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One TEF concept does not fit all: The case for human risk assessment of polychlorinated biphenyls

Majorie B. M. van Duursen, Karin I. van Ede and Martin van den Berg

Abstract

Human risk assessment of dioxins and dioxin-like compounds relies heavily on toxic equivalency factors (TEFs) that are mainly based on *in vivo* rodent studies. However, especially for the PCBs there are many uncertainties with respect to the actual dioxin-like activities and subsequent health effects in humans. For example, the relative effect potencies (REPs) for PCB126 are consistently up to two orders of magnitude lower in human cell models than in rodents and rodent cell cultures. For other dioxin-like (DL) PCBs, REPs can often not be obtained in human models due to a lack of AHR-mediated responses. In addition, DL-PCB-related effects such as thyroid disruption are largely attributed to mechanisms that are not (directly) AHR-mediated. Consequently, the AHR-mediated risk in humans for DL-PCBs is likely overestimated in the current TEF concept. The increasing availability of *in vitro* models using human cells will provide great opportunities to determine human-specific REP/TEFs based on toxicologically relevant endpoints. A better understanding of human-specific responses should lead to more reliable potency estimates of human effects and ultimately improved human risk assessment for DL-PCBs.

Addresses

Division of Toxicology and Pharmacology, Institute for Risk Assessment Sciences, Utrecht University, Yalelaan 104, P.O. Box 80177, NL-3508 TD, Utrecht, The Netherlands

Corresponding author: van Duursen, Majorie BM (m.vanduursen@uu.nl)

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Dioxins, PCBs, Human risk assessment, Toxic equivalency factor.

1. PCBs and the WHO-TEF concept

Human risk assessment of exposure to dioxin-like compounds (DLCs) remains a continuous challenge for toxicologists and regulators. This is especially true for polychlorinated biphenyls (PCBs) that have been used until mid-70s as coolants, coatings and lubricants. While production and use of PCBs has been prohibited for over forty years, they are still abundantly present in the environment and in tissues of birds, fish and mammals, including humans, due to their highly persistent and bioaccumulative properties. In addition, disposal of PCB-containing equipment and materials still contributes to PCB release in the environment leading to accumulation in the food chain. Consequently, human exposure to PCBs remains an issue of concern [1,2]. There are 209 possible PCBs, of which 12 are traditionally considered to have dioxin-like properties via AHR-mediated processes and have been assigned a toxic equivalency factor (TEF). TEFs reflect the potency of a specific congener to produce an AHR-mediated biological effect relative to the most potent AHR agonist known, *i.e.* 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Total toxic equivalents (TEQs) can then be calculated by multiplying the concentration of a congener with its TEF to perform cumulative risk assessment of the mixture.

During the 1990's, TEF values were harmonized in several expert meetings organized by the World Health Organization (WHO). The first WHO-endorsed TEF values were published in 1998 [3]. In subsequent WHO expert meetings, these TEFs were reevaluated and some TEFs were adjusted based on scientific data that was gathered in a relative effect potency (REP) database [4–6]. Most REPs are established in experimental studies using rats and mice or rodent-based cell models. These rodent-derived TEFs are applied world-wide for human risk assessment purposes. This in spite of the fact that upon AHR activation, a wide variety toxic and biological effects can occur that display clear differences in rodent and human responses [7]. Some of the species-differences in AHR-mediated responses can be attributed to genetic variations. Generally, the human AHR is considered to be relatively low responsive to DLCs [8]. In the 2005 TEF reevaluation, concern was expressed by the WHO about interspecies variability in REPs, specifically for the toxicologically relevant PCB126. Yet, the TEF value of PCB126 was retained, because too limited (human) relevant information was available at that time to adjust the 1998 WHO TEF [5]. Similar uncertainties were expressed for *non-ortho* PCB77, PCB81 and PCB169. In addition, the WHO expressed serious concern for the wide variation in REPs of the environmentally abundant *mono-ortho* PCBs. It was deemed highly likely that these variations were due

to contamination of the compounds with more potent DLCs. Because of the lack of sufficient experimental data, the median of REP distribution was used to set the TEF of all *mono-ortho* PCBs uniformly at 0.00003. Clearly, the lack of sufficient data to set a scientifically balanced TEF value hampers accurate human risk assessment of DL-PCBs.

