REVIEW

Polybrominated Dibenzo-*p*-Dioxins, Dibenzofurans, and Biphenyls: Inclusion in the Toxicity Equivalency Factor Concept for Dioxin-Like Compounds

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In 2011, a joint World Health Organization (WHO) and United Nations Environment Programme (UNEP) expert consultation took place, during which the possible inclusion of brominated analogues of the dioxin-like compounds in the WHO Toxicity Equivalency Factor (TEF) scheme was evaluated. The expert panel concluded that polybrominated dibenzo-*p*-dioxins (PBDDs), dibenzofurans (PBDFs), and some dioxin-like biphenyls (dl-PBBs) may contribute significantly in daily human background exposure to the total dioxin toxic equivalencies (TEQs). These compounds are also commonly found in the aquatic environment. Available data for fish toxicity were evaluated for possible inclusion in the WHO-UNEP TEF scheme (van den Berg et al., 1998). Because of the limited database, it was decided not to derive specific WHO-UNEP TEFs for fish, but for ecotoxicological risk assessment, the use of specific relative effect potencies (REPs) from fish embryo assays is recommended. Based on the limited mammalian REP database for these brominated compounds, it was concluded that sufficient differentiation from the present TEF values of the chlorinated analogues (van den Berg et al., 2006) was not possible. However, the REPs for PBDDs, PBDFs, and non-ortho dl-PBBs in mammals closely follow those of the chlorinated analogues, at least within one order of magnitude.

Therefore, the use of similar interim TEF values for brominated and chlorinated congeners for human risk assessment is recommended, pending more detailed information in the future.

Key Words: dioxin; halogenated hydrocarbon; persistent organic chemicals; polychlorinated biphenyls; regulatory/policy; biomarkers.

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The Toxicity Equivalency Factor (TEF) methodology has been evolving since it was first proposed by the Ontario Ministry of the Environment in 1983. The World Health Organization (WHO) has been the lead regulatory agency involved in the development of this methodology (Ahlborg *et al*., 1994; van den Berg *et al*., 1998, 2006). Initially, this TEF method included only polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs). As exposure, mechanistic, and toxicological data accumulated, the dioxin-like polychlorinated biphenyls (dl-PCBs) were included in the TEF approach for risk assessment. When the TEF methodology was refined further, other individual chemicals or chemical classes were evaluated or proposed for inclusion. The polybrominated dioxins (PBDDs) and dibenzofurans (PBDFs) are classes of chemicals that were recently proposed for inclusion (van den Berg *et al*., 2006). This review represents the outcome of a joint WHO and United Nations Environment Programme (UNEP) expert consultation. It discusses the available information on exposure,

mechanism of action, and toxicity of the brominated analogues of the PCDDs, PCDFs, and PCBs and provides arguments for including these compounds in the existing WHO TEF scheme.

OCCURRENCE AND EXPOSURE

Over the last decade, there has been accumulating evidence that PBDDs and PBDFs can be found in a variety of biological matrices in addition to their well-known presence in abiotic matrices such as fly ash. One of the most noticeable occurrences is their presence as impurities in commercial mixtures of brominated flame retardants (BFRs), such as polybrominated diphenylethers (PBDEs), and subsequent occurrence in household products and house dust. PBDFs are major contaminants of PBDEs with congener profiles dependent on the degree of bromination of the commercial mixture. PBDDs are usually not detected above the analytical limit of detection in these flame retardant mixtures, and based on the production of these commercial mixtures, the potential global emissions of PBDFs in 2001 were estimated to be several thousands of kilograms (Hanari *et al.*, 2006).

In addition, during combustion processes, such as in poorly run municipal waste incinerators, significant amounts of PBDDs and PBDFs can be formed, including those with the most toxic dioxin-like properties (Tu *et al.*, 2011). Open burning of domestic waste and accidental fires can produce similar or higher amounts of PBDDs and PBDFs compared with their chlorinated congeners. The origin of these PBDFs may partly be explained by their presence as contaminants in the commercial PBDE flame retardant mixtures in existing (household) materials, but *de novo* synthesis can not be excluded (Gullett *et al.*, 2010; Lundstedt *et al.*, 2011). Furthermore, thermal degradation, photochemical transformation, sunlight exposure, and recycling of (other) BFRs, such as tetrabromobisphenol A, can also yield significant amounts of PBDFs (Ortuño *et al.*, 2011).

The formation of TrBDDs and TeBDDs in the marine environment via natural processes is also well established, and reactive bromophenols and hydroxylated PBDEs are assumed to be precursors of the naturally occurring PBDDs. Although several TeBDD isomers have been measured in the marine environment, the highly toxic 2,3,7,8-TeBDD has not been detected, and only congeners with bromine atoms on two or three lateral positions have been observed (Haglund, 2010; Haglund *et al.*, 2007). TrBDDs have been detected in mussels, algae, and sponges (Löfstrand *et al.*, 2010; Malmvärn *et al.*, 2005a,b, 2008; Unger *et al.*, 2009). In view of their common occurrence in the marine food chain, more toxicological studies are required to determine whether these lower brominated congeners also meet the criteria for inclusion in the WHO TEFs approach for dioxin-like compounds (van den Berg *et al.*, 1998). The congener-specific patterns of PBDDs and PBDFs from the marine environment and combustion processes are quite distinct with higher chlorinated and more toxic congeners dominating from

