Toxic Equivalency Factors (TEFs) for PCBs, PCDDs, PCDFs for Humans and Wildlife

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An expert meeting was organized by the World Health Organization (WHO) and held in Stockholm on 15–18 June 1997. The objective of this meeting was to derive consensus toxic equivalency factors (TEFs) for polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) and dioxinlike polychlorinated biphenyls (PCBs) for both human, fish, and wildlife risk assessment. Based on existing literature data, TEFs were (re)evaluated and either revised (mammals) or established (fish and birds). A few mammalian WHO-TEFs were revised, including 1,2,3,7,8-pentachlorinated DD, octachlorinated DD, octachlorinated DF, and PCB 77. These mammalian TEFs are also considered applicable for humans and wild mammalian species. Furthermore, it was concluded that there was insufficient *in vivo* evidence to continue the use of TEFs for some di-*ortho* PCBs, as suggested earlier by Ahlborg et al. [Chemosphere 28:1049–1067 (1994)]. In addition, TEFs for fish and birds were determined. The WHO working group attempted to harmonize TEFs across different taxa to the extent possible. However, total synchronization of TEFs was not feasible, as there were orders of a magnitude difference in TEFs between taxa for some compounds. In this respect, the absent or very low response of fish to mono-*ortho* PCBs is most noticeable compared to mammals and birds. Uncertainties that could compromise the TEF concept were also reviewed, including nonadditive interactions, differences in shape of the dose–response curve, and species responsiveness. In spite of these uncertainties, it was concluded that the TEF concept is still the most plausible and feasible approach for risk assessment of halogenated aromatic hydrocarbons with dioxinlike properties. *Key words*: dioxins, humans, PCBs, polychlorinated biphenyls, TEFs, toxic equivalency, uncertainties, wildlife. *Environ Health Perspect* 106:775–792 (1998). [Online 10 November 1998]
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Polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and biphenyls (PCBs) constitute a group of persistent environmental chemicals. Due to their hydrophobic nature and resistance towards metabolism, these chemicals have been found in fatty tissues of animals and humans. Several PCDDs, PCDFs, and PCBs have been shown to cause toxic responses similar to those caused by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the most potent congener within these groups of compounds. These toxic responses include dermal toxicity, immunotoxicity, carcinogenicity, and adverse effects on reproduction, development, and endocrine functions.

PCDDs, PCDFs, and PCBs exist in environmental and biological samples as complex mixtures of various congeners whose relative concentrations differ across trophic levels. These differences are caused by environmental degradation, which refers to the different environmental fates of congeners with different solubilities, volatilities, and rates of degradation/metabolism. As a result, these mixtures change spatially and temporally into the environment and are very different from the technical mixtures originally released into the environment.

The complex nature of PCDD, PCDF, and PCB mixtures complicates the risk evaluation for humans, fish, and wildlife. For this purpose, the concept of toxic equivalency factors (TEFs) has been developed and introduced to facilitate risk assessment and regulatory control of exposure to these mixtures. To apply this TEF concept, a fundamental understanding of the mechanism of action is a prerequisite. At present, there is sufficient evidence available that there is a common mechanism for these compounds, involving binding to the aryl hydrocarbon (Ah) receptor as an initial step. When applying the TEF concept, the toxicity of these compounds relative to that of 2,3,7,8-TCDD is determined on the basis of available *in vivo* and *in vitro* data. However, it should also be understood that the TEF concept is based on a number of

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assumptions and has limitations. In this respect, the most basic assumption is that the combined effects of the different congeners are dose or concentration additive, and results of many studies support this assumption.

In the last decade, several different TEF schemes have been developed for PCDDs, PCDFs, and dioxinlike PCBs. Recognizing the need for a harmonized approach in setting internationally agreed upon TEFs, the European Centre of Environmental Health of the World Health Organization (ECEH-WHO) and the International Programme on Chemical Safety (IPCS) have initiated a program to derive TEFs for these compounds for assessing the impact of these compounds on human and environmental health.

At an initial WHO consultation on the derivation of TEFs for wildlife for dioxinlike compounds, held on 9–10 August 1996 in Bilthoven, The Netherlands, the question was addressed whether it is appropriate to use TEFs derived for the purpose of human risk assessment to estimate the risk for fish and wildlife species. The meeting concluded that there was a need to derive separate sets of TEFs for fish and wildlife, and recommended to combine this effort with a reevaluation and possible update of existing TEFs for human health risk assessment. In addition, it was recommended that the same TEFs for human health and wildlife should be used as much as possible.

For the (re)evaluation process, experimental data on the relative potencies (REPs) of PCDDs, PCDFs, and dioxinlike PCBs for mammalian, avian, and fish species have been collected by the Institute of Environmental Medicine, Karolinska Institute, Stockholm, Sweden, and inserted into a database. Following collection of all available information, an ECEH-WHO/IPCS meeting was held at the Karolinska Institute on 15–18 June 1997. The objective of the meeting was to assess the REP values in the database and to derive consensus TEFs for PCDDs, PCDFs, and dioxinlike PCBs for both human, fish, and wildlife risk assessment. The results of this process are presented in this paper.

TEF Concept and Data Requirements

Toxic Equivalency Factors, Relative Potencies, and Toxic Equivalents

In the literature, there is some confusion regarding the definition of the term toxic equivalency factor (TEF). One reason for confusion is the fact that the term TEF has been used in two different ways: 1) as a relative potency value that is based on the results of several *in vivo* and *in vitro* studies; or 2) as the relative potency of a compound relative to TCDD to cause a particular toxic or biological effect in a single study. Furthermore, TEF is frequently used to refer to an end point that is not a toxic response per se, such as binding affinity to the aryl hydrocarbon (Ah) receptor or induction of cytochrome P4501A1, although these biochemical effects may in some way be associated with subsequent toxic responses.

It is important to realize that in this paper and associated WHO publications TEF indicates an order of magnitude estimate of the toxicity of a compound relative to TCDD. This consensus TEF value has been derived using careful scientific judgment after considering all available scientific data. However, when the potency of a compound relative to TCDD has been obtained in a single *in vivo* or *in vitro* study, it will be referred to as a REP value.

TEF values, in combination with chemical residue data, can be used to calculate toxic equivalent (TEQ) concentrations in various environmental samples, including animal tissues, soil, sediment, and water. TEQ concentrations in samples are calculated using the following equation:

$$TEQ = \Sigma_{n1}[PCDD_i \times TEF_i] + \Sigma_{n2}[PCDF_i \times TEF_i] + \Sigma_{n3}[PCB_i \times TEF_i]. \quad (1)$$

TEFs and TEQs are used for risk characterization and management purposes, e.g., to help prioritize areas of concern for cleanup. However, in relation to the use of TEFs for abiotic compartments, the biological meaning of these values is obscure. This is caused by the fact that the assumed biological or toxicological effect is influenced by many physicochemical factors before the actual uptake of the compounds by the organism takes place. Nevertheless, TEQ values can be used as relative measures between different abiotic samples, e.g., sediment and soil, to prioritize remedial actions. In relation to the initial transport of these compounds from the abiotic to biotic compartment, it was recognized that congener-specific biota-sediment accumulation factors (BSAFs) can be used to predict the concentrations in fish tissue, after which these can be converted to TEQs using TEFs.

The TEFs presented in this paper apply only to AhR-mediated responses, and this concept assumes a model of dose additivity. In relation to this prerequisite, we decided that for PCDDs, PCDFs, and some planar PCBs there is sufficient evidence from both *in vivo* and *in vitro* studies supporting the dose or concentration-additivity model for Ah receptor-mediated responses. In addition, it should be emphasized that the TEF concept cannot be applied to effects that are not Ah receptor-mediated, and it does not consider modulating effects of compounds that are not Ah receptor ligands.

TEF values were determined for the following classes of vertebrates: mammals, fish, and birds. TEFs that were determined for mammalian species were also considered to be applicable for human risk assessment purposes. It was concluded that, to date, not enough information was available to determine REP values in amphibians and reptiles, such that TEFs could not be proposed for these classes of vertebrates. At this time, development of TEFs for invertebrates is not recommended because there is limited evidence for ligand activation of AhR or for TCDD-like toxicity in invertebrates.

The criteria for including a compound in a fish and wildlife TEF scheme are the same criteria as those used for the derivation human TEFs (*1*). These are 1) a compound must show a structural relationship to the PCDDs and PCDFs; 2) a compound must bind to the Ah receptor; 3) a compound must elicit Ah receptor-mediated biochemical and toxic responses; and 4) a compound must be persistent and accumulate in the food chain.

Compilation of the Database

Since 1993, the data of all available mammalian, bird, and fish studies on relative toxicity of dioxinlike compounds that meet the criteria of inclusion in a TEF scheme (*1*) have been collected, and this information has been stored in a database at the Karolinska Institute in Stockholm (Sweden). These publications were analyzed and the data to be included in the database were selected using the following criteria:

1. At least one PCDD, PCDF, or PCB congener and a reference compound must be included in the study.
2. Either TCDD or PCB 126 must be included as a reference compound in the same experiment or studied with the same experimental design by the same authors in another experiment.
3. The relevant end point should be affected by the congener studied as well as the reference compound.

PCB 126 was used as reference in some cases, with an assigned REP of 0.1 for mammals and birds or 0.005 for fish, based on a variety of *in vivo* studies covering various end points. The use of PCB 77 and PCB 169 as reference compounds was no longer included in this evaluation due to the limited comparative information available for these two compounds. Only dioxin-specific end points were included in the database (see criteria 3).

However, it is recognized that some end points such as liver enlargement and tumor promotion are affected by both dioxinlike and nondioxinlike halogenated aryl hydrocarbons (HAHs; e.g., mono- or di-*ortho* PCBs). In addition, several biological or toxic effects have been described for PCBs, for example, which are probably not related to an Ah receptor-mediated mechanism of action. Among others, these effects include a decrease in dopamine levels (*2,3*), alterations in retinoid and thyroid hormone levels (*4,5*), and binding to the estrogen receptor (*6*). These effects show distinctly different structure–activity relationships for PCBs than those observed for Ah receptor binding involving multiple *ortho* chlorine substitution or hydroxylated metabolites. Based on the four criteria mentioned earlier, these effects, how biologically relevant they might be, are not covered in this TEF concept. For these compounds and their associated effects, a different approach for risk assessment is needed, which might possibly involve the development of an alternative toxic equivalency concept.

