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REVIEW

The 2005 World Health Organization Reevaluation of Human and Mammalian Toxic Equivalency Factors for Dioxins and Dioxin-Like Compounds

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In June 2005, a World Health Organization (WHO)-International Programme on Chemical Safety expert meeting was held in Geneva during which the toxic equivalency factors (TEFs) for dioxin-like compounds, including some polychlorinated biphenyls (PCBs), were reevaluated. For this reevaluation process, the refined TEF database recently published by Haws *et al.* (2006, *Toxicol. Sci.* 89, 4–30) was used as a starting point. Decisions about a TEF value were made based on a combination of unweighted relative effect potency (REP) distributions from this database, expert judgment, and point estimates. Previous TEFs were assigned in increments of 0.01, 0.05, 0.1, etc., but for this reevaluation, it was decided to use half order of magnitude increments on a logarithmic scale of 0.03, 0.1, 0.3, etc. Changes were decided by the expert panel for 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) (TEF = 0.3), 1,2,3,7,8-pentachlorodibenzofuran (PeCDF) (TEF = 0.03), octachlorodibenzo-*p*-dioxin and octachlorodibenzofuran (TEFs = 0.0003), 3,4,4′,5-tetrachlorbiphenyl (PCB 81) (TEF = 0.0003), 3,3′,4,4′,5,5′-hexachlorobiphenyl (PCB 169) (TEF = 0.03), and a single TEF value (0.00003) for all relevant mono-*ortho*–substituted PCBs. Additivity, an important prerequisite of the TEF concept was again confirmed by results from recent *in vivo* mixture studies. Some experimental evidence shows that non-dioxin–like aryl hydrocarbon receptor agonists/antagonists are able to impact the overall toxic potency of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds, and this needs to be investigated further. Certain individual and groups of compounds were identified for possible future inclusion in the TEF concept, including 3,4,4′-TCB (PCB 37), polybrominated dibenzo-*p*-dioxins and dibenzofurans, mixed polyhalogenated dibenzo-*p*-dioxins and dibenzofurans, polyhalogenated naphthalenes, and polybrominated biphenyls. Concern was expressed about direct application of the TEF/total toxic equivalency (TEQ) approach to abiotic matrices, such as soil, sediment, etc., for direct application in human risk assessment. This is problematic as the present TEF scheme and TEQ methodology are primarily intended for estimating exposure and risks via oral ingestion (e.g., by dietary intake). A number of future approaches to determine alternative or additional TEFs were also identified. These included the use of a probabilistic methodology to determine TEFs that better describe the associated levels of uncertainty and "systemic" TEFs for blood and adipose tissue and TEQ for body burden.

Key Words: **dioxins; dibenzofurans; PCBs; TEFs; reevaluation; WHO.**

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Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs) are persistent organic pollutants that are omnipresent in the global environment. Many of these hydrophobic and lipophilic compounds are highly resistant to metabolism in vertebrate species, including humans. As a result of these properties, biomagnification occurs through the food chain, and high tissue concentrations can often occur in top predator species. Most, if not all, toxic and biological effects of these compounds are mediated through the aryl hydrocarbon receptor (AhR), a cytosolic receptor protein present in most vertebrate tissues with high affinity for 2,3,7,8–substituted PCDD/Fs and some non-*ortho*–substituted PCBs (Poland *et al.*, 1985; Safe, 1986; Safe *et al.*, 1985). Hundreds of congeners are formed during synthetic processes such as combustion and certain industrial activities (Hutzinger *et al.*, 1985). Thus, human exposure either through food or the environment results in the uptake of a large number of these compounds. As a result, humans retain dozens of PCB congeners in their tissues, blood, and milk (Liem *et al.*, 2000; Schecter *et al.*, 1994). Most PCDD and PCDF congeners with a 2,3,7,8-chlorine substitution pattern are also strongly retained (Van den Berg *et al.*, 1994). Thus, risk assessment of these compounds involves a complex mixture of PCDD, PCDF, and PCB compounds that are AhR agonists sharing a common mechanism of action and should not be done for only one specific congener.

During the last few decades, data from many experimental studies with mixtures of these compounds are consistent with an additive model, although deviations up to a factor of two, and sometimes more, from additivity are not uncommon (Barnes, 1991; Barnes *et al.*, 1991; Birnbaum and DeVito, 1995; Safe, 1986, 1997, 1998; Safe *et al.*, 1985; Van den Berg *et al.*, 1998; Zabel *et al.*, 1995). As a result of this generally accepted additivity, the toxic equivalency concept was developed during the mid 1980's (Barnes, 1991; Barnes *et al.*, 1991; Safe, 1986; Safe *et al.*, 1985). It uses the relative effect potency (REP) determined for individual PCDD, PCDF, and PCB compounds for producing toxic or biological effects relative to a reference compound, usually 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). The total toxic equivalent (TEQ) is operationally defined by the sum of the products of the concentration of each compound multiplied by its TEF value and is an estimate of the total 2,3,7,8-TCDD–like activity of the mixture.