the latter source (Tu *et al.*, 2011; Wyrzykowska *et al.*, 2009). Another source of direct human exposure to PBDDs and PBDFs is house and office dust, which may originate from wear and tear processes of common household products, e.g., polyurethane foam, TV sets, computers, and other electronic and electrical equipment, containing flame retardants such as PBDEs. 2,3,7,8-Substituted PBDDs and PBDFs are detected at significant quantities, e.g., in house dust and sewage sludge (Brorstrom-Lunden *et al.*, 2010; Suzuki *et al.*, 2010). If similar TEF values are applied for the 2,3,7,8-substituted PBDDs and PBDFs as for the chlorinated congeners, the brominated congeners can contribute up to 17% of the total amount of TEQs in Japanese house dust (Suzuki *et al.*, 2010). Due to the widespread occurrence of 2,3,7,8-substituted PBDDs and PBDFs, particularly in the aquatic environment, it is not surprising that these compounds were detected in seafood for human consumption. Recent studies from various countries indicate that PBDDs and PBDFs can contribute significantly to the total amount of TEQ in seafood items compared with their chlorinated congeners (Ashizuka *et al.*, 2005; Fernandes *et al.*, 2008, 2009a; Lam *et al.*, 2008). A comparable situation is observed for the presence of 2,3,7,8-substituted PBDFs in dairy products and meat (Fernandes *et al.*, 2009b). In the present WHO TEF/TEQ scheme, certain dl-PCBs contribute significantly to the total TEQs in many biological or food samples (van den Berg *et al.*, 1998, 2006). The TEQ contribution of dioxin-like polybrominated biphenyls (dl-PBBs) is yet not clarified sufficiently; however, limited recent data indicate that non-ortho dl-PBBs (i.e., 81, 77, 126, and 169) are found in human milk and shellfish, albeit at very low concentrations (M. Rose (personal communication)). Future studies must show whether these dl-PBB congeners indeed contribute significantly to the total amount of TEQ in human milk and food. As a result of their presence in the human food chain and abiotic environment, e.g., house dust and combustion particles, it could be predicted that 2,3,7,8-substituted PBDDs and PBDFs are found in human tissues. Although this information is still limited, dioxin-like PBDDs and PBDFs have indeed been found at concentrations, which warrant further studies with respect to their contribution to the total amount of TEQ in the human body (Choi *et al.*, 2003; Ericson Jogsten *et al.*, 2010).

KINETICS AND METABOLISM

Absorption

A limited number of studies have been conducted with PBDDs and PBDFs, providing comparative data with their chlorinated congeners. 2,3,7,8-TeBDD and -TeBDF have been studied in some detail. Based on these data, it can be assumed that gastrointestinal, pulmonary, and dermal uptake does not vary significantly between the chlorinated and brominated congeners (Banks and Birnbaum, 1991; Brewster *et al.*, 1989; Diliberto *et al.*, 1993; Nagao *et al.*, 1996). As observed with chlorinated

congeners, the amount of absorption/bioavailability in the body depends significantly on the number of bromine substituents.

Tissue Distribution

PBDDs and PBDFs are primarily retained in liver and adipose tissue as reported earlier for chlorinated analogues. Distribution between these tissues is dose dependent and similar for 2,3,7,8-TeBDD and 2,3,7,8-TeCDD. Differences observed after dermal absorption are attributed to the higher lipophilicity and/or molecular size of 2,3,7,8-TeBDD (Diliberto *et al*., 1993; Kedderis *et al*., 1991a, b, 1992, 1993, 1994). Comparative studies between 2,3,7,8-TeCDD and TeBDD indicate comparable liver/adipose tissue ratios (Nagao *et al*., 1996; WHO, 1998). The tissue distribution of 1,2,3,7,8-PeCDD and -PeBDD in the rat is also comparable (Golor *et al*., 1993). Toxicokinetic information for PBDFs is even more limited than for the PBDDs. A study with 1,2,7,8-TeBDF indicated an affinity for the liver and adipose tissue in the rat comparable with TeBDD (Kedderis *et al*., 1994). The tissue distribution of 2,3,4,7,8-PeBDF was also studied in the marmoset monkey with liver and adipose tissue being major storage sites (Schulz *et al*., 1993). However, 2,3,4,7,8-PeBDF and -PeCDF have a much higher affinity for the liver than 2,3,7,8-TeCDD or -TeBDD. This indicates similar congener-specific differences in hepatic affinity for the brominated and chlorinated congeners (van den Berg *et al*., 1994).

Metabolism

Like 2,3,7,8-TeCDD, elimination of 2,3,7,8-TeBDD is relatively slow in rodents, and metabolic transformation to mono- and dihydroxy metabolites occurs for both congeners, but oxygen bridge cleavage appears predominant for TeCDD (De Jongh *et al*., 1993; Poiger and Buser, 1984). At present, information regarding metabolic pathways of 2,3,7,8-substituted PBDFs is lacking, and limited information is only available for 1,2,7,8-TeBDF (Kedderis *et al*., 1994). 1,2,7,8-TeBDF was rapidly metabolized in the rat, which is consistent with the presence of two adjacent unsubstituted carbons facilitating rapid metabolism of PCDF (van den Berg *et al*., 1994).