Compounds included in the database are the 2,3,7,8-substituted PCDDs and PCDFs and those PCBs with established dioxinlike activity, especially the non- and mono-*ortho* PCBs. We agreed that there are a large number of other halogenated compounds that meet the criteria for inclusion in the TEF concept and could contribute to the total concentration of TEQs in environmental samples. These include any or all of the following classes of polychlorinated compounds: naphthalenes, diphenyl ethers, diphenyl toluenes, phenoxy anisoles, biphenyl anisoles, xanthenes, xanthones, anthracenes, fluorenes, dihydroanthracenes, biphenyl methanes, phenylxylylethanes, dibenzothiophenes, quaterphenyls, quaterphenyl ethers, and biphenylenes. In addition to the chlorinated compounds, brominated and chloro/bromo-substituted analogues of PCDDs (PBDDs) and PCDFs (PBDFs) have been found in the environment (*7–10*) and are known to induce CYP1A1 activity *in vivo* and *in vitro* (*11*). PBDDs and PBDFs have also been shown to cause developmental toxicity, and REPs have been determined in mammals and fish (*12–14*). However, it was decided that, at present, insufficient environmental and toxicological data are available to establish a TEF value for any of the above compounds.

Calculation of REPs

The following methods were used to derive REPs from the available data:

- REPs were used as reported in each publication; if experimental data were also reported, these were used to calculate the REPs using one of the methods below.
- REPs were calculated by comparing dose–response curves or by using linear interpolation of log-doses, comparing the same effect level; if necessary, corrections were made for different control levels.
- REPs were determined from ratios of medium effective dose (ED_{50}), median lethal dose (LD_{50}), and median effective concentration (EC_{50}) values, tumor promotion indexes, dissociation constant (K_d) values for Ah receptor binding, or directly estimated from the graphs presented.

All the data were compiled into a spreadsheet format using Quattro Pro (version 6.0) for Windows (Corel Corporation Limited). The database used for the derivation of TEF values is available from The European Centre for Environment and Health, World Health Organization, Bilthoven, The Netherlands.

Basis for the Review Process

TEFs for Mammalian Wildlife

The TEF values, which are mainly based on rodent studies, should be suitable for both human risk assessment and for estimating the risk for species of mammalian wildlife.

Based on two semichronic studies with mink and TCDD and PCB 169, the REP of PCB 169 was evaluated. In the first study with adult male mink, a 28-day oral LD_{50} value (single dose) of 4.2 µg TCDD/kg body weight (bw) was reported (*15,16*), and a 28-day dietary median lethal concentration (LC_{50}) of 4.3 ppb was calculated for female mink. In the second study, PCB 169 was given to mink via the diet (*17*). Exposure of adult females to dietary concentrations of 0.05 mg PCB 169/kg bw for 131 days resulted in 50% mortality. Assuming a daily feed consumption of 150 g and a body weight of 1 kg, the total intake of PCB 169 was about 1,000 µg/kg bw. By using the LD_{50} values of 4.2 for TCDD and 1,000 for PCB 169, a value of 0.0042 can be calculated as a first estimate for the relative potency of PCB 169 in mink. Based on these calculations, the previous assigned TEF value of 0.01 for PCB 169 for human risk assessment seems fairly reasonable for mink and gives some support to the use of similar TEF values for humans and wild mammals.

The TEF concept is further supported in mink by comparison of the no observed adverse effect level (NOAEL) derived from a meta-analysis of mink reproductive toxicity data (*18*) with the NOAEL calculated from a mink laboratory reproductive study in which the diets were subject to complete chemical characterization (*19*). These two independent analyses of the TEF approach, utilizing different effects data sets and the same set of international TEFs (*1,20*) for the assessment of mink reproductive toxicity, confirmed one another. Overall these results suggest that the relative potencies of the PCBs, PCDDs, and PCDFs toward mink reproductive toxicity are not different from those of the rodent models from which most of the data were derived.

One argument that might be considered to refine future risk assessment of mammalian wildlife is the substance-specific biotransformation capacity of different mammalian species. Certain marine mammals might have a higher capacity to metabolize dioxins and dioxinlike congeners than certain terrestrial mammals, but due to their position in the foodchain and strictly aquatic diet, body burdens can still be high. Due to these differences in metabolic CYP1A1 capacities, it has been suggested that terrestrial mammals, when compared to highly exposed marine mammals, may experience a greater threat from dioxinlike compounds, e.g., PCB 126, than mammalian top predators in the aquatic food webs (*21–25*).

Furthermore, pharmacokinetic differences will undoubtedly contribute to the interspecies variability observed with different congeners. At present, the mammalian TEF scheme is based on administered dose. Although it has been discussed whether mammalian TEFs should be derived based on target tissue concentrations, this is at present considered not feasible due to the limited amount of scientific information available.

TEFs for Ah Receptor-mediated Toxicity for Fish

Fish, including all salmonids that have been studied, express CYP4501A activity (*26,27*), which is an Ah receptor-mediated process and can be induced by many (halogenated) aromatic hydrocarbons (*28–31*). This mechanism indicates that fish should be sensitive to the effects of Ah receptor-active compounds; but compared to mammals, fish are less responsive to mono-*ortho* PCBs. The hepatic cytochrome P450-dependent enzymes in fish are considered to be functionally similar to those of mammals (*32*). In those instances where adequate data are available, it appears that fish enzymes are influenced by some of the same factors that affect these enzymes in mammals: species (*32*), strain (*33*), size and/or age (*34*), developmental stage (*35*), sex (*36*), reproductive state (*37*), nutrition (*38*), and exposure to certain types of organic xenobiotic inducers as well as environmental conditions (*39*).

The inclusion of fish in the TEF concept is justified by the common Ah receptor-mediated mechanism of response; this was confirmed by many toxicological and

biochemical studies during the last decades. Fish-specific REPs have been derived from mortality in early life stages in rainbow trout (*40–42*) and from *in vivo* CYP1A induction in adult rainbow trout (*43*) and *in vitro* in rainbow trout liver (RTL-W1) (*44–46*) and gonadal cell lines (*47*). These have been developed for ecological risk assessments involving fish exposed to complex mixtures of dioxinlike PCBs, PCDDs, and PCDFs. Generally, the REPs derived from CYP1A induction either *in vivo* or *in vitro* are higher than those derived from early life stage mortality (*48*). Injection of trout eggs with TCDD has been shown to result in the same effects and LD_{50} as with maternal transfer of the chemical (*41*) and has confirmed the application of the TEF concept in fish.

In addition, it has been established that REPs determined for mortality in rainbow trout sac fry following waterborne exposure are nearly identical to those determined for the same end point after egg injection (*40,42,49*). Thus, the REPs based on sac fry mortality were not influenced by the route of exposure. Zabel et al. (*50*) also showed that the REP for PCB 126 in producing lake

Table 1. Toxic equivalency factors (TEFs) for human risk assessment and mammals

Congener	Description	Old TEF	WHO TEF
Dioxins			
1,2,3,7,8-PentaCDD	Based on new *in vivo* tumor promotion data and CYP1A1/A2 induction potencies from subchronic studies (*79–81*); a value closer to 1.0 is recommended.	0.5	1
1,2,3,4,7,8-HexaCDD	Limited *in vivo* data are available, but more recent LD_{50} values continue to support the current value (*82,83*); no revision is recommended.	0.1	0.1
1,2,3,6,7,8-HexaCDD	No new *in vivo* data. Recent *in vitro* data support the current value (*84*); no revision is recommended.	0.1	0.1
1,2,3,7,8,9-HexaCDD	No new *in vivo* information; no revision is recommended.	0.1	0.1
1,2,3,4,6,7,8-HeptaCDD	Two recent semichronic studies indicate values between 0.02 and 0.03 for various end points (*85,86*); no revision is recommended.	0.01	0.01
OctaCDD	A recalculation of the old data in which exposure versus tissue concentrations were compared suggests that a lower value is more appropriate (*87*).	0.001	0.0001
Furans			
2,3,7,8-TetraCDF	Recent semichronic studies generally support a value of 0.1 (*88,89*); less emphasis was put on LD_{50} values; no revision is recommended.	0.1	0.1
1,2,3,7,8-PentaCDF	Although new semichronic data suggest that mice are more responsive (*81,90*), the combined potency values indicate the current value is adequately protective; no revision is recommended.	0.05	0.05
2,3,4,7,8-PentaCDF	Most REPs dealing with relevant toxic end points [e.g., (*79*)] support the continued use of a value of 0.5. Due to the fact that this congener is a major contributor to total TEQs in biological samples, confirmation of recent immunotoxicity data indicating a higher value is strongly recommended (*91*); at present, no revision is recommended.	0.5	0.5
1,2,3,4,7,8-HexaCDF	Additional *in vivo* potency values on nonspecific biological end points (liver, thymus, body weight alterations) are in the range of the current TEF (*92*); no revision is recommended.	0.1	0.1
1,2,3,6,7,8-HexaCDF	No new information; no revision is recommended.	0.1	0.1
1,2,3,7,8,9-HexaCDF	Very limited data (*93*) and no new *in vitro* and QSAR data that suggest a change in value is warranted; no revision is recommended.	0.1	0.1
2,3,4,6,7,8-HexaCDF	Limited new *in vitro* and QSAR information indicate a change of value is not warranted (*93*); no revision is recommended.	0.1	0.1
1,2,3,4,6,7,8-HeptaCDF	Recent values greater than 0.01 are under discussion due to possible impurity of test compound and the fact that no reference (TCDD/PCB126) was included at the time of studies (*94*). In addition, the same study reports approximately the same response with 1,2,3,4,7,8,9-heptaCDF, whereas this congener is known to be less persistent *in vivo* (*95*). Confirmation of REPs >0.01 is required; no revision is recommended.	0.01	0.01
1,2,3,4,7,8,9-HeptaCDF	A very limited data set is available and the same comment holds as for 1,2,3,4,6,7,8-heptaCDF; no revision is recommended.	0.01	0.01
OctaCDF	New *in vivo* EROD induction potency values (*81*) and an expected structural similarity with octaCDD for the *in vivo* situation support a change in analogy with octaCDD.	0.001	0.0001
Non-*ortho* PCBs			
3,3′,4,4′-TetraCB (77)[a]	Recent subchronic studies with EROD induction and hepatic retinol decreases suggest a value around or below 0.0001 (*96–98*), which is lower than the previous value based on tumor promotion (*99*); however, impurity and background contamination (e.g, PCB 126) is an identified problem with this congener. A change in value to 0.0001 is recommended.	0.0005	0.0001
3,4,4′,5-TetraCB (81)	Based on Ah receptor binding, *in vitro* CYP1A induction, and QSAR calculations, a value similar to PCB 77 was suggested (*100–102*). It should be noted that *in vitro* REPs possibly overestimate the potency of this compound, as in analogy with PCB 77, it is likely to be metabolized efficiently by higher organisms; however, this congener is detectable in certain wildlife species and human samples. A value of 0.0001 is recommended.	–	0.0001
3,3′,4,4′,5-PentaCB (126)	Multiple *in vivo* end points in semichronic studies with rats, including CYP1A1/A2 induction, tumor promotion, and hepatic retinol decreases, support the current value (*89,103,104*); no revision is recommended.	0.1	0.1
3,3′,4,4′,5,5′-HexaCB (169)	Recent *in vivo* EROD induction and thymic atrophy values support a conservative value of 0.01 for this congener. Confirmation of recent immunotoxicity data possibly indicating a higher REP is strongly recommended (*91,105*); no revision is recommended.	0.01	0.01