2. Human health effects

The health effects of PCBs have been studied in humans exposed through diet, work, and industrial accidents. In humans, PCB exposure is associated with cancer [9], skin lesions (including typical AHR-mediated chloracne), disrupted thyroid hormone action, altered menstrual cycles, as well as damage to the nervous, immune, and cardiovascular systems [10]. PCB exposure in the womb or during lactation is associated with developmental toxicity, including decreased IQ, impaired psychomotor development and immune function [11]. Obviously, human studies are complicated by the mixture nature of PCB exposure to both DL- and NDL-PCBs and their possible interactions with other (dioxin-like) chemicals [12]. Consequently, although PCBs may contribute to adverse health effects in human study populations, it cannot be determined with certainty which congeners may have caused the effects, whether these are DL-PCBs and what their relative contributions have been.

2.1. Thyroid hormone

PCB exposure is often linked to disruption of thyroid hormone actions in animal and human studies. The thyroid gland produces triiodothyronine (T3), but predominantly its prohormone thyroxine (T4). T4 production is stimulated by thyroid stimulating hormone (TSH) from the pituitary and transported in the blood by transporters such as thyroxine-binding globulin (TBG) and transthyretin (TTR). Thyroid hormones play important roles in regulating human growth and development, especially development of the brain and disruptions of normal thyroid function have been shown to impair physical growth and mental development. Generally, PCBs are considered disruptors of thyroid hormone actions via three putative mechanisms: (1) PCBs directly alter the structure of the thyroid gland, (2) PCBs can displace T4 from thyroid hormone binding proteins, leading to release of free T4 and subsequent enhanced metabolism and excretion, and (3) PCBs alter T4 metabolism via AHR-mediated increase of CYP1A1 or uridine diphosphate glucuronosyl-transferases (UDP-GTs) activity, which leads to enhanced clearance of circulating T4 [13].

Studies on TEQs and thyroid function in humans are quite inconsistent [14,15]. One explanation might be the huge diversity in TEQ composition with respect to the relative contribution of PCDD/F and DL-PCBs in different study populations. In babies born from mothers that were exposed to predominantly to TCDD in the Seveso accident in Italy 1976, higher TSH blood levels were found [16]. In contrast, studies on predominantly PCB-exposed populations show mostly no effect on TSH/T3 but a decrease in free T4 levels and decrease thyroid volume that is correlated with DL-PCB exposure [17,18]. While several studies describe associations between thyroid hormone disruption and DL-PCB exposure, only a few have attempted to calculate congener-specific REPs from these human data. In a Slovak PCB-exposed population, Trnovec et al. calculated REPs for PCBs based on two thyroid outcomes, *i.c.* thyroid volume and serum free thyroxine (fT4) concentration. In this study, only REPs for PCB105 (0.0000019 for fT4) and PCB81 (0.01 for both thyroid volume and fT4) could be calculated, which are respectively lower and higher than their WHO-TEFs [19]. In addition, some REPs could be calculated based on CYP1A1 and 1B1 gene expression in blood peripheral lymphocytes in the same population [20]. For PCB81, the CYP1A1-based REP was 0.00093 and CYP1B1-based REP was 0.2057. CYP1B1-based REPs for PCB126 and 169 were 0.0171 and 0.0371, respectively. REPs for the *mono-ortho* DL-PCBs varied between 0.000034 and 0.00067. The difference in CYP1A1 and 1B1-derived REPs might be explained by differential regulation of expression. Although both CYP1A1 and CYP1B1 induction are considered AHR-mediated effects, CYP1B1 regulation is considered to be more multifactorial. Besides AHR, key transcription factors for CYP1B1 include steroid Sp1 transcription factor, cyclic AMP (cAMP)—response element—binding protein (CREB), estrogen receptor (ER) as well as epigenetic factors and post-transcriptional modifications [21].