Elimination and Excretion

Tissue-specific and whole-body half-life has only been reported for 2,3,7,8-TeBDD in rodents. Comparable with 2,3,7,8-TeCDD, there are significant differences in elimination from the liver and adipose tissue, with more rapid elimination from the liver. Half-lives of 2,3,7,8-TeBDD in liver, adipose tissue, and whole body of the rat varied between 15–25, 40–55, and 15–20 days, respectively (Kedderis *et al*., 1991b; Nagao *et al*., 1996). Thus, elimination rates are comparable with those reported in rodents for 2,3,7,8-TeCDD (van den Berg *et al*., 1994). Nevertheless, differences between the disposition of 2,3,7,8-TeBDD and -TeCDD in mice following subchronic exposure have also been reported with TeBDD retained less than TeCDD, i.e., 6.9 versus 23.5% of the dose, respectively.

In addition, dose-dependent hepatic sequestration of TeBDD was observed in these mice, with a four- to fivefold induction of CYP1A2 activity (DeVito *et al*., 1998). The elimination rate of 1,2,3,7,8-PeCDD from the liver and adipose tissue in the rat was similar to 2,3,7,8-TeBDD (Golor *et al*., 1993) and is comparable with that observed for 2,3,7,8-TeCDD and 1,2,3,7,8-PeCDD (van den Berg *et al*., 1994).

Blood from an industrial cohort has provided information about the half-lives of 2,3,7,8-TeBDD and 2,3,7,8-TeBDF in humans, which was reported to be 2.9–10 (mean value 5.9) and 1.1–1.9 (mean value 1.5) years, respectively (Zober *et al*., 1992). Thus, in humans, the half-life of 2,3,7,8-TeBDD is comparable to 2,3,7,8-TeCDD (5–11 years) (Pirkle *et al*., 1989; Poiger and Schlatter, 1986; Wong *et al*., 2011). Furthermore, observed toxicokinetic differences between TeBDD and TeBDF are similar to those observed for both chlorinated congeners in several laboratory species and humans (van den Berg *et al*., 1994).

In conclusion, the available toxicokinetic information for PBDDs and PBDFs indicates properties comparable with the chlorinated congeners. This applies to tissue distribution, metabolism, and excretion. However, the number of brominated congeners that has been studied remains limited. These results suggest that the weaker carbon-bromine bond compared with the carbon-chlorine bond does not lead to more rapid elimination of the brominated congeners in experimental animals and humans. Thus, experimental data provide evidence that a comparable persistence in biota, including humans, for the 2,3,7,8-substituted brominated and chlorinated congeners exists.

BIOLOGICAL AND TOXIC EFFECTS IN MAMMALS AND FISH

Because PBDDs, PBDFs, and PBBs occur in the abiotic environment and in the human food chain, risks should be determined for both human and wildlife populations. Such an approach is consistent with the 1998 WHO TEF reassessment, which differentiated TEF values for humans (mammals), birds, and fish (van den Berg *et al*., 1998). A similar approach was chosen for this joint WHO-UNEP evaluation to determine TEF values that are useful for human and ecotoxicological risk assessment. As expected, the majority of the data is available from mammalian *in vivo* and *in vitro* studies and the expert panel thought that reliable, but limited, data are available for relative potencies of PBDDs and PBDFs in fish. However, information for avian models is lacking. It was also found that information for birds and dl-PBBs is not sufficient to determine REPs. Therefore, this WHO-UNEP consultation only evaluated relative potencies for humans and fish.

Structure-Activity Relationships in Mammalian Models

The structure-activity relationships (SARs) of PCDDs and PCDFs are among the best examined from any group of environmental contaminants. Numerous publications

have summarized these SARs for various toxicological, biochemical, and molecular endpoints using a wide variety of *in vivo* and *in vitro* systems, including human tissues. From these studies, there is scientific consensus that congeners with a 2,3,7,8-substituted chlorine pattern can elicit dioxin-like toxicity (Safe, 1986, 1990, 1994). Based on this scientific information, the WHO developed consensus TEFs for the 2,3,7,8-substituted PCDDs, PCDFs, and some PCBs based upon a similar dioxin-like mechanism of action in humans and wildlife (Ahlborg *et al*., 1994; van den Berg *et al*., 1998, 2006). There is general agreement that similar SARs can be applied for the 2,3,7,8-substituted PBDDs and PBDFs (Birnbaum *et al*., 2003; Weber and Greim, 1997; WHO, 1998). Information that supports this view follows in the paragraphs below.

Biochemical Effects in Mammalian Models

Exposure to 2,3,7,8-TeCDD, the prototypical and most potent persistent dioxin-like polyhalogenated aromatic hydrocarbon (dl-PHAH), and related congeners like PCDDs, PCDFs, and dl-PCBs produce a wide variety of species and tissue-specific toxic and biological effects (Denison *et al*., 2011; Furness *et al*., 2007; Hankinson, 1995; Poland and Knutson, 1982; Safe, 1990). Furthermore, the majority, if not all, of the biological and toxic effects of these compounds in vertebrates are mediated by the Ah receptor (AhR), a soluble intracellular ligand-activated transcription factor. AhR-dependent ("dioxin") toxicity involves persistent activation of the AhR signaling pathway by metabolically stable (persistent) dl-PHAHs and subsequent alterations in gene expression (Denison *et al*., 2011; Poland and Knutson, 1982; Safe, 1990). There is much experimental evidence that demonstrates the ability of 2,3,7,8-substituted PBDDs and PBDFs to bind to the AhR, activate the AhR signaling pathway, and produce classical dioxin-like toxic and biological effects (Andres *et al*., 1983; Dannan *et al*., 1983; Render *et al*., 1982; Robertson *et al*., 1982, 1983). The routine detection of 2,3,7,8-substituted PBDDs and PBDFs in biological and environmental matrices, including those of the human food chain, by itself warrants inclusion in the WHO-UNEP TEF concept. For this WHO-UNEP evaluation, the scientific literature was reviewed for published relative potencies (REPs) of PBDDs, PBDFs, and dl-PBBs and compared with their chlorinated congeners.