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trout early life stage mortality is similar to the REP for the same response in rainbow trout. These results are significant because they suggest that TCDD-like congeners show similar REPs in rainbow trout and lake trout and support both the use of rainbow trout REPs in assessments of risks to lake trout and, perhaps, other fish species. While this agreement in REPs for PCB 126 between trout species is encouraging, further comparisons of REPs for other potent congeners and more disparate fish species are needed. In relation to environmental risk assessment, the use of early life stage mortality-specific TEFs and the egg toxicity equivalent additivity model predicted that PCDDs, PCDFs, and PCBs in Great Lakes lake trout are currently below levels that cause early life stage mortality (*51*). This information is in agreement with recent observations of juvenile lake trout survival in this area of the Great Lakes and gives further support for the use of the TEF concept in fish.

One of the major differences between the TEFs determined for mammals and fish is the lack or low response to mono-*ortho* PCBs in the latter taxa. Even with an extremely sensitive Ah receptor-mediated effect, the induction of CYP1A1, it was found that fish are very insensitive, if sensitive at all, to mono-*ortho*-substituted PCBs (*45,48,52–55*). Similar lack of responses toward mono-*ortho* PCBs have been observed for early life stage mortality in rainbow trout (*40,42*). Based on the above considerations, we decided that fish should be included in the TEF concept, but due to the deviations found for PCBs, fish should be treated as a separate taxa in this TEF evaluation.

TEFs for Birds

Although many studies with young or adult birds have shown dioxinlike toxic and biochemical effects of TCDD and PCBs, these studies were not designed for REPs of compounds to be reliably derived (*56–63*). At present, TEFs for birds can only be derived from egg injection studies, studies with cultured avian hepatocytes, and studies with cultured thymus cells.

The end points mentioned above have been studied in a number of avian species. These species include domestic chicken, duck, domestic goose, turkey, pheasant, gull, common tern, double-crested cormorant, and American kestrel (*64–68*). As with fish and mammals, Ah receptor-mediated CYP1A1 induction has been found in a large number of avian species, clearly indicating an Ah receptor responsiveness in this taxa. This justifies the inclusion of birds in the TEF concept. The induction of CYP1A1 in avian systems has been studied for most of the environmentally relevant PCDDs, PCDFs, and dioxinlike PCBs. However, the number of studies that could be used for this evaluation was limited. REPs for Tier 1 studies were not available for PCDDs and PCDFs, but for some PCBs, Tier 1 studies (*in ovo* lethality) were available. In general, REPs for ethoxyresorufin-*O*-deethylase (EROD) induction for dioxins, furans, and non-*ortho*-PCBs in birds are in the same range as those reported from mammalian systems. The major exception is TCDF, which can be more potent than TCDD in hepatocyte cultures from several species of birds (*65,66,69*).

Based on studies with chicken embryos, it has been concluded that birds can be highly responsive toward non-*ortho* PCBs, although much of the information available concerns induction of CYP1A1 and not distinct toxic

Table 1. (*continued*)

Congener	Description	Old TEF	WHO TEF
Mono-*ortho* PCBs	For this group of congeners, more than one mechanism of action (not only Ah receptor specific) is involved. A variety of *in vivo* mammalian experiments have demonstrated distinct Ah receptor-mediated biochemical and toxic effects. Among these are CYP1A1/1A2 induction and reproductive and teratogenic effects similar to TCDD.		
2,3,3′,4,4′-PentaCB (105)	The majority of potency values from subchronic bioassays support a value of less than 0.0002, including those for tumor promotion and thymus atrophy (*97,106*). The immunotoxicity data suggest a TEF between 10^{-5} and 10^{-7} while acute *in vivo* values for nonspecific end points, e.g., liver weight gain and body weight decrease, are in the range of 10^{-3} and 10^{-4}; no revision is recommended.	0.0001	0.0001
2,3,4,4′,5-PentaCB (114)	There are no new data which suggest that the current value is not appropriate. Older Ah receptor binding and enzyme induction data need confirmation regarding the present value (*100,101,107*); at present no revision is recommended.	0.0005	0.0005
2,3′,4,4′,5-PentaCB (118)	New data support the original value (*91,96,108*); no revision is recommended.	0.0001	0.0001
2′,3,4,4′,5-PentaCB (123)	No new data are available; no revision is recommended.	0.0001	0.0001
2,3,3′,4,4′,5-HexaCB (156)	Recent data from semichronic studies suggest that the old value is appropriate (*109*). Higher REPs measuring nonspecific end points, e.g., effects on thyroid hormones and body and thymus weight, were not included in the evaluation. Some concern was raised about identity and purity of test compounds in some studies; no revision is recommended.	0.0005	0.0005
2,3,3′,4,4′,5′-HexaCB (157)	No new relevant *in vivo* data available; no revision is recommended.	0.0005	0.0005
2,3′,4,4′,5,5′-HexaCB (167)	No new data are available; no revision is recommended.	0.00001	0.00001
2,3,3′,4,4′,5,5′-HeptaCB (189)	New data for immunotoxicity (*91*) are within the range of the current value; no revision is recommended.	0.0001	0.0001
Di-*ortho* PCBs	The limited data set as recognized in 1993 still exists. *In vivo* subchronic assay results with relevant end points, including CYP1A1/A2 induction and reproductive toxicity, with the structurally similar congener PCB 153 (*98,104,110–112*) do not confirm the weak Ah receptor agonist properties reported from *in vitro* experiments (*11,113,114*); as a result, the previous TEF values for di-*ortho* PCBs have been withdrawn.		
2,2′,3,3′4,4′,5 HeptaCB (170)	Withdrawn.	0.0001	–
2,2′,3,4,4′,5,5′-OCB (180)	Withdrawn.	0.00001	–

Abbreviations: WHO, World Health Organization; CDD, chlorinated dibenzodioxin; CDF, chlorinated dibenzofurans; CB, chlorinated biphenyls, LD_{50}, median lethal dose; REPs, relative potencies; QSAR, quantitative structure–activity relationship; EROD, ethoxyresorufin-*O*-deethylase; Ah, aryl hydrocarbon; PCBs, polychlorinated biphenyls.

[a]International Union of Pure and Applied Chemistry numbers are shown in parentheses.

end points (*70–72*). Birds, in contrast with fish, show a more pronounced response to mono-*ortho* PCBs, and REPs resemble those found in mammalian systems. In addition, studies with cultured chicken embyo hepatocytes indicate that some di-*ortho* PCBs, e.g., PCBs 128, 138, 170, and 180, are also EROD inducers (*69,73,74*). However, *in ovo* studies with either biochemical or toxic end points have not been done with these di-*ortho* PCBs, which could confirm an Ah receptor-mediated response by these congeners.

When REPs for CYP1A1 induction and embryo mortality in avian systems are compared, it can be concluded that no significant