3. Human PCB *in vitro* REPs

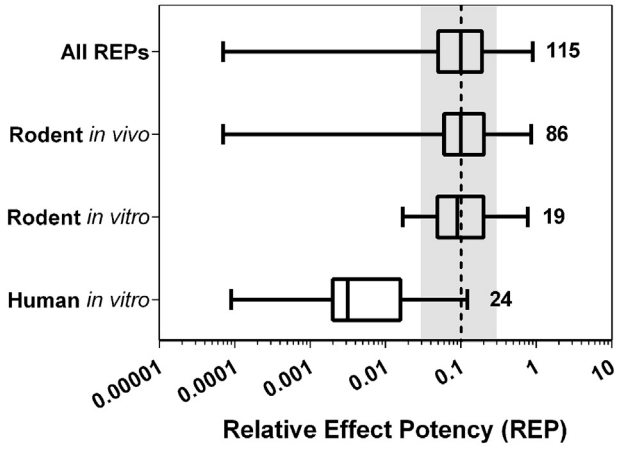
Obtaining human *in vivo* REPs is clearly challenging, but useful human *in vitro* data is also strikingly scarce for PCBs in the 2006 REP database [4]. Human *in vitro* REPs of PCBs that were retained in the latest TEF evaluation mostly concerned PCB77 (6 retained REPs) and PCB126 (8 retained REPs). Besides REPs to induce CYP1A1-mediated EROD activity (2 for PCB77 and 4 for PCB126), the endpoints on which the TEFs of these congeners were based also included inhibition of aromatase (CYP19) activity for PCB126 [22], and 2-methoxyestrogen formation in human mammary [23] and hepatoma cells [24] (Table 1). For PCB77, human REPs included anti-estrogenic effects in human breast cancer cells and prostate-specific antigen (PSA) secretion and proliferation of human prostate cancer cells. Although an AHR-independent mechanism was suggested for the latter effect [25]. Since the compilation of the 2004 REP database and the 2005 WHO expert meeting, several studies have been published that derived REPs for PCBs in human cell types. Here,

Table 1 Relative effect potencies for PCB126 derived from human cell-based studies.

Human cell model	Tissue	Endpoint	REP	Reference
JEG-3	Placenta	aromatase (CYP19) activity	0.00069	[22],[a]
JEG-3	Placenta	EROD activity	0.015	[22],[a]
MCF-10A	Breast	2-MeOE1/2 formation	0.00269	[50],[a]
MCF-10A	Breast	EROD activity	0.00357	[50],[a]
MCF-7	Breast	2-MeOE1/2 formation	0.00657	[50],[a]
MCF-7	Breast	EROD activity	0.01739	[50],[a]
MCF-7	Breast	CYP1A1 mRNA	0.1221[c]	[27]
Primary hepatocytes	Liver	EROD activity	0.003	[43]
Primary hepatocytes	Liver	CYP1A1 mRNA	0.00009	[43]
Primary hepatocytes	Liver	EROD activity	0.0042	[29]
Primary hepatocytes	Liver	Genomics, geometric mean	0.002	[51]
HepG2	Liver	EROD activity	0.001	[28]
HepG2	Liver	EROD activity	0.03	[52]
HepG2	Liver	EROD activity	0.02	[24],[a]
HepG2	Liver	2-MeOE2 formation	0.011	[24],[a]
HepG2	Liver	EROD activity	0.002	[43]
HepG2	Liver	CYP1A1 mRNA	0.002	[43]
HepG2-luciferase	Liver	EROD activity	0.016	[52]
HepG2-luciferase	Liver	AHR activation	0.0033	[29]
PBMCs	Blood	EROD activity	0.056	[53]
PBMCs	Blood	AhRR mRNA	0.002	[54]
PBMCs	Blood	EROD activity	0.003	[54]
PBMCs	Blood	CYP1B1 mRNA	0.0171[b]	[20]
Primary keratinocytes	Skin	CYP1A1 mRNA	0.0024	[29]
Primary keratinocytes	Skin	CYP1A1 mRNA	0.003	[41]
Median human REP			**0.0033**	

2-MeOE1/2, 2-methoxyestrone/-estradiol; CYP1A1, cytochrome P450 1A1; EROD, ethoxyresorufin-O-deethylase; PBMCs, peripheral blood mononuclear cells.
[a] Data retained in the 2004 REP database [4].
[b] REPs based on *in vivo* exposed humans.
[c] REP is uncertain as it was based on curves fitted to 100% TCDD response without description of absolute maximal PCB126 induction.