Figure 1 shows REP ranges for brominated compounds compiled from a range of *in vitro* and *in vivo* studies and compares them with their respective chlorinated compounds, and the numerical value ranges for these REPs are presented in Supplementary table 1. These values have been calculated on a molar basis to correct for the large differences in molecular weight that are associated with the brominated congeners. In general, REP ranges of the chlorinated versus brominated congeners reveal some minor differences, but a general overlap is present for the most toxic and relevant congeners. One of the most noticeable differences observed is the fact that PBB 77 appears significantly more potent than PCB 77.

For mono-ortho dl-PBBs, very limited information is available and does not allow quantitative evaluation of their REP ranges compared with their chlorinated analogues. However, these studies indicated that the SARs may be comparable with their chlorinated analogues (Dannan *et al*., 1983; Parkinson *et al*., 1983; Robertson *et al*., 1984).

Toxicity in Mammalian Models

In vivo toxicity studies with PBDDs and PBDFs are limited compared with those with PCDDs and PCDFs. However, there is general consensus from a qualitative point of view that the 2,3,7,8-substituted brominated congeners produce a similar toxic spectrum as TeCDD, albeit with congener-specific differences in potency (Kimbrough and Jensen, 1989; WHO, 1998). In rodents, the major overt toxic effects of 2,3,7,8-TeBDD are observed on thymus, body weight, and liver, whereas teratogenic effects such as hydronephrosis and cleft palate are identical to that caused by 2,3,7,8-TeCDD.