Table 2. Toxic equivalency factors (TEFs) for fish

Congener	Description	WHO TEF
Dioxins		
1,2,3,7,8-PentaCDD	In a rainbow trout egg injection study, this compound was found to be 0.7 times as potent as 2,3,7,8-TCDD at causing early life stage mortality (*40*); a value of 1.0 is recommended.	1
1,2,3,4,7,8-HexaCDD	In a rainbow trout egg injection study, this compound was found to be 0.3 times as potent as 2,3,7,8-TCDD at causing early life stage mortality (*40*); a value of 0.5 is recommended.	0.5
1,2,3,6,7,8-HexaCDD	In a rainbow trout egg injection study, this compound was found to be 0.02 times as potent as 2,3,7,8-TCDD at causing early life stage mortality (*42*); a value of 0.01 is recommended.	0.01
1,2,3,7,8,9-HexaCDD	No *in vivo* studies have been carried out with this compound. It was determined to be 0.02 times as potent as 2,3,7,8-TCDD at inducing EROD in PLHC-1 cells (*115*); a value of 0.01 is recommended.	0.01
1,2,3,4,6,7,8-HeptaCDD	In a rainbow trout egg injection study, this compound was found to be 0.002 times as potent as 2,3,7,8-TCDD at causing early life stage mortality (*42*); a value of 0.001 is recommended.	0.001
OctaCDD	A two week study in carp using a single ip dose of this compound failed to detect any dose or hepatic concentration relationship with CYP1A induction (*53*). Based on structure homology with OCDF, a value <0.0001 is recommended.[a]	<0.0001
Furans		
2,3,7,8-TetraCDF	In a rainbow trout egg injection study, this compound was found to be 0.03 times as potent as 2,3,7,8-TCDD at causing early life stage mortality (*40*); a value of 0.05 is recommended.	0.05
1,2,3,7,8-PentaCDF	In a rainbow trout egg injection study, this compound was found to be 0.03 times as potent as 2,3,7,8-TCDD at causing early life stage mortality revision (*40*); a value of 0.05 is recommended.	0.05
2,3,4,7,8-PentaCDF	In a rainbow trout egg injection study this compound was found to be 0.3 times as potent as 2,3,7,8-TCDD at causing early life stage mortality (*40*); a value of 0.5 is recommended.	0.5
1,2,3,4,7,8-HexaCDF	In a rainbow trout egg injection study, this compound was found to be 0.2 times as potent as 2,3,7,8-TCDD at causing early life stage mortality (*40*); a value of 0.1 is recommended.	0.1
1,2,3,6,7,8-HexaCDF	At present, available data are only from QSAR studies (*93,116*). Also based on structural similarity between the hexaCDF isomers generally observed in experiments, a value of 0.1 is recommended.	0.1
1,2,3,7,8,9-HexaCDF	Data from EROD induction in PLHC-1 cells and QSAR studies indicate an REP between 0.04 and 0.09 (*116*); a value of 0.1 is recommended.	0.1
2,3,4,6,7,8-HexaCDF	Based on the structural similarity of this compound with other hexaCDFs and supported by QSAR modeling (*93,116*); a value of 0.1 is recommended.	0.1
1,2,3,4,6,7,8-HepaCDF	At present, no *in vivo* or *in vitro* data are available from fish studies, but based on structure homology with 1,2,3,4,7,8,9-heptaCDF, a value of 0.01 is recommended.	0.01
1,2,3,4,7,8,9-HeptaCDF	Data from EROD induction in PLHC-1 cells indicate an REP of 0.01 (*116*); a value of 0.01 is recommended.	0.01
OctaCDF	A two week study in carp using a single ip dose of this compound failed to detect any dose or hepatic concentration relationship with CYP1A induction (*53*). In the PLHC-1 cell line, this compound was 0.0008 times as potent as 2,3,7,8-TCDD at inducing EROD activity (*115*). However, both studies do not accurately reflect the environmental *in vivo* situation in which extreme low solubility and large molecular size limiting transport over the gill membranes (*117*) should reduce the octaCDF potency for fish significantly. A value <0.0001 is recommended.[a]	<0.0001
Non-*ortho* PCBs		
3,4,4′,5-TetraCB (81)[b]	In a rainbow trout egg injection study, this compound was found to be 0.0006 times as potent as 2,3,7,8-TCDD at causing early life stage mortality (*42*); a value of 0.0005 is recommended.	0.0005
3,3′,4,4′-TetraCB (77)	In a rainbow trout egg injection study, this compound was found to be 0.0002 times as potent as 2,3,7,8-TCDD at causing early life stage mortality (*40*); a value of 0.0001 is recommended.	0.0001
3,3′,4,4′,5-PentaCB (126)	In a rainbow trout egg injection study this compound was found to be 0.005 times as potent as 2,3,7,8-TCDD at causing early life stage mortality (*40*); a value of 0.005 is recommended.	0.005
3,3′,4,4′,5,5′-HexaCB (169)	In a rainbow trout egg injection study, this compound was found to be 0.00004 times as potent as 2,3,7,8-TCDD at causing early life stage mortality (*42*); a value of 0.00005 is recommended.	0.00005
Mono-*ortho* PCBs		
2,3,3′,4,4′-PentaCB (105)	In a rainbow trout egg injection study, this compound did not cause early life stage mortality at the highest dose administered (*40*); a value of <0.000005 is recommended.	<0.000005
2,3,4,4′,5-PentaCB (114)	A value of <0.000005 was assigned based on the similarity of the structure of this compound with other mono-*ortho* pentaCBs.	<0.000005
2,3′,4,4′,5-PentaCB (118)	In a rainbow trout egg injection study, this compound did not cause early life stage mortality at the highest dose administered (*40*); a value of <0.000005 is recommended.	<0.000005
2′,3,4,4′,5-PentaCB (123)	A value of <0.000005 was assigned based on structural similarity of this compound with other mono-*ortho* pentaCBs.	<0.000005
2,3,3′,4,4′,5-HexaCB (156)	An *in vitro* study with the rainbow trout RTG-2 cell line supports the earlier assigned value of <0.000005 for the other mono-*ortho* PCBs (*47*). In another *in vitro* study with the rainbow trout cell line RTL-2, this compound was 0.00003 times as potent as TCDD in inducing EROD activity (*45*); a value of <0.000005 is recommended.	<0.000005
2,3,3′,4,4′,5′-HexaCB (157)	A value of <0.000005 was assigned based on structural similarity of this compound with other mono-*ortho* pentaCBs; this value is also supported by QSAR modeling (*118*).	<0.000005
2,3′,4,4′,5,5′-HexaCB (167)	A value of <0.000005 was assigned based on structural similarity of this compound with other mono-*ortho* pentaCBs.	<0.000005
2,3,3′,4,4′,5,5′-HeptaCB (189)	A value of <0.000005 was assigned based on structural similarity of this compound with other mono-*ortho* pentaCBs.	<0.000005

Abbreviations: WHO, World Health Organization; CDD, chlorinated dibenzodioxins; ip, intraperitoneal; EROD, ethoxyresorufin-*O*-deethylase; CDF, chlorinated dibenzofurans; QSAR, quantitative structure–activity relationship; REP, relative potency; CB, chlorinated biphenyl.

[a]After a postmeeting consultation in February 1998, we agreed upon a revision of the TEF values for octaCDD and octaCDF in fish and birds, which were presented at the 17th International Symposium on Chlorinated Dioxins and Related Compounds on 25–29 August 1997 in Indianapolis, Indiana.

[b]International Union of Pure and Applied Chemistry numbers are shown in parentheses.

differences have been found between these values. Thus, the relative potencies of these compounds, as determined by CYP1A1 induction in cultured avian hepatocytes, appear to be predictive for the relative toxicity in the developing embryo (66).

However, some toxic end points, such as porphyrin accumulation in cultured avian hepatocytes, should be approached with caution because structure–activity relationships differ significantly from those of CYP1A induction (*74–76*). Based on this information, it is unclear if porphyrin accumulation in birds is uniquely an Ah receptor-mediated effect. Therefore, it is unclear whether it should be included in the TEF concept. This case again stresses the importance to include only those end points in which the Ah receptor-mediated mechanism is shown to be involved and is the major determining factor.

Review of TEFs

Approach for Deriving TEFs

For reevaluation of the existing mammalian TEFs for PCDDs, PCDFs, and PCBs, both previously reviewed and new data were examined. These included all congeners that have been selected before and also published data of multiple *ortho*-substituted PCBs (*1,11,77,78*). For prioritization, a rank order was followed as was suggested at an earlier WHO/IPCS TEF meeting (*1*). The TEFs for humans and mammals were primarily derived from *in vivo* toxicity data, which were given more weight than *in vitro* and/or quantitative structure–activity relationship (QSAR) data. *In vivo* toxicity data were prioritized according to the following ranking scheme: chronic > subchronic > subacute > acute. In the final TEF selection, different Ah receptor-specific end points were also ranked according to toxic > biochemical (e.g., enzyme induction) response. For revision of the existing mammalian TEFs, we agreed that if the available information was considered insufficient to warrant a change, the existing value would remain. The suggested TEFs for humans and mammals, including a short description, are given in Table 1.

A tiered approach was followed in deriving TEFs for fish and birds. Following this approach, we noted that the number of bird and fish studies, which could be used for the derivation of a TEF, was often very limited when compared with mammalian data. For all congeners, the studies that were given the most weight were tier 1 studies, followed by tiers 2, 3, and 4. The tiers were as follows:

- Tier 1: Overt toxicity observed in developing embryos; the only end point used was the LD_{50}
- Tier 2: Biochemical effects observed in developing embryos; the only end point used was the relative potency to induce CYP1A
- Tier 3: Biochemical effects observed in *in vitro* systems; the only endpoint used was the REP to induce CYP1A in cultured cells
- Tier 4: Estimates from QSAR studies.

Fish studies that determined early life stage mortality were considered to be most useful for determining TEF values (*40,42*). In addition, there was a preference to use results from egg injection studies in which the dose to the egg was known, rather than results from waterborne studies in which this was not determined. Furthermore, TEF values for octaCDD, octaCDF, and the mono-*ortho* PCBs for fish were given a "lower than" value. This approach was chosen because fish appear to be very insensitive, if sensitive at all, toward an Ah receptor-mediated response of octaCDD, octaCDF, or these types of PCBs. Nevertheless, we recognized that some regulatory agencies would like to have some directions for possible TEF values of these compounds in fish. Therefore, based on the present state of the science, a "smaller than" TEF value was given for these compounds, which should be considered to be the expected upper limit of such a compound in fish. The suggested TEFs for fish, including a short description, are given in Table 2.

Few studies have been carried out to determine the overt toxic effects of PCBs in bird embryos after injection of compounds into eggs. Where data from tier 1 studies were available, they were used to derive TEFs, but TEF values of PCDDs and PCDFs for birds were mainly derived from tier 2, 3, or 4 studies. These data include mainly studies that measured EROD induction in cultured hepatocytes or derived QSAR. The suggested avian TEFs, including a short motivation, are given in Table 3.

In line with the already existing TEF values, new TEFs were rounded to a value of either 1 or 5, irrespective of the order of magnitude difference with the reference compound, TCDD.

Discussion

Molecular Basis for TEFs across Species

A strict criterion for application of the TEF concept is that a compound must be demonstrated to bind to the Ah receptor since most (if not all) biological effects of these compounds appear to be mediated by the Ah receptor (*128,129*). Studies of biological effects of 2,3,7,8-TCDD and related polyhalogenated polyaromatic hydrocarbons have made it apparent that there are extensive and important species differences in the functional responses elicited by these compounds (*130*). However, for a number of common biological effects, many species can respond at dose levels that are within one order of magnitude (*131*).

With regard to the ligand binding properties of the Ah receptor, most data on receptor interaction with PCDDs, PCDFs, and PCBs have been generated using rodent experimental model systems. In addition, there are data on the ligand binding specificity of the human Ah receptor (*129,132–134*). The mouse has been studied in most detail with regard to determinants in Ah receptor structure of ligand binding activity. Four differently sized allelic variants of the receptor have been described, which show extended C-terminal parts as well as point mutations that affect binding of 2,3,7,8-TCDD and other polyhalogenated aromatic hydrocarbons (*135,136*).

With regard to humans, the Ah receptor is detected in a wide variety of tissues (e.g., placenta, liver, lung) and primary/established human-derived cell lines. Moreover, the Ah receptor can be activated both *in vivo* and *in vitro* into a DNA binding state by a variety of halogenated aromatic hydrocarbons (*134,137,138*). As in mice, a similar situation with several different allelic variants of the receptor might be present in humans. Although the mean 2,3,7,8-TCDD binding affinity (K_d) for the human Ah receptor may be lower than that observed in responsive mouse strains (C57BL/6J), there exisits a range of K_d values similar in magnitude to the range observed between responsive and nonresponsive mouse strains (*135*).

