1×10^{-12}	1.3×10^{-11}	81.2 ± 9.5
	5	3.2×10^{-11}	8.9×10^{-11}	99.1 ± 3.4		5	2.0×10^{-11}	4.2×10^{-11}	$67 \pm 5.0^{*}$
	10	1.8×10^{-11}	4.5×10^{-11}	$70.8 \pm 1.3^{*}$		10	3.2×10^{-11}	7.1×10^{-11}	$48.3 \pm 4.0^{*}$
PCB-167	0.1	8.9×10^{-12}	2.5×10^{-11}	97.7 ± 8.4	PCB-167	0.1	1.6×10^{-11}	3.8×10^{-11}	77 ± 12.7
	1	1.12×10^{-11}	3.55×10^{-11}	87 ± 12.2		1	1.6×10^{-11}	4.0×10^{-11}	$74.8 \pm 4.8^{*}$
	5	1×10^{-11}	3.55×10^{-11}	89.7 ± 6.4		5	1.6×10^{-11}	4.0×10^{-11}	86.2 ± 3.4
Effects of mono-*ortho*-PCBs in TCDD-induced AhR-expression and EROD activity in rat H4G1.1c2 cells									
PCB-105	1	1×10^{-11}	3.2×10^{-11}	87 ± 10.4	PCB-105	1	1.3×10^{-11}	2.7×10^{-11}	97.0 ± 14
	5	4.0×10^{-13}	4.5×10^{-11}	51.8 ± 16.9		5	1.2×10^{-11}	3.9×10^{-11}	68.0 ± 12.2
	10	1.3×10^{-13}	2.1×10^{-11}	76.0 ± 7.2		10	4.5×10^{-13}	3.2×10^{-11}	96.0 ± 5.3
PCB-118	1	7.1×10^{-12}	1.4×10^{-11}	$37.7 \pm 7.6^{*}$	PCB-118	1	1.1×10^{-11}	1.8×10^{-11}	94.0 ± 18
	5	2.8×10^{-12}	1.8×10^{-11}	42.0 ± 21		5	1.1×10^{-11}	2.5×10^{-11}	72.0 ± 17.9
	10	6.7×10^{-12}	4.2×10^{-11}	66.0 ± 5.4		10	1.2×10^{-11}	3.5×10^{-11}	65.0 ± 12.6
PCB-156	1	1.4×10^{-10}	1.6×10^{-10}	54.2 ± 7.8	PCB-156	1	1×10^{-10}	1.5×10^{-10}	105.0 ± 17.3
	5	2.2×10^{-11}	1.3×10^{-10}	75.7 ± 4.2		5	2.2×10^{-12}	1×10^{-10}	129.0 ± 20.1
	10	1×10^{-10}	2.0×10^{-10}	$64.8 \pm 1.3^{*}$		10	1×10^{-11}	5×10^{-11}	85.0 ± 3.7
PCB-167	0.1	5.6×10^{-12}	1.1×10^{-11}	59.2 ± 22.8	PCB-167	0.1	7.9×10^{-12}	1.5×10^{-11}	123.0 ± 34
	1	5.6×10^{-12}	1.4×10^{-11}	$40.2 \pm 14.3^{*}$		1	7.9×10^{-12}	1.4×10^{-11}	86.0 ± 17.1

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Compound	Concentration (μM)	EC_{25} (nM)	EC_{50} (nM)	AhR-EGFP	Compound	Concentration (μM)	EC_{25} (nM)	EC_{50} (nM)	EROD
	5	5×10^{-12}	1.5×10^{-11}	83.1 ± 10.6		5	7.6×10^{-11}	1.4×10^{-11}	131.0 ± 2.7

The data are presented as one representative experiment (±S.E.M.), conducted in triplicate.

*Statistically significant compared to the positive control (TCDD 1 nM) ($p < 0.05$).