Toxicity studies have been reported for different brominated congeners using oral, ip, and sc administration (Ao *et al*., 2009; Haijima *et al*., 2010; Ivens *et al*., 1992,1993; Mason *et al*., 1987a; Moore *et al*., 1979; Nagao *et al*., 1990; White *et al*., 2012). These studies indicate that the toxic effects in rodents for 2,3,7,8-TeBDD are in the same dose range as that of 2,3,7,8-TeCDD, indicating a comparable potency for both congeners. Furthermore, the relative potencies of PBDDs for reduction in body weight gain and thymic atrophy followed the order 2,3,7,8-TeBDD > 1,2,3,7,8-PeBDD > 1,2,4,7,8- PeBDD > 1,3,7,8-TeBDD, similar to that of the chlorinated congeners (Mason *et al*., 1987a). A study with mice examined the relative teratogenic effects of 2,3,7,8-TeBDD, 2,3,7,8-TeBDF, 1,2,3,7,8-PeBDF, and 2,3,4,7,8-PeBDF and compared the effects with their chlorinated congeners. Although the ranking order was similar for the brominated and chlorinated congeners, relative potencies were different between the groups of congeners (Birnbaum *et al*., 1991). In a subchronic mouse study, the potency of 2,3,7,8-TeBDD for enzyme induction compared with TeCDD was also evaluated. Based on administered dose, the relative potencies for CYP1A1 induction in liver, lung, and skin ranged from 0.03 to 0.15 for 2,3,7,8-TeBDD compared with 2,3,7,8-TeCDD. However, if this difference was evaluated based on tissue concentrations, the relative potencies increased from 0.63 to 4.5 (DeVito *et al*., 1997). Possible toxic effects of PBDDs and PBDFs on immune functions and developmental brain functions have recently been studied in more detail (Ao *et al*., 2009; Haijima *et al*., 2010; White *et al*., 2012). In mice, 2,3,7,8-TeCDD or 2,3,7,8-TeBDD had nearly identical potencies for immunological endpoints, such as thymus weight and cell numbers, spleen weight and cell numbers, and IL-5 production (Ao *et al*., 2009). Another recent study presented relative potencies for immune suppression in mice of several PBDDs and their chlorinated congeners. The PBDDs produced a comparable suppression of the plaque-forming cell response in mice compared with their chlorinated congeners. Of note is
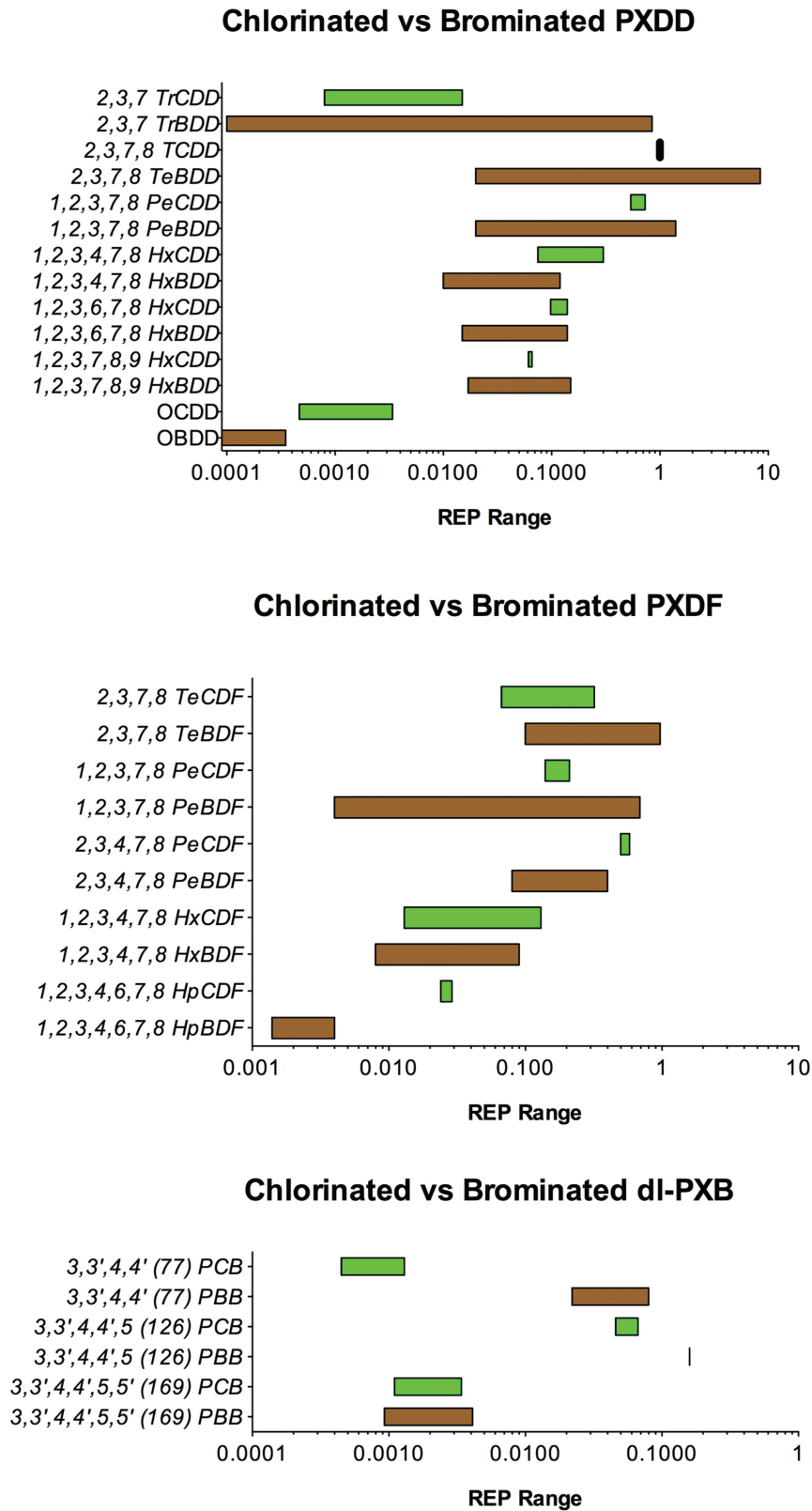
FIG. 1. Relative potency ranges of PXDDs, PXDFs, and dl-PXBs compiled from *in vitro* and *in vivo* studies. Polychlorinated compounds are indicated in green bars and polybrominated compounds indicated in brown bars.

that the relative potency of the 2,3,7,8-TeBDF was significantly higher than its chlorinated analogue, whereas the 2,3,7-TrBDD did not cause this immunosuppression at all (White *et al.*, 2012). Furthermore, mice exposed to TeCDD or TeBDD *in utero* and via lactation showed nearly identical responses to fear conditioning tests and had deficits in contextual and auditory fear memory, indicating that both TeCDD and TeBDD disrupt memory and emotional functions (Haijima *et al.*, 2010). A total of 32 different *in vivo* and *in vitro* REP values of 2,3,7,8-TeBDD were evaluated. From this wide range of experiments, an average REP value of 1.02 (median: 0.64, min: 0.02, max: 8.45) could be derived. Large differences in outcome of 2,3,7,8-TeBDD studies were observed compared with results for 2,3,7,8-TeCDD. Nevertheless, average and median REPs of 2,3,7,8-TeBDD are similar or close to 2,3,7,8-TeCDD. Thus, use of similar potencies for both congeners (TEF = 1) is suggested as an interim approach pending more detailed chronic studies with 2,3,7,8-TeBDD.

If the one order of magnitude estimated uncertainty of the assigned WHO TEF values is taken into consideration (van den Berg *et al.*, 2006), relative potencies between brominated and chlorinated congeners are in general comparable. With respect to differences in species sensitivity to acute toxicity, brominated and chlorinated congeners also induce comparable effects in the guinea pig. The similar toxicity of 2,3,7,8-TeBDD and 2,3,7,8-TeBDF as previously observed for 2,3,7,8-TeCDD and 2,3,7,8-TeCDF in the guinea pig is associated with the inability of this species to rapidly metabolize and eliminate the 2,3,7,8-halogenated dibenzofurans (van den Berg *et al.*, 1994).