The extension of the TEF concept to other classes of vertebrates such as fish and birds is supported by data on ligand binding properties of the Ah receptor in these species. In this respect it should be noted that the Ah receptor has been detected in both bony and cartilagenous fish (*139–141*). Thus, it appears that the Ah receptor is phylogenetically very old, which is supported by the fact that development of these fish groups diverged from one another about 450–550 million years ago. Moreover, there exist homologs of both the receptor gene and the gene encoding the dimerization partner of the receptor, Arnt, in the nearly entirely sequenced genome of the nematode worm *Caenorhabditis elegans*. Studies using species-specific recombinant cells and primary cultures from birds also suggest that the ligand binding specificity of the Ah receptor may not be identical between species (*66,142*). Furthermore, one study determined the specific binding characteristics of TCDD to the Ah receptor in four different avian species and found differences in binding affinity of over an order of magnitude (*67*). Consequently, some caution in

the absolute homology of ligand binding properties of the Ah receptor between species may be justified.

In conclusion, the Ah receptor and Arnt have been very well conserved during evolution, indicating that they may have important physiological functions, possibly in development (*27*). This evolutionary conservation, in combination with the ligand binding properties of the Ah receptor, support the use of the TEF concept across taxa.

The Role of Pharmacokinetics and Food Chain Transport in the TEF Concept

The pharmacokinetic behavior of dioxinlike compounds and PCBs is largely governed by three major factors: 1) lipophilicity, 2) binding to CYP1A2 leading to hepatic sequestration, and 3) relative rates of metabolism. Lipophilicity controls the rate and extent of absorption, tissue distribution, and passive elimination. In addition, the chlorine substitution pattern determines hepatic sequestration and rate of metabolism. Pharmacokinetic properties have been shown to play a role in the determination of TEF values for a number

Table 3. Toxic equivalency factors (TEFs) for birds

Congener	Description	WHO TEF
Dioxins		
1,2,3,7,8-PentaCDD	In a chicken embryo egg injection study, this compound was found to be approximately equipotent to 2,3,7,8-TCDD as an inducer of EROD (*119*). In addition, a study with chick embryo hepatocytes from several bird species determined that the potency of this compound to induce EROD is similar or even higher than that of TCDD (*68,69*); a value of 1 is recommended.	1.0
1,2,3,4,7,8-HexaCDD	In a chicken embryo egg injection study this compound was found to be approximately 0.05 times less potent than 2,3,7,8-TCDD as an inducer of EROD (*119*); a value of 0.05 is recommended.	0.05
1,2,3,6,7,8-HexaCDD	In a chicken embryo egg injection study, this compound was found to be approximately 0.01 times less potent than 2,3,7,8-TCDD as an inducer of EROD (*119*); a value of 0.01 is recommended.	0.01
1,2,3,7,8,9-HexaCDD	In two chicken embryo egg injection studies, this compound was found to be approximately 0.1–0.4 times less potent than 2,3,7,8-TCDD as an inducer of EROD (*119,120*); a value of 0.1 is recommended.	0.1
1,2,3,4,6,7,8-HeptaCDD	In a chicken embryo egg injection study, this compound was found to be a poor inducer of EROD (*119*); a lower than value of 0.001 is recommended.	<0.001
OctaCDD	At present, no *in vivo* or *in vitro* data are available for this compound in birds; a value of 0.0001 was recommended based on data from mammalian studies.[a]	0.0001
Furans		
2,3,7,8-TetraCDF	Chicken embryo egg injection studies and one study with chicken embryo hepatocytes of several species of birds found this compound to be approximately equipotent or even more potent than 2,3,7,8-TCDD as an inducer of EROD (*66,119,121*); a value of 1 is recommended.	1.0
1,2,3,7,8-PentaCDF	In a chicken embryo egg injection study, this compound was found to be approximately 0.3 times less potent than 2,3,7,8-TCDD as an inducer of EROD (*119*); a value of 0.1 is recommended.	0.1
2,3,4,7,8-PentaCDF	Studies with chicken embryo egg injections and chick embryo hepatocytes from several bird species indicated that this compound is approximately equipotent to 2,3,7,8-TCDD as an inducer of EROD (*68,119*); a value of 1.0 is recommended.	1.0
1,2,3,4,7,8-HexaCDF	This compound was reported to be 0.01 times as potent as 2,3,7,8-TCDD as an inducer of EROD in chicken embryos after egg injection (*119*). However, examination of the dose–response curves indicates problems with rigorous comparison to that of 2,3,7,8-TCDD. Based on QSAR calculations with other existing bird data (M. Tysklind and P.L. Anderson, University of Umea, Sweden), a value of 0.1 is recommended.	0.1
1,2,3,6,7,8-HexaCDF	In a chicken embryo egg injection study, this compound was found to be approximately 0.4 times less potent than 2,3,7,8-TCDD as an inducer of EROD (*119*). In combination with QSAR calculations with other existing bird data (M. Tysklind and P.L. Anderson, University of Umea, Sweden), a value of 0.1 is recommended.	0.1
1,2,3,7,8,9-HexaCDF	No *in vivo* or *in vitro* data are available. Based on QSAR calculations with other existing bird data (M. Tysklind and P.L. Anderson, University of Umea, Sweden), a value of 0.1 is recommended.	0.1
2,3,4,6,7,8-HexaCDF	No *in vivo* or *in vitro* data are available. Based on QSAR calculations with other existing bird data (M. Tysklind and P.L. Anderson, University of Umea, Sweden); a value of 0.1 is recommended.	0.1
1,2,3,4,6,7,8-HeptaCDF	At present no data are available for this compound in birds; a value of 0.01 is recommended based on similar homolog class REPs across taxa for the hexaCDFs and heptaCDFs.	0.01
1,2,3,4,7,8,9-HeptaCDF	At present, no data are available for this compound in birds; a value of 0.01 is recommended based on similar homolog class REPs across taxa for the hexaCDFs and heptaCDFs.	0.01
OctaCDF	At present, no *in vivo* or *in vitro* data are available for this compound in birds; a value of 0.0001 was recommended based on data from mammalian studies.[a]	0.0001
Non-*ortho* PCBs		
3,3′,4,4′-TetraCB (77)	Studies with chickens and wild avian species reported *in vitro* and *in ovo* effects, with REPs ranging from <0.0003 to 0.15 depending on the species (*66,68,69,122,123*). A value of 0.05 is recommended.	0.05
3,4,4′,5-TetraCB (81)	Egg injection studies have not been carried out with this compound. Two studies with chick embryo hepatocytes from several bird species indicate a wide range of REPs (0.001– 0.5) of this compound to induce EROD relative to TCDD (*66,68*). As for most species, REPs were close to a value of 0.1, which is the recommended value.	0.1
3,3′,4,4′,5-PentaCB (126)	A 24-hr study with chickens determined a REP of 0.07 (*124*); a value of 0.1 is recommended.	0.1
3,3′,4,4′,5,5′-HexaCB (169)	One study determined the LD_{50} in chicken embryos after injection of the compound into the egg, and a REP of 0.002 was reported (*125,126*). A value of 0.001 is recommended.	0.001

continued, next page

of dioxinlike chemicals. These factors can include alterations in absorption, tissue distribution, and metabolism between individual congeners.

The importance of pharmacokinetic factors has been illustrated with studies using octaCDD in rodents. This compound initiated dioxinlike responses following subchronic exposure to rats, with an estimated relative potency of 0.01 based on hepatic levels correlated with the induction of CYP1A1 (*87*). However, earlier acute toxicity studies had suggested that octaCDD was essentially nontoxic, with a TEF value less than 0.000001. These subchronic studies also showed that octaCDD is extremely poorly absorbed and absorption from the gastro-intestinal tract decreased with increasing dose (*143*). Consequently, very little is stored from a (single) high dose of octaCDD in tissues of animals. In contrast, repeated exposure to relatively low doses can lead to significant tissue accumulation and hence a biological response. Based on these findings, it can be discussed whether future TEF values should be based on intake or tissue level values to bridge differences between species.

Differences in tissue distribution can also significantly influence TEF values based on tissue concentrations. The liver/adipose tissue distribution can vary significantly between the species and dose levels used (*95*). A number of highly toxic congeners such as 2,3,4,7,8-pentaCDF, 2,3,7,8-TCDD, and PCB 126 bind very tightly to CYP1A2 and subsequently concentrate in the liver in many rodent species, even at very low dose levels (*88,131,144–148*). In this respect, it should be noted that the structure–activity relationship for binding to the Ah receptor and to CYP1A2 are not identical (*149*). As the level of CYP1A2 is increased, dioxinlike compounds redistribute from the adipose tissue back to the liver. Thus, depending on the species and dose level, the binding of an Ah receptor agonist to CYP1A2 can alter tissue distribution. As a result of this CYP1A2 binding, hepatic REPs for these compounds are lower than those based on intake because of disproportionately high liver concentrations. However, if other tissues are considered, e.g., skin and lung, the REP values would be higher because the tissue concentrations are lower than expected based on administered dose (*81*).

The distribution of PCBs in laboratory animals differs significantly from that of the 2,3,7,8-substituted PCDFs and PCDDs with respect to liver distribution. Highly biopersistent PCBs, e.g, PCB 153, are predominantly stored in adipose tissue and skin and not in the liver (*150*). Nevertheless, PCB congeners that are isostereomers of TCDD, such as PCB 126, attain a high liver concentration. With the addition of one *ortho* chlorine, the hepatic accumulation decreases dramatically, as was shown in comparative semichronic studies with rats and PCBs 126 and 156 (*103,109*). These differences again appear to be related to induction of and binding to CYP1A2. It has also been shown that coadministration of PCDDs, PCDFs, or dioxinlike PCBs with the nondioxinlike congener PCB 153 can result in modulation of the hepatic disposition and elimination (*151–153*). These toxicokinetic interactions are quantitatively limited, compound and dose dependent, and probably the result of multiple mechanisms involving, among others, *de novo* synthesis of both the Ah receptor and CYP1A2 (*151*). To some extent, these toxicokinetic interactions could explain the nonadditive effects that were observed when combinations of dioxins and PCB 153 were used in rodent studies (*95*). To what extent these toxicokinetic interactions are also relevant at low-level environmental exposure is still unknown.

The position and degree of halogenation determines the rate and extent of metabolism, which is the key determinant of excretion or bioaccumulation of these compounds (*95,154*). Full lateral halogenation at the 2,3,7,8-positions produces PCDDs and PCDFs that lack two adjacent unsubstituted carbon atoms. These congeners tend to be very resistant to metabolism, as these positions are also preferentially oxidized by the cytochrome P450 system, most likely by the CYP1A enzymes. Because of the stress on the furan ring, PCDFs are more susceptible to biochemical degradation than PCDDs. In addition, the positions adjacent to the oxygen bridge in the PCDF molecule (positions 4 and 6) are more sensitive to metabolic attack than those in the PCDD molecule (*95*).