Figure 1



Boxplot comparison of *in vivo* and *in vitro*-derived effect potencies for PCB126, relative to TCDD. All REPs, rodent *in vivo* and rodent *in vitro* REPs are from the 2004 REP database [4], human REPs are from the 2004 REP database supplemented with more recently published human REPs (listed in Table 1). The numbers indicate the number of REPs that are represented by the boxplots. The black dashed line and gray area represent the current WHO-TEF value of 0.1 for PCB 126 ± half log uncertainty. Modified after [26].

especially the REP of PCB126 has been the focus of much debate, since this REP is consistently up to two orders of magnitude lower in human cell types compared with those of rodents (Figure 1) [26–28]. The median REP from human cell-based assays for PCB126 is 0.0033 (Table 1). Only a few newer studies described REPs for other DL-PCBs in human cell types. This is not surprising considering the general lack of AHR-mediated responses like CYP1A1 induction by the *mono-ortho* substituted congeners. For example, Larsson and coworkers could not determine human REPs for PCB77, 169, 105, 118, 156, 167, 189 due to lack of response in an extensive *in vitro* screening evaluation using human primary hepatocytes (EROD activity) and keratinocytes (CYP1A1 and AhRR mRNA levels) and a human AHR-reporter gene assay [29]. Wall et al. showed some induction of CYP1A1 gene expression in human mammary tumor MCF-7 cells and calculated REPs for PCB126 (0.1221), PCB105 (<0.00005), PCB118 (<0.00005) and PCB156 (0.0001) [27]. Finally, in a sensitive human luciferase reporter gene assay, human AHR was poorly activated by PCB77 (REP ~ 0.00001) and PCB126 (REP could not be determined) and was unaffected by the *mono-ortho* substituted PCB157 [30].

Several human *in vitro* studies have investigated the effects of DL-PCBs on non-CYP1A1-mediated endpoints, often in combination with NDL-PCBs. Transcriptomic analysis of human peripheral blood mononuclear cells *in vitro* exposed to PCB118 in a mixture with NDL-PCBs 138, 153, 170 and 180 showed correlations with markers that are related to metabolic disorders (including obesity and diabetes) cardiovascular, endocrine disruption, and cancers [31]. An *in vitro* test battery based on human liver and colon cell lines showed that a mixture containing DL-PCBs 77, 81, 105, 114, 118, 126 and 169 modulated gene expression of nuclear receptors AHR, ER-beta, PPAR-gamma, AR, RAR-alpha and THR-alpha [32]. One study described relatively strong anti-androgenic activities for PCB118 (IC50 0.47 ± 0.01 μM) and PCB126 (IC50 0.53 ± 0.14 μM) using an AR-reporter gene assay [33]. This is not surprising, considering that the AHR is

known to interfere with steroid hormone regulation through a number of different mechanisms, including direct cross-talk between AHR and the ER [34] and AR [35]. Together, these results provide biological plausibility for a number of effects that have been observed for DL-PCBs in epidemiological studies.

4. Setting the right TEF

TEFs are applied world-wide to assess risk for humans and wildlife health, related to the presence of dioxins and DLCs in the food chain and environment. Obviously, setting the right TEF is crucial for correct human and environmental risk assessment, but also has significant implications from an economical point of view, *e.g.* for setting safe levels for food and feed and guidance for clean-up of contaminated sites. At present, *non-ortho* PCBs, dominated by PCB126, are the main contributors to the total TEQs in food [36,37]. Globally, DL-PCBs make up about 50% of total TEQs in human breast milk [11]. This percentage is similar to the PCB contribution to blood TEQs in the US population [38]. It should be noted, however, that TEQ blood profiles and relative PCB contributions are very population and exposure dependent. Also, a global decrease in absolute PCB levels is observed in human blood. Yet, the relative contribution of PCBs to persistent organic pollutant blood levels is high and even seems to increase [39], stipulating a continuing need for reliable risk assessment using human-relevant TEFs. There is increasing evidence that the WHO-TEF for PCB126 might be overly conservative for human risk assessment and causes an overestimation of health risks. A similar situation may be present for the other PCBs in the TEF concept.