Toxicity in Fish Models

There is only one detailed *in vivo* toxicity study examining the relative potencies (REPs) of PBDD, PBDF, and dl-PBB congeners in fish (Hornung *et al.*, 1996a). In this study, the early life stage toxicity was assessed at the larval stage of development in different strains of rainbow trout. The toxic endpoints observed were yolk sac edema, pericardial edema, hemorrhages, craniofacial malformation, and growth retardation prior to death. This pattern of toxic effects is referred to as blue sac syndrome and is commonly observed in the larval stage of rainbow trout exposed to individual dioxin-like PCDDs, PCDFs, and non-ortho dl-PCBs. Relative potencies for individual dioxin-like PBDD, PBDF, and dl-PBB congeners determined in these rainbow trout studies are presented in Table 1 (Hornung *et al.*, 1996a; Walker *et al.*, 1996; Zabel *et al.*, 1995a,c).

The mechanism of blue sac syndrome associated mortality in fish larvae, exposed as embryos to dioxin-like chemicals, is mediated by the AhR2 and ARNT1 proteins and induces alterations in gene expression comparable with the Ah-receptor mechanism in mammals (Heideman *et al.*, 2004; Peterson *et al.*, 2003, 2006). All polychlorinated and -brominated congeners shown in Table 1 produced this blue sac syndrome–related mortality and share the same AhR2-mediated mechanism and

identical endpoints of toxicity across several different fish species (King-Heiden *et al.*, 2011).

Table 1 compares the "fish-specific" REPs of PCDDs, PCDFs, and dl-PCBs with those of PBDDs, PBDFs, and dl-PBBs based on larval mortality in rainbow trout. The REP of each congener is expressed as the ratio of $LD_{egg\ 50}$ of 2,3,7,8-TeCDD (pmol/g egg) to $LD_{egg\ 50}$ of a PCDD, PDCF, non-ortho dl-PCB, PBDD, PBDF, or non-ortho dl-PBB congener (pmol/g egg). For the polychlorinated congeners, their REPs follow the classic SAR with PCDDs and PCDFs having greater REP values than non-ortho dl-PCBs. This is also the case for PBDDs and PBDFs compared with non-ortho dl-PBBs. When

TABLE 1

Comparison of Relative Potencies of PXDD, PXDF, and dl-PXB Congeners in Causing Larval Mortality in Different Strains of Rainbow Trout[a,b]

Congener	REPs chlorinated congeners	REPs brominated congeners	Trout strain
PXDD			
2,3,7		0.017[c]	Erwin
		0.018[c]	McConoughy
1,3,7,8		0.013[c]	Erwin
2,3,7,8	1[d]		Shasta
	1[e]	2.54[c]	Arlee
	1[d]	2.22[c]	Erwin
	1[d]	1.90[c]	Eagle Lake
		1.14[c]	Eagle Lake
1,2,3,7,8	0.730[e]	0.082[c]	Arlee
		0.140[c]	Eagle Lake
1,2,3,4,7,8	0.319[e]	0.009[c]	Arlee
1,2,3,6,7,8	0.024[d]		Shasta
1,2,3,4,6,7,8	0.002[d]		Shasta
PXDF			
2,3,7,8	0.028[e]	0.250[c]	Erwin
1,2,3,7,8	0.034[e]	0.041[c]	Erwin
2,3,4,7,8	0.359[e]	0.071[c]	Erwin
1,2,3,4,7,8	0.280[e]	0.002[c]	Erwin
dl-PXB			
3,3′,4,4′ (77)	0.0002[e]	0.002[c]	Erwin
		0.001[c]	Eagle Lake
		0.001[c]	Shasta
		0.002[c]	Arlee
3,4,4′,5 (81)	0.001[d]		Eagle Lake
3,3′,4,4′,5 (126)	0.005[e,f]		Arlee
3,3′,4,4′,5,5′ (169)	0.00004[d]		Erwin
		0.0001[c]	Arlee

Note. [a]2,3,7,8-TeCDD-like endpoints of early life–stage toxicity, grossly identical to blue sac syndrome and characterized by yolk sac edema, pericardial edema, multifocal hemorrhages, craniofacial malformation, and growth retardation prior to mortality, were observed during the larval stage of development for all congeners shown in this table.

[b]Calculated as ratio of LD50 of 2,3,7,8-TeCDD (pmol/g egg) to LD50 of a PBDD, PBDF, or dl-PBB or PCDD, PCDF, or dl-PCB congener.

[c]Hornung *et al.* (1996a).

[d]Zabel *et al.* (1995a).

[e]Walker and Peterson (1991).

[f]PCB 126 relative potency in causing larval mortality in lake trout is 0.0030 (Zabel *et al.*, 1995c).

individual congeners within each class (PCDDs to PBDDs; PCDFs to PBDFs; non-ortho dl-PCBs to non-ortho dl-PBBs) are compared, the REPs for many brominated congeners with similar substitution to the chlorine congeners are distinctly different. 2,3,7,8-TeBDD, 1,3,7,8-TeBDD, and PBB 77 caused a higher mortality in the rainbow trout early life stage assay than their corresponding chlorinated congeners. Thus, SARs for the brominated compounds appear to be different from that observed for their chlorinated analogues in the same fish bioassay. The most notable difference is the observation that trilateral substituted PBDDs are weak AhR2 agonists in fish, providing the rationale to include these congeners in future aquatic risk assessment.