For PCBs the presence of two adjacent unsubstituted carbon atoms also facilitates the metabolic conversion to more polar metabolites. As with PCDFs and PCDDs, the cytochrome P450 1A enzyme(s) seems to play an important role in metabolism of

Table 3. (*continued*)

Congener	Description	WHO TEF
Mono-*ortho* PCBs		
2,3,3′,4,4′-PentaCB (105)	One study determined the LD_{50} in chicken embryos after injection of the compound into the egg, and a REP value of 0.00014 was reported (*125,126*). A value of 0.0001 is recommended.	0.0001
2,3,4,4′,5-PentaCB (114)	At present, available data are only from QSAR studies (*74,102,127*); based on these calculations, a value of 0.0001 is recommended.	0.0001
2,3′,4,4′,5-PentaCB (118)	One study was carried out to determine the LD_{50} in chicken embryos after injection of the compound into the egg; at the highest doses, no lethality was observed (*126*). This compound is also a poor inducer of EROD in avian hepatocyte cultures (*66*); a value of 0.00001 is recommended.	0.00001
2′,3,4,4′,5-PentaCB (123)	At present, available data are only from QSAR studies (*74,102*); based on these calculations, a value of 0.00001 is recommended.	0.00001
2,3,3′,4,4′,5-HexaCB (156)	One study determined the LD_{50} in chicken embryos after injection of the compound into the egg and reported a REP of 0.0002 (*126*). A value of 0.0001 is recommended.	0.0001
2,3,3′,4,4′,5′-HexaCB (157)	One study determined the LD_{50} in chicken embryos after injection of the compound into the egg, and reported a REP of 0.0001 (*126*). A value of 0.0001 is recommended.	0.0001
2,3′,4,4′,5,5′-HexaCB (167)	One study determined the LD_{50} in chicken embryos after egg injection and reported a REP <0.00008 (*126*). A value of 0.00001 is recommended.	0.00001
2,3,3′,4,4′,5,5′-HeptaCB (189)	No *in vivo* or *in vitro* data are available. Based on QSAR calculations with other existing bird data (M. Tysklind and P.L. Anderson, University of Umea, Sweden), a value of 0.00001 is recommended.	0.00001

Abbreviations: WHO, World Health Organization; CDD, chlorinated dibenzodioxins; EROD, ethoxyresorufin-*O*-deethylse; CDF, chlorinated dibenzofurans; QSAR, quantitative structure–activity relationship; REPs, relative potencies; CB, chlorinated biphenyl, LD_{50}, median lethal dose.

[a]After a postmeeting consultation in February 1998, we agreed upon a revision of the TEF values for octaCDD and octaCDF in fish and birds, which were presented at the 17th International Symposium on Chlorinated Dioxins and Related Compounds on 25–29 August 1997 in Indianapolis, Indiana.

those PCB congeners that are more or less isosteric with 2,3,7,8-TCDD (*155*). This is based on a study with rats, marine mammals, and wild bird species in which it was observed that the metabolic degradation of PCB 77 correlated well with EROD activity. However, the same study indicated that for fish species this isoenzyme might be less effective in metabolizing these PCBs (*156*). In addition, several other P450 isoenzymes, e.g., P450 2B and 3A, might be involved in the metabolism of PCBs that have an *ortho*-substitution pattern. With an increasing *ortho* chlorine substitution pattern, the induction of P450 2B1 and 2B2 isoenzymes increases significantly (*155,157*). Studies with PCB patterns and enzyme activities in wild mammals indicate that, at least in seals and polar bears, the involvement of CYP2B isoenzymes in metabolism of PCBs cannot be excluded (*158,159*).

The role of metabolism can also be important when comparing acute and subchronic studies. In rodent studies using both 2,3,7,8-TCDF and TCDD, it was demonstrated that the REP of 2,3,7,8-TCDF for enzyme induction was almost equal to that of TCDD after acute exposure. However, when subchronic studies were done, 2,3,7,8-TCDF was much less potent than TCDD due to its rapid metabolism and lack of bioaccumulation *(88)*. A similar explanation was suggested when comparing the relative potencies for the induction of cleft palate in mice by 1,2,3,7,8-pentaCDF versus 2,3,4,7,8-pentaCDF (*160*). When comparing the brominated dioxins and dibenzofurans with the chorinated ones, it appears that at least some brominated compounds can be more resistant to metabolism than their corresponding chlorinated analogs (*12*). Species differences in metabolism might also be one of the causes for the observed differential TEFs between fish, birds, and laboratory mammals. For example, some fish and birds may have limited ability to metabolize PCB 77 and 2,3,7,8-TCDF. However, both lay eggs and embryos have little depuration capability compared to adults. The respective avian and mammalian PCB 77 and 2,3,7,8-TCDF TEFs demonstrate more potency *in ovo* than *in vivo*. The PCB 77 and 2,3,7,8-TCDF TEFs are higher in bird eggs/embryos without a postconception maternal influence than in mammalian embryos with maternal metabolic protection. Conversely, a similar fish and mammalian comparison shows almost identical fish egg/embryo and mammalian TEFs. The role of metabolism, subsequent elimination, and reproductive strategy differences, especially through maternal egg deposition for most nonmammalian vertebrate species, points to the need to focus future REP/TEF determinations on *in vivo* or *in ovo* studies with comparisons between target organ or tissue exposure measurements and toxicity end points. In addition, it points to the need to base future TEF values on *in vivo* studies in which steady-state conditions are obtained or at least have been approached. Utilizing this approach will greatly increase the value of laboratory-determined exposure measurements for data interpretation in similar environmental samples regardless of taxonomic group.

As illustrated above, many PCBs, PCDFs, and PCDDs are relatively resistant to metabolism and can therefore accumulate in different trophic levels, causing biomagnification and toxicity higher in the foodchain (*161*). For PCDDs and PCDFs, the structure–activity relationships for bioaccumulation are relatively simple and involve primarily the 2,3,7,8-substituted congeners for most vertebrate species. Only in some invertebrates and the guinea pig has the accumulation of non-2,3,7,8-substituted PCDDs and PCDFs been found to be significant, but the guinea pig has been found to have low metabolic capacities for these compounds (*95*). Thus, from a pharmacokinetic point of view, only the 2,3,7,8-substituted congeners should be considered when TEFs must be determined for ecotoxicological risk assessment of vertebrates.

For PCBs the situation is more complex, as structure–activity relationships are less well defined for metabolism and associated bioaccumulation. The degree of accumulation is strongly determined by the capability of the organism to metabolize the various PCB congeners (*22,162*). This can differ qualitatively as well as quantitatively between species. In general, it can be stated that fish are less capable of metabolizing PCBs than most birds and mammals. In spite of the complexity of the above processes, the lack of chlorine substitution on both the *meta* and *para* positions in the PCB molecule appears to facilitate the metabolism of these compounds in most higher vertebrate species (*21,156,158,163*). As a result of this "biofiltering" by metabolism at different levels in the foodchain, the qualitative PCB pattern in top predators shows some resemblance, which allows selection of PCB congeners of major concern for risk assessment (*164*).

Qualitative and quantitative changes occur in PCDD, PCDF, and PCB patterns among different trophic levels and between the abiotic and biotic components of different ecosystems. This is especially confounded when attempts are made to perform risk and exposure assessments from a contaminated soil or sediment. It is preferable and indeed desirable to model Ah receptor-active congeners from abiotic to biotic components of ecosystems because of differential partitioning across these matrices, which are influenced by physicochemical properties, physiological differences, food chain position, and different reproductive strategies.

Depending on the assessment and toxicity end points identified in a particular ecological risk assessment, application of the appropriate TEFs for fish, birds, or mammals, as well as resulting TEQs, should only be determined in biotic matrices after the quantitative estimates of congener concentrations are made in a particular target species or generic food chains. Conversely, TEQs in abiotic matrices could be misused and have obscure value. For example, calculating TEQs in sediment or soil does not harm or place any risk on these abiotic matrices, regardless of TEF scheme. The application of the TEF concept for foodchain transport out of the abiotic compartments is clearly an area for future research.

Application of *in Vitro* Assays in the Derivation of TEFs

A number of *in vitro* assays, either in primary cultures or immortalized cells, have been used to establish REPs for mammals, birds, and fish. These include assays for Ah receptor competitive ligand binding, CYP1A1 gene induction, cell differentiation, plaque-forming cell (PFC) response, and porphyrin accumulation.

The most extensively used biological *in vitro* response studied so far is the induction of the *CYP1A1* gene. *CYP1A1* gene induction has been measured by monitoring changes in mRNA (*165,166*), protein levels (*66,167*), and/or enzyme activity [i.e., EROD or aryl hydrocarbon hydroxylase (AHH)] (*168,169*).

In addition, permanently transfected cells have also been established that contain CYP1A-regulated reporter genes. These cell lines contain a permanently incorporated reporter gene that is regulated by the mouse CYP1A1 regulatory region (*55,142, 170,171*). These cell lines have a number of advantages over conventional enzyme induction assays such as 1) a sensitive and more easily measurable enzyme activity; 2) chemical inducers do not act as competitive inhibitors of the reporter gene; and 3) a larger number of available species- and tissue-specific assays.

Only two studies have compared endogenous EROD induction to reporter gene induction (*55,172*). The structure–activity relationship of the tested chemicals was comparable between wild-type and recombinant H4IIE cells. Dose–response relationships exhibited comparable dynamic ranges, and binary mixtures of TCDD, PCB 126, and PCB 77 did not depart from additivity in either cell line. In addition, PCB 153 significantly antagonized induction by

TCDD and PCB 126 in both cell lines. The recombinant cell line was found to be generally more sensitive to PCDDs, PCDFs, and PCBs when compared to the wild-type cells (*172*). Additionally, Richter et al. (*55*) recently reported that a recombinant trout cell line (RLT 2.0) could be used as an approximate predictor of responses in fish. However, the use of this assay was not advocated to develop REPs for ecological risk assessment since it was still plagued by the same drawbacks that limit the utility of other *in vitro* assays.