Since the early 1990's, the World Health Organization (WHO) has organized expert meetings with the objective to harmonize the toxic equivalency factors (TEFs) for dioxin and dioxin-like compounds on the international level, thereby giving recommendations to national regulatory authorities. Prior to 2005, two WHO (re)evaluations of the TEFs were conducted. In 1993, the first evaluation was done that resulted in human and mammalian WHO TEFs for all 2,3,7,8-PCDDs and PCDFs but also a recommended TEF value for several PCBs (Ahlborg *et al.*, 1994). A WHO TEF (re)evaluation was again done in 1997, which led to the revision of several mammalian TEF values of important congeners and withdrawal of the di-*ortho* PCBs from the TEF concept for dioxin-like compounds. In addition, the first WHO TEF values for birds and fish were proposed during this meeting (Van den Berg *et al.*, 1998). To support this meeting, the Karolinska Institutet in Stockholm (Sweden) prepared a database with results from all studies for which REP values were known at that time, and they were used to determine the WHO 1998 TEF values. This REP database was recently used as a starting point to compile a much more extensive database for REP values (Haws *et al.*, 2006). In June 2005, a third WHO expert meeting to reevaluate current mammalian TEF values was held in Geneva, Switzerland. Preceding this meeting, a 1-day public hearing took place with stakeholders, interested parties, and members of the expert panel, during which the panel members were able to discuss various aspects of the TEF and TEQ concept with the participants and use this information during the actual reevaluation process. Besides the reevaluation of the WHO 1998 TEF values, the validity, criteria and correct use of the TEF/TEQ concept, and methods for proper identification of TEF values and possible compounds for future inclusion were discussed. This report presents the results of this meeting, including the TEF values that now are proposed as WHO 2005 TEFs for human risk assessment of these compounds.

VALIDITY AND CRITERIA OF THE TEF CONCEPT

During the 2005 WHO reevaluation of the 1998 WHO TEF values, both the general TEF concept and REP criteria were extensively discussed. The criteria for inclusion of a compound in the TEF concept at this meeting were similar to those used at the two earlier WHO expert meetings (Ahlborg *et al.*, 1994; Van den Berg *et al.*, 1998). These criteria are that for inclusion in the TEF concept a compound must

- show a structural relationship to the PCDDs and PCDFs;
- bind to the AhR;
- elicit AhR-mediated biochemical and toxic responses;
- be persistent and accumulate in the food chain.

It was recognized that the vast amount of literature available in this field provides many examples of uncertainties associated with the determination of REPs. In addition, high variation can sometimes be found in REP values for the same congener and for similar endpoints in different species (e.g., rats vs. mice).

The 2005 WHO reevaluation of the TEF values made extensive use of the review and REP database of Haws *et al.* (2006) in which a set of criteria was developed to identify, include, or exclude REPs for dioxin-like compounds. Extensive consultations between the compilers of this database with the WHO represented by M. van den Berg and R. E. Peterson took place. However, it must be emphasized that for this 2005 TEF reevaluation, the expert panel used all available REPs, either included or excluded in this database, and made their own assessment (Haws *et al.*, 2006). Studies published since the 1997 reevaluation were also fully evaluated.

When reviewing the database of mammalian REPs for dioxin-like compounds, it was observed that even for the most thoroughly studied congeners like 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) and PCB 126, significant gaps in knowledge exist (Haws *et al.*, 2006). Reasons for significant differences in REPs for the same congener can be caused by the use of different dosing regimens (acute vs. subchronic), different endpoints, species, and mechanisms (e.g., tumor promotion caused by at least two different mechanisms as for mono-*ortho*–substituted PCBs), as well as different methods used for calculating REPs. Thus, different methodological approaches used in different studies clearly provide uncertainties when deriving and comparing REPs. If future study designs to derive REPs were more standardized and similar, the variation in REPs when using the same congener, endpoint, and species might be expected to be smaller.

At this 2005 meeting, the ''ideal'' REP study design was discussed as previous WHO TEF evaluations did not provide sufficient information regarding the criteria that needed to be met to establish an REP value and give an expert panel the greatest degree of confidence in a particular REP. The following general guidelines for a future ideal dose-response study used to determine an *in vivo* REP resulted from the workshop:

- A full dose-response curve for both the congener and for 2,3,7,8-TCDD should be determined.
- The congener and 2,3,7,8-TCDD should be administered by the same route to animals of the same species, strain, sex, and age, and the animals should be housed, fed the same diet, and maintained under the same conditions in the same laboratory.
- Ideally, the absolute maximal response (efficacy) should be similar for both the congener and for 2,3,7,8-TCDD and their dose-response curves should be parallel, but in practice, this is often not observed for various reasons.
- If the above dose-response criteria are met, the REP should be calculated by dividing the effective dose 50% (ED_{50}) of 2,3,7,8-TCDD by the ED_{50} of the congener.
- If full dose-response relationships are not attained and determination of ED_{50}'s is not possible, lowest observed effect doses or concentrations or benchmark doses could be used to determine the REP. However, such an REP has more uncertainty than if ED_{50}'s were used.