In conclusion, the present information for relative potencies of PBDDs, PBDFs, and dl-PBBs in fish is more or less limited to one particular bioassay system but with multiple species. The REPs provided in Table 1 provide a basis for ecotoxicological risk assessment in the aquatic environment and are clearly different from those of mammals. However, the (limited) data for fish derived from this bioassay show a significant deviation in REPs between the brominated and chlorinated congeners in fish. In fact, these data provide evidence that the SARs for PBDDs, PBDFs, and dl-PBBs may be different from the chlorinated congeners. Due to the limited database available for the brominated compounds, a recommendation for general fish-specific TEFs for the brominated congeners can not be made. However, if regulatory agencies need to determine ecotoxicological risks for aquatic organisms, the relative potencies presented in Table 1 may be used to estimate the total toxicity equivalency of PBDDs, PCDFs, and dl-PBBs with or without the PCDDs, PCDFs, and dl-PCBs.

Mixture Interaction Between PBDDs, PBDFs, Non-ortho dl-PBBs, and Their Chlorinated Congeners

Although there is a wealth of information regarding the additivity of PCDDs, PCDFs, and dl-PCBs (van den Berg *et al.*, 1998, 2006), there is a paucity of experimental data involving mixture toxicity studies with the brominated congeners. Most information is available from mixture experiments using early life stage assays in fish. Using fish-specific REPs, the hypothesis was tested if pairs of PBDD, PBDF, and dl-PBB congeners interact additively to cause mortality in fish embryos. Four congener pairs were tested across a wide range of different dose combinations (2,3,7,8-TeBDD/1,2,3,7,8-PeBDD), (2,3,7,8-TeBDD/1,2,3,7,8-PeBDF), (1,2,3,7,8-PeBDD/2,3,4,7,8-PeBDF), and (3,3′,4,4′-TBB/2,3,4,7,8-PeBDF). In all cases, interactions between these congener pairs were additive (Hornung *et al.*, 1996b), which is comparable with earlier observations made for pairs of chlorinated congeners in the same fish larvae bioassay. Nevertheless, these mixture toxicity experiments showed some deviations from additivity for mixtures of 2,3,7,8-TeCDD/PCB 77 and 2,3,7,8-TeCDD/PCB 126. However, these limited deviations from additivity toward synergism are not sufficient to warrant a change from the TEF prerequisite of additivity (Zabel *et al.*,

1995c). The same fish larvae bioassay has been used to test a complex and toxicologically relevant mixture of PCDDs, PCDFs, and PCBs, as measured in Great Lakes fish and found not to act in a strictly additive manner. However, these data are still within the one order of magnitude uncertainty factor estimated for individual TEF values (van den Berg *et al.*, 2006; Walker *et al.*, 1996).

In conclusion, the fish bioassays provide sufficient support for the TEF additivity model for 2,3,7,8-substituted PBDDs, PBDFs, and non-ortho dl-PBBs. In addition, it should be recognized that the common mechanism of a single receptor-mediated effect for both the chlorinated and brominated congeners warrants the use of additivity for mixtures. Based on common mechanistic considerations from mammals and fish, it can be assumed that these conclusions can be generalized to other classes of vertebrates, including humans. However, it should be noted that quantitative differences in REPs and TEFs for individual brominated congeners can differ between classes of vertebrates, as concluded earlier by WHO for the chlorinated congeners (van den Berg *et al.*, 1998).

DISCUSSION

In 1998, the WHO published an extensive review of the biological and toxic properties of PBDDs and PBDFs and concluded that brominated and chlorinated 2,3,7,8-substituted congeners elicit a similar spectrum of effects (WHO, 1998). As with PCDDs, PCDFs, and some non-ortho dl-PCBs, the brominated congeners show comparable biological and toxic effects, such as immune suppression, enzyme induction, thyroid hormone and vitamin A perturbations, antiestrogenicity, teratogenicity, and neurobehavioral deficits. These effects of brominated congeners are found at similar low doses as 2,3,7,8-substituted PCDDs and PCDFs, indicating comparable potencies for the 2,3,7,8-brominated congeners (for review, see WHO (1998)). Because the 1998 WHO review of PBDDs and PBDFs, several other studies determined the relative potencies of these compounds in various experimental systems (Ao *et al.*, 2009; Behnisch *et al.*, 2003; Guruge *et al.*, 2009; Haijima *et al.*, 2010; Olsman *et al.*, 2007; Samara *et al.*, 2009). Studies performed for this purpose used either AhR-mediated induction of CYP1A1/1B1 activity or luciferase expression, developmental effects in fish, or immunoassays. On the other hand, changes in immune, memory, and emotional functions have also been studied *in vivo*. If the assumed uncertainty of one order of magnitude in WHO TEFs is taken into account, it can be concluded that the relative potencies of 2,3,7,8-substituted PBDDs and PBDFs are comparable to their corresponding chlorinated congeners in mammalian systems. However, it should also be noted that the limited mammalian database for REPs of PBDDs, PBDFs, and dl-PBBs does not allow sufficient differentiation for a TEF compared with their chlorinated congeners. In Figure 1, REP ranges for PBDDs and PBDFs are

presented and can be compared directly with their chlorinated counterparts. The results in this figure also show differences in the REP ranges between some chlorinated and brominated congeners, with dl-PBBs significantly different from the dl-PCBs. Depending on the relevance in the present exposure of humans to dl-PBBs, it must be decided whether more detailed chronic studies with these congeners are warranted.