In vitro CYP1A induction has also been used to assess the level of dioxin equivalents (TEQs) in a number of environmental samples (*69,173–177*). In general, the TEF/TEQ approach using CYP1A induction accurately predicted dioxin equivalents within a complex mixture when compared to gas chromatography-mass spectrometry analysis or *in vivo* assessments, but may overestimate the TEQs when using other responses such as PFC (*91*). The accuracy of this *in vitro* approach also depends on the composition of the sample. The presence of high amounts of Ah receptor agonists that are not covered by the TEF concept (e.g, polycyclic aromatic hydrocarbons) can result in a considerable overestimation of TEQs in environmental samples. Furthermore, these *in vitro* studies have shown that some responses (i.e., PFC, reporter gene, and AHH/EROD induction) and cell lines (H4IIE, recombinant reporter cells) are susceptible to nonadditive interactions. This is especially true if the complex mixture contains PCB congeners that are partial Ah receptor agonists (*91,142,178,179*).

In summary, a single *in vitro* assay based on a single surrogate species may not accurately predict the toxicity of a chemical or complex mixture following exposure to other species. Nevertheless, the use of *in vitro* assays provides a general tool as a prescreening method of TEQs in environmental samples. However, it does not replace *in vivo* experiments when determining TEFs for dioxinlike compounds.

The Use of QSARs for Identification and Screening of Dioxinlike Compounds

PCDDs, PCDFs, and PCBs consist of a large number of possible congeners with varying degrees of chlorination and substitution patterns. Thus, from a practical point of view, it does not seem feasible to test extensive numbers of congeners for their biological or toxic properties. The determination of QSARs could facilitate future risk assessment procedures significantly. Based on a limited number of compounds, structure–activity relationships have been developed for Ah receptor-mediated effects, e.g., induction of CYP1A1 and binding to this protein (*11,129,180*). In addition, structure–activity relationships have also been determined for effects that are not directly related to the Ah receptor and involve both parent compounds and their hydroxylated metabolites. These non-Ah receptor-mediated structure–activity relationships include binding to transthyretin and thyroxin-binding globulin (*6,181,182*), binding to the estrogen receptor (*183,184*), and decrease in dopamine levels (*2,3*). Based on these studies it can be concluded that the structure–activity relationships of the latter effects deviate significantly from those observed for the Ah receptor-mediated effects. As only Ah receptor-mediated effects are considered in the present TEF concept, these structure–activity relationships will not further be considered in this evaluation (see criteria above).

Most biological systems are complex, and it is unlikely that only one or a few chemical properties will suffice to describe them. Thus, it is necessary to characterize these compounds with a multitude of physicochemical descriptors. Such a broad chemical characterization may capture the underlying, hidden factors that correlate with the response of interest. This information can then be used as the future base for the selection of congeners for biological testing (*185*).

Within the TEF concept, the critical question is how to identify the physicochemical properties that determine if a compound can be expected to be dioxinlike or not. The planar structure of the PCDDs and the PCDFs is characteristic for high Ah receptor binding affinity. Furthermore, the 2,3,7,8-chlorine substitution pattern is of great importance as well. PCBs show a larger chemical variation due to the possible rotation of the two phenyl rings. The number of chlorine atoms in the *ortho*-position will, in this case, define the degree of dioxinlike properties. However, from a chemical point of view, it is difficult to define the point at which the compounds lose their dioxinlike properties. As a consequence of this, no single PCB congener can be identified as being structurally representative for the whole class of compounds. Hence, a number of congeners must be investigated in order to include the many facets of chemical structure within the PCB class of chemicals.

The use of multivariate chemical characterization (*118*) in combination with factorial design provides a tool by which small sets of structurally representative congeners can be selected. This tool can be used in the design of *in vitro* and *in vivo* experiments in order to introduce systematic structural variation in the congeners to be tested. In studies of complex environmental mixtures, indicator congeners can be selected from the structural variation found in such matrices as flue gases from incineration plants or Aroclor mixtures. The use of these systematic and balanced sets of congeners in the experimental protocol will provide increased knowledge of the biological behavior of the compounds studied.

Small sets of congeners, representative for the PCDDs, PCDFs, and PCBs, have been suggested (*186,187*). Twenty congeners were selected based only on their physicochemical properties and not on expected biological activity. As a first screening of possible dioxinlike effects, PCDFs and PCBs were tested in different *in vitro* systems using primary hepatocytes from different species as well as rat and fish hepatoma cell lines. Based on these results, QSARs have been established to define the correlation between chemical properties and a dioxinlike biochemical response. As can be seen in Table 4, these studies predict that a large number of PCBs, and also PCDFs, exhibit dioxinlike activity measured as *in vitro* induction of CYP1A(1) activity.

The systematic selection approach makes it possible to make interpolations and predictions of not yet tested congeners. In this way, all congeners within the chemical range in which the selection of test congeners were made can be ranked according to expected dioxinlike activity and the congeners of special interest can be identified and investigated further. This method has so far mainly focused on CYP1A1 induction, but could also be used in the screening of other dioxin-linked effects or other groups of halogenated aromatics, e.g., polychlorinated naphthalenes and polybrominated diphenylethers.

Based on the information presented in Table 4 about the predicted dioxinlike activity of PCBs, it should be noted that experimental data were used from *in vitro* experiments. Therefore, the structure–activity relationships determined with this multivariate characterization await further confirmation from *in vivo* experiments, including the role of pharmacokinetics, before these dioxinlike PCBs can be included in the TEF concept. Nevertheless these QSAR data indicate that more PCBs might be considered in the future for inclusion in the TEF concept. In relation to the large number of PCDFs that are predicted to induce CYP1A 1 *in vitro* activity, it should be noted that in *in vivo* situations only the 2,3,7,8-substituted PCDFs exhibit a significant tissue retention. In this case, a pharmacokinetic factor (metabolism) seems to dominate the toxicodynamic aspects of these non-2,3,7,8-PCDFs (*in vitro* Ah receptor binding and CYP1A1 induction).

Uncertainties Associated with the TEF Concept

Toxic equivalency factors were initially developed for calculating the TEQs in mixtures of PCDDs and PCDFs. The TEF

approach for hazard assessment of reconstituted PCDD/PCDF mixtures has been validated using standardized TEF or response-specific TEF values. In a limited number of validation studies using mixtures, a good correlation was found between the observed *in vivo* or *in vitro* response and TEQ values calculated from the relative concentrations of individual congeners in the mixture (*84,188–190*). The non-*ortho* and mono-*ortho* PCBs also elicit Ah receptor-mediated responses. As a consequence, TEFs have been assigned to these PCBs (*1,11,77*). From a risk assessment point of view this was a logical decision, as most environmental matrices contain PCDDs, PCDFs, and PCBs. In fact, in some environmental samples, the overall contributions of PCBs to TEQs exceed that of the PCDDs and PCDFs (*191*). When concentrations of TEQs in complex mixtures from environmental matrices were determined *in vitro* and compared to TEQs by using REPs from the same *in vitro* system, the results were generally within a factor of 2 (*192*).

However, the inclusion of PCBs in the TEF concept also poses a problem due to the nonadditive effects, which have been observed in laboratory studies with mixtures containing PCDDs, PCDFs, and PCBs. Ah receptor antagonist activities of certain PCB congeners, including the major environmental contaminant PCB 153, have been reported in several experimental systems. Recently, these nonadditive interactions of PCB on dioxinlike effects have been reviewed (*193*). In summary, the following antagonistic effects of nondioxinlike PCBs were described: induction of EROD activity in chick embryo hepatocytes (*194*), splenic PFC response to sheep erythrocytes in mice (*195*), splenic PFC response to trinitrophenyl-lipopolysaccharide in mice (*196*), serum IgM units in mice (*91*), mouse fetal cleft palate (*110,195*), and chick embryo malformations and edema and liver lesions (*197*). The apparent antagonism by PCB 153 of the TCDD-induced immunosupression is due to the enhanced immune response induced by PCB 153 (*198*). In addition, synergistic interactions have also been reported between PCBs and dioxins in the development of porphyria in rats (*199*) and mice (*89*), the induction of CYP1A1 (*152,178*) and thyroid hormone levels and associated enzyme activities (*200,201*). These multiple nonadditive interactions between dioxinlike and nondioxinlike HAHs require further investigation to establish the extent to which they compromise the TEF concept.

Several reports have also questioned the relative contributions of TEQs associated with dioxinlike compounds versus the substantial daily intakes of natural nonchlorinated Ah receptor agonists in cooked foods and vegetables (*193,202,203*). The Ah receptor agonist and antagonist activities of indole-3-carbinol have been reported (*204–206*). Perinatal exposure of pregnant rats to indole-3-carbinol resulted in reproductive abnormalities in male rat offspring, which were also elicited by TCDD in the same study. However, when comparing the effects caused by indole-3-carbinol or TCDD, both similar and different responses were observed (*205*). In contrast, a recent study with TCDD or indole-3-carbizole in rats did not find characteristic TCDD-like responses, e.g., hypophagia, body weight loss, and CYP1A1 induction (*207*). These results suggest that at least in some animal and cell models, the potential effects of the natural Ah receptor agonists could be significant. However, it has been suggested that the difference in pharmacokinetics between natural and halogenated Ah receptor agonists may decrease the potential impact of the natural agonists in *in vivo* situations. These possible differences between halogenated and natural Ah receptor agonists, such as indole-3-carbinol, in pharmacokinetics and toxicodynamics should be examined in more detail in *in vivo* experiments.

In view of the nonadditive effects mentioned above, a question has been raised as to which effect would compromise the TEF concept more: antagonism or synergism. From the available experimental data, it appears that antagonism is the most commonly reported nonadditive effect between individual dioxinlike compounds and complex mixtures (*195,208–212*). However, it should be noted that the occurrence of either antagonism or synergism is ratio and dose dependent. With respect to nonadditive effects between TCDD and PCB 153 on CYP1A1 induction in rodents, it was observed that synergism prevailed at the lower dose levels, while antagonism dominated at higher dose levels (*95*). Mechanistically, this antagonism can be explained by the fact that less potent congeners still have Ah receptor binding affinities and therefore are effective competitors for binding the site (*195,209*). This reduces the probability of the more toxic dioxinlike compounds to bind to the Ah receptor. However, the less active congeners do not bind with such a high affinity that they would effectively induce EROD activity or cause other Ah receptor-mediated adverse effects (*11*). In this respect, some results of interactive studies with these compounds are equivocal. For instance, 3,3′,4,4′-tetraCB and TCDD caused greater than additive induction of AHH activity in the liver of rainbow trout at doses calculated to produce 50% or less of the maximum response. However, in rainbow trout at greater doses, the same mixture was found less than additive (*213*). In addition, PCB 153 had an antagonistic effect on the induction of EROD activity by TCDD (*214*), but was found to be synergistic in another study (*215*).