Ideally, human-derived TEFs should be applied to evaluate human risk. For the present TEF concept, however, it was decided that rodent *in vivo* studies should be the primary source to determine TEFs for human risk assessment, because they include toxicokinetic and toxicodynamic aspects [5,6]. With an increasing amount of data from human-relevant *in vitro* models, we now have to realize that this approach may not be best suited for reliable human risk assessment. Multiple studies consistently show that human cells are up to 10−1000 times less sensitive for TCDD-induced effects than those of rodents and monkeys [40−43]. Also, clear compound-selective differences in both the relative potency and efficacy can be observed in AHR-dependent gene expression between human, rat, mouse and guinea pig cells [29,30,41,44]. Although the AHR ligand binding domain is fairly well conserved, the numerous amino acid differences in the ligand binding domains between different species have been suggested to explain some of the species − as well as congener-specific differences observed [8,45]. Another significant uncertainty in DLC risk assessment is presented by the fact that TEFs are globally used to calculate human risks based on blood, serum or plasma levels. Several studies have now shown that for some DLCs, the systemic, blood-based REPs are different from REPs that are calculated based on oral doses [46]. In one of our recent papers, it was argued that *in vitro*-derived REPs may better reflect the actual potency of a DLC determined at the target tissue based on systemic concentrations [26]. Taken together, this means that *in vitro* REPs from studies especially using human cells can very well be used to determine human relevant, congener-specific AHR-mediated effects. In addition, human cell-based studies can provide insight into the mechanisms underlying DLC-mediated effects.

This leaves the question if PCB-mediated effects should only be attributed to AHR-mediated actions, a prerequisite for inclusion in the present TEF concept. Consistently, studies that describe AHR activation in human cell-based reporter assays or hallmark AHR-mediated effects (*i.e.* upregulation of CYP1A1 expression and activity) show that DL-PCBs are partial AHR agonists at best. Several human health effects such as thyroid hormone disruption have been associated with DL-PCB exposure. However, mechanistic studies imply that this can at least partially be attributed to actions of PCBs and/or its metabolites that are not or indirectly linked to AHR. For example, TTR binding and T4 displacement by PCB metabolites is obviously not an AHR-mediated action, but indirectly the AHR is involved via cytochrome P450-mediated metabolite formation. Also, studies from Zoellers group showed that PCBs 105, 118, 126 can act as agonists on the thyroid receptor, only if they are metabolized by CYP1A1 [47]. This indirect correlation between TH-regulated genes and CYP1A1 mRNA was also demonstrated in human placenta samples [48]. These studies on thyroid disruption and non-CYP1A1 endpoints clearly show that REPs/TEFs for PCBs are not necessarily directly or solely linked to AHR actions. Similar results have been found for studies on neurotoxicity of PCBs [49]. This issue is also relevant for other persistent halogenated aromatics, *e.g.* hexachlorobenzene and brominated naphthalenes, which are considered candidates for inclusion in a TEF concept. While the TEF concept is applicable to assess the dioxin-like potential of a congener or mixture, obviously, non- or indirect AHR-mediated effects should also be considered when performing a full hazard or toxicological risk assessment of a specific chemical.

In conclusion, it is evident that crucial gaps and uncertainties still exist in the present TEF concept for DLCs, especially PCBs, despite the tremendous amount of experimental data and related scientific expert meetings since the discovery of the AHR. We still face significant challenges on how to deal with multiple mechanisms of action, from which some are not (directly) AHR-related, of the 12 DL-PCBs to perform reliable human risk assessment. Based on recent human data, a human-TEF for PCB126 of 0.003 seems more

appropriate than the current rodent-based WHO-TEF of 0.1, at least for AHR-mediated effects. The increasing availability of *in vitro* models using human cells will provide great opportunities to determine human-specific REP/TEFs based on toxicologically relevant endpoints. A better understanding of human-specific responses should lead to more reliable potency estimates of human effects and ultimately improved human risk assessment for DL-PCBs.

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- • of special interest
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