Consequently, the limited *in vivo* and *in vitro* data from mammalian systems for PBDDs, PBDFs, and non-ortho dl-PBBs support the use of similar TEFs as interim values for human risk assessment, pending more research with (semi)chronic studies. Clearly, dissimilarities between both groups of congeners exist, but possible consequences should again be placed in the context of the assumed uncertainty of one order of magnitude for WHO mammalian TEFs of the chlorinated congeners. Based on some of the limited data, it is possible that 2,3,7,8-TeBDF and 1,2,3,7,8-PeBDF may have higher relative potencies than their chlorinated analogues. This could be due to the longer half-lives of both brominated congeners in experimental animals and humans. New toxicity studies can provide more clarity on this issue in the future. For further comparison, the present WHO TEFs for chlorinated congeners and data from recent publications with the range of REP values for both the chlorinated and brominated congeners are given in Supplementary table 1 (Behnisch *et al*., 2003; Olsman *et al*., 2007; Samara *et al*., 2009).

The expert panel also reviewed the available evidence with respect to human exposure to PBDDs, PBDFs, and dl-PBBs. It was concluded that these compounds may contribute significantly to the total TEQs in daily human background exposure. As a result, inclusion of 2,3,7,8-substituted PBDDs and PBDFs in the WHO-UNEP TEF concept is essential for improving human risk assessment for dioxin-like compounds. Although, very little information is available on dl-PBBs, there are indications that the more toxic non-ortho dl-PBBs are still present in the environment and human food chain. Therefore, from a precautionary principle, it is recommended that the non-ortho dl-PBBs 77, 81, 126, and 169 are also included in the WHO-UNEP TEF concept for dioxin-like compounds. In contrast, there seems to be an almost complete lack of exposure and toxicity information concerning the mono-ortho dl-PPBs. Future studies may provide more clarity about the brominated mono-ortho dl-PCBs with respect to inclusion in the WHO-UNEP TEF concept. In view of the significant uncertainty already present in the TEF values for the mono-ortho PCBs (van den Berg *et al*., 2006), it appears premature to assign similar values to the mono-ortho PBBs until more information about REPs has become available. Similarly, very limited information is available for the polybrominated and polychlorinated naphthalenes (PBNs and PCNs) and their occurrence in the human food chain (Isosaari *et al*., 2006; Schiavone *et al*., 2009), in spite of the fact that these compounds are known to have dioxin-like activity. The latter aspect by itself could warrant inclusion in the TEF method if future studies show substantial occurrence in the human food chain (Behnisch *et al*., 2001, 2003).

The mixed halogenated dibenzo-*p*-dioxins (PXDDs), dibenzofurans (PXDFs), and biphenyls (PXBs) are other dl-compounds that may be considered in the WHO-UNEP TEF concept in the near future. During the last decade, their occurrence in the environment and human food chain has been studied only to a limited extent. However, it was concluded that PXDDs, PXDFs, and non-ortho dl-PXBs may significantly contribute to overall dioxin-like exposure. Unfortunately, complicated analytical methodology at present hampers a regular and adequate human and environmental exposure assessment. With respect to *de novo* formation of PXDDs, PXDFs, and PXBs, it is noticeable that these compounds may be formed during combustion processes (Falandysz *et al*., 2012; Gómara *et al*., 2011; Ohta *et al*., 2008; Myers *et al*., 2012; Weber and Kuch, 2003; Terauchi *et al*., 2009).

Many different PBDD, PBDF, and dl-PBB congeners have now been detected in the aquatic environment. For this reason, the available data for fish and wildlife were evaluated for possible inclusion in the WHO-UNEP TEF concept, along with the chlorinated congeners as published earlier (van den Berg *et al*., 1998). For this purpose, only REPs from fish larvae studies are available for the brominated compounds. These indicate that there may be significant differences between the SARs of REPs for fish early life stage toxicity for both brominated and chlorinated congeners. Because of this limited database, it was decided not to derive specific WHO-UNEP TEFs for fish. Instead, it is recommended that regulatory agencies consider the use of REPs provided in Table 1 for the use of ecotoxicological risk assessment in the aquatic environment.

CONCLUSIONS

At present, there is sufficient evidence to conclude that concentrations of 2,3,7,8-substituted PBDDs, PBDFs, and possibly some non-ortho dl-PBBs in human food, tissue, and milk can contribute significantly to the total amount of TEQ. In mammalian systems, the mechanism of action and type of toxicity of 2,3,7,8-substituted PBDDs, PBDFs, and non-ortho dl-PBBs are similar to their chlorinated congeners. The available evidence also demonstrates that many REPs for PBDDs and PBDFs in mammals are similar to those of their chlorinated analogues or at least within one order of magnitude (see Fig. 1). This is comparable to the inherent uncertainty of one order of magnitude in the present WHO TEF approach for dl-like chlorinated compounds. Based on the considerations discussed above, the use of similar interim TEF values for brominated and chlorinated congeners for human risk assessment is recommended by the WHO and UNEP, pending availability of future studies on these compounds.

FUNDING

Technology, Industry and Economics, and the Intramural Research Program of the National Institutes of Health, NIEHS, and NCI.

SUPPLEMENTARY DATA

Supplementary data are available online at http://toxsci.oxfordjournals.org/.

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