In conclusion, there has been much discussion about the possible interactions between and among individual congeners in complex technical mixtures and extracts of environmental matrices (*216*). Based on receptor theory and the proposed mechanism of action of Ah receptor-active compounds, an additive model for the prediction of TCDD TEQs still seems most plausible in spite of the also observed nonadditive interactions. It is unlikely that the use of additivity in the TEF concept will result in a great deal of error in predicting the concentrations of TEQs due to synergism or antagonism.

Table 4. Screening of compounds with a relative potency (REP) for PCDFs of at least 10^{-3} TCDD and for PCBs of 10^{-5} of PCB 126 in different *in vitro* systems

Compounds and assay	Number of dioxinlike congeners	Reference
PCDFs in H4IIE rat hepatoma bioassay	40 out of 87 tetra- through octaCDFs	(*93*)
PCDFs in PLHC-1 fish hepatoma bioassay	47 out of 87 tetra- through octaCDFs	(*127*)
PCBs in primary hepatocytes from chicken	56 out of 154 tetra- through heptaCBs	(*102*)
PCBs in primary hepatocytes from monkey	79 out of 154 tetra- through hepta CBs	(*102*)
PCBs in primary hepatocytes from pig	101 out of 154 tetra- through heptaCBs	(*102*)

Abbreviations: PCDFs, chlorinated dibenzofurans; PCBs, chlorinated biphenyls. Congeners tested and creating the quantitative structure–activity relationship models were selected based on multivariate chemical characterization in combination with experimental design.

Validation of the TEF Concept for Environmental Risk Assessments

A range of validation studies with fish and birds have examined the suitability of an additive model of toxicity for these compounds. These span from isobolographic studies of several pairs of Ah receptor agonists (and/or antagonists) to the testing of complex environmental mixtures found in the environment.

In rainbow trout and lake trout embryos, binary mixtures of Ah receptor agonists were tested following the isobolographic method (*14,50,217*). These interactions on embryo lethality between congener pairs were, in general, found to be additive. However, for the combinations of TCDD and some non-*ortho* PCBs, deviations from additivity that were dependent on the ratio of the congeners were also reported (*49,213,218*). With respect to these nonadditive interactions,

Table 5. World Health Organization toxic equivalency factors (TEFs) for humans, mammals, fish, and birds

Congener	TEF		
	Humans/mammals	Fish[a]	Birds[a]
2,3,7,8-TCDD	1	1	1
1,2,3,7,8-PentaCDD	1	1	1[b]
1,2,3,4,7,8-HexaCDD	0.1[a]	0.5	0.05[b]
1,2,3,6,7,8-HexaCDD	0.1[a]	0.01	0.01[b]
1,2,3,7,8,9-HexaCDD	0.1[a]	0.01[c]	0.1[b]
1,2,3,4,6,7,8-HeptaCDD	0.01	0.001	<0.001[b]
OctaCDD	0.0001[a]	<0.0001	0.0001
2,3,7,8-TetraCDF	0.1	0.05	1[b]
1,2,3,7,8-PentaCDF	0.05	0.05	0.1[b]
2,3,4,7,8-PentaCDF	0.5	0.5	1[b]
1,2,3,4,7,8-HexaCDF	0.1	0.1	0.1[b,d]
1,2,3,6,7,8-HexaCDF	0.1	0.1[d]	0.1[b,d]
1,2,3,7,8,9-HexaCDF	0.1[a]	0.1[c,d]	0.1[d]
2,3,4,6,7,8-HexaCDF	0.1[a]	0.1[d,e]	0.1[d]
1,2,3,4,6,7,8-HeptaCDF	0.01[a]	0.01[e]	0.01[e]
1,2,3,4,7,8,9-HeptaCDF	0.01[a]	0.01[c,e]	0.01[e]
OctaCDF	0.0001[a]	<0.0001[c,e]	0.0001[e]
3,4,4′,5-TetraCB (81)	0.0001[a,c,d,e]	0.0005	0.1[c]
3,3′,4,4′-TetraCB (77)	0.0001	0.0001	0.05
3,3′,4,4′,5-PentaCB (126)	0.1	0.005	0.1
3,3′,4,4′,5,5′-HexaCB (169)	0.01	0.00005	0.001
2,3,3′,4,4′-PentaCB (105)	0.0001	<0.000005	0.0001
2,3,4,4′,5-PentaCB (114)	0.0005[a,d,e,f]	<0.000005[e]	0.0001[g]
2,3′,4,4′,5-PentaCB (118)	0.0001	<0.000005	0.00001
2′,3,4,4′5-PentaCB (123)	0.0001[a,d,f]	<0.000005[e]	0.00001[g]
2,3,3′,4,4′,5-HexaCB (156)	0.0005[d,e]	<0.000005	0.0001
2,3,3′,4,4′,5′-HexaCB (157)	0.0005[d,e,f]	<0.000005[d,e]	0.0001
2,3′,4,4′,5,5′-HexaxCB (167)	0.00001[a,f]	<0.000005[e]	0.00001[g]
2,3,3′,4,4′,5,5′-HeptaCB (189)	0.0001[a,d]	<0.000005	0.00001[g]

Abbreviations: CDD, chlorinated dibenzodioxins; CDF, chlorinated denzofurans; CB, chlorinated biphenyls; QSAR, quantitative structure–activity relationship.

[a]Limited data set.

[b]*In vivo* CYP1A induction after *in ovo* exposure.

[c]*In vitro* CYP1A induction.

[d]QSAR modeling prediction from CYP1A induction (monkey, pig, chicken, or fish).

[e]Structural similarity.

[f]No new data from 1993 review (*1*).

[g]QSAR modeling prediction from class specific TEFs.

deviations from strict additivity were less than a factor of two in the LD_{50} values (*50,217*). In addition, brominated analogs of 2,3,7,8-TCDD and other dioxin, furan, and biphenyl congeners also showed additive interactions (*14*).

When testing synthetic or complex environmental mixtures of chemicals in fish, additivity also appears to be the general case (*219*). This was shown with a synthetic mixture of Ah agonists and non-Ah receptor compounds in rainbow trout early life stage mortality tests in which results simply followed an additive model (*40*). The additivity model has also been investigated in fish through the use of environmentally derived mixtures. One study used the organic extract made from lake trout, which was injected into eggs of hatchery-reared rainbow trout (*220*) and lake trout (*221*). Additive toxicity of PCDDs, PCDFs, and planar PCBs to developing trout embryos was also evaluated through the direct injection of environmentally derived mixtures into newly fertilized eggs (*220*). The good agreement between $TEQs_{egg}$ calculated with the trout early life stage mortality TEFs for concentrations of PCDDs, PCDFs, and PCBs measured in Lake Michigan lake trout and $TEQs_{egg}$ measured on the basis of toxicity of the lake trout extract to rainbow trout sac fry following egg injection again suggests that $TEQs_{egg}$ are strictly additive and TEFs for all significant Ah receptor agonists present were included (*220*).

The results from these studies showed again that the mixture of these compounds present in lake trout acted in an additive fashion when compared with experiments using single congeners (*40*). The additive model of toxicity is also supported by studies of embryotoxicity in birds. The toxicity of an environmentally derived mixture of chemicals, including dioxinlike chemicals, was tested in chicken and found to be additive (*222*).

Additionally, the TEF approach could also be validated by using TEF factors derived from experiments in chickens that were successfully used to predict the embryo lethality for double crested cormorant eggs (*175,223*). The TEF and TEQ approaches were also succesfully applied in a biological monitoring study with great blue herons and double crested cormorants (*224,225*). Thus, as with fish, the additivity in the TEF concept is also supported by studies with (wild) birds. Based on the use of more environmentally relevant species, it can be concluded that the type of interaction that is most prevalent among Ah receptor agonists and non-Ah receptor compounds is additivity. However, more studies to validate the additivity in fish and wildlife are required to better understand the limitations of the TEF and TEQ approachs. Yet, evidence from fish and bird studies indicates that the hazards of not using such an approach are greater than the uncertainties currently observed with the TEF/TEQ approaches.

Conclusions

Based on an extension of the existing database (*1*), TEFs for PCDDs, PCDFs, and PCBs were reevaluated and either revised (mammals) or established (fish and birds). A limited number of existing mammalian TEFs for HAHs were revised based on new scientific information or reevaluation of existing data. These HAHs included 1,2,3,7,8-pentaCDD, octaCDD, octaCDF, and PCB 77. In addition, we decided that there was insufficient *in vivo* evidence to support Ah receptor agonist activity and thus determine TEF values for some di-*ortho* PCBs. Therefore, we recommended the withdrawal of the TEF values for PCB 170 and 180 that were assigned earlier (see Table 1) (*1*).

The mammalian TEFs established by this WHO expert meeting and presented here are considered to be applicable for the human situation as well as for wild mammalian species. In addition, TEFs for fish and birds were determined, which could be used in ecotoxicological risk assessments of these vertebrate classes.

When deriving TEFs for humans/mammals, fish, and birds, we attempted to harmonize the TEFs across different taxa to the extent possible, as this would have a clear advantage from a risk assessment and management perspective. However, total synchronization of TEFs between mammals, birds, and fish was not feasible, as there were obvious indications of orders of a magnitude difference in TEFs between the taxa for some compounds. In this respect the absent or very low response of fish to mono-*ortho* PCBs compared to mammals and birds is most noticeable. It is also important to note that mammalian TEFs are based on intake (administered dose) while fish and bird TEFs are based on residue analysis (tissue concentration and administered dose in egg injection studies).

We also reviewed a number of uncertainties that could compromise the TEF concept when used for risk assessment purposes. These uncertainties include nonadditive interactions, differences in shape of the dose–response curve, and species responsiveness. This was based on the proposed Ah receptor mechanism of action for PCDDs, PCDFs, and dioxinlike PCBs, but was also based on a number of combination studies with mammals, birds, and fish that predicted the measured TEQ adequately according to the dose additive model.

Therefore, the prediction of TEQs according to the TEF model is considered to be plausible and to be the most feasible approach for risk assessment of HAHs with dioxinlike properties. In view of the available scientific evidence from studies with mixtures, it was concluded that it is unlikely for the use of this additive model to result in a great deal of error in predicting the concentrations of TCDD TEQs or responses at environmentally relevant levels due to nonadditive interactions.

A summary of the suggested WHO TEFs for PCDDs, PCDFs, and dioxinlike PCBs is shown in Table 5.

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