For studies that are designed to determine REPs, it is clear that *in vivo* studies have the highest priority because they combine both toxicokinetic as well as toxicodynamic aspects. Therefore, *in vivo* studies should preferably be used for setting TEFs. Nevertheless, *in vitro* studies can contribute significantly to establish the AhR-mediated mechanism of action of a compound and explain possible differences in species sensitivity, especially with respect to that of humans versus experimental animal species. For *in vitro* studies, stricter criteria should be applied as these are from an experimental design point of view usually easier to accommodate than *in vivo* studies. For *in vitro* studies, the following experimental design is suggested to determine an REP:

- A vehicle group and at least four graded concentrations of a congener and four graded concentrations of 2,3,7,8-TCDD should be selected.
- For congener and 2,3,7,8-TCDD treatment groups, three of these concentrations should elicit a response that falls between the EC_{20} and EC_{80} for the congener and for 2,3,7,8-TCDD.
- At least one concentration should elicit a maximal response (EC_{100}), and the concentration-response curves should be parallel.
- The REP should be based on the EC_{50} of 2,3,7,8-TCDD and the EC_{50} of the congener.

In general, 2,3,7,8-TCDD has been used as the reference compound of choice, but in several studies, PCB 126 has been used instead of 2,3,7,8-TCDD. Based on available data from the literature, it was concluded that PCB 126 could indeed be used as a reference compound in rat studies with an REP value of 0.1. Recent studies have confirmed this value for multiple endpoints (Toyoshiba *et al.*, 2004; Walker *et al.*, 2005). However, it should be examined in more detail if the same REP for PCB 126 is applicable as a reference compound for mouse studies. The REP values for some endpoints such as enzyme induction tend to be significantly lower in mice than in rats (Birnbaum and DeVito, 1995; DeVito *et al.*, 2000; Harper *et al.*, 1993; van Birgelen *et al.*, 1996a). In this respect, it should be noted that mice studies in which PCB 126 was used as a reference compound were excluded from the database and from further consideration because of other methodological reasons (Haws *et al.*, 2006).

Literature data also indicate that the PCB 126 REP for enzyme induction in human cell systems, including primary hepatocytes, breast cancer cell lines, and primary lymphocytes, may be one or two orders of magnitude lower (van Duursen *et al.*, 2003; Zeiger *et al.*, 2001). In addition, the apparent binding affinity of 2,3,7,8-TCDD to the human AhR is generally 1/10th that of the AhR of the more sensitive rodent species, but significant variation among individual humans occurs (Ema *et al.*, 1994; Harper *et al.*, 2002; Poland *et al.*, 1994; Ramadoss and Perdew, 2004; Roberts *et al.*, 1990). It has been suggested that on average, humans are among the more dioxin-resistant species, but the human data set is too limited to be conclusive (Harper *et al.*, 2002; Okey *et al.*, 2005). A study with AhR-humanized mice may indicate lower responsiveness toward toxic effects of 2,3,7,8-TCDD (Moriguchi *et al.*, 2003). Taken together, this information warrants more research into

REP values in human systems to establish if the present TEFs based on rodent studies are indeed also valid for humans.

Additivity is an important prerequisite of the TEF concept, and this aspect was revisited in detail by the 2005 expert panel. It was concluded that results from recent *in vivo* mixture studies with dioxin-like compounds are consistent with additivity and support the TEF approach (Fattore *et al.*, 2000; Gao *et al.*, 1999; Hamm *et al.*, 2003; Walker *et al.*, 2005). Gao *et al.* (1999) studied the REP and additivity of 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, and 1,2,3,4,7,8-HxCDD in a rat ovulation model; their results confirmed both parallel dose-response curves and mixture additivity. Fattore *et al.* (2000) measured hepatic vitamin A reduction in rats after subchronic dietary exposure to a low-dose mixture containing 1,2,3,7,8-PeCDD, 2,3,4,7,8-PeCDF, and 1,2,3,6,7,8-HxCDF to test additivity. The effects of this mixture showed that the predicted results based on WHO 1998 TEFs were approximately twofold higher. Hamm *et al.* (2003) studied a mixture of nine dioxins, furans, and coplanar PCBs and looked at developmental reproductive endpoints in rats, comparing results of the mixture to that of 2,3,7,8-TCDD alone. The results showed that the experimental estimated TEQ was within a factor of two of that predicted from the WHO 1998 TEFs. A mixture study from the National Toxicology Program was also examined by the expert panel, and again the results generally supported additivity and parallel dose-response curves for complex and long-term neoplastic and nonneoplastic endpoints (Walker *et al.*, 2005).

Thus, results in these recent mixture studies could be predicted rather well with the WHO 1998 TEFs, within a factor of two or less. This degree of accuracy was somewhat surprising in view of the complicated experimental situation present in subchronic toxicity studies, where congener-specific toxicodynamics and kinetics are intermingled and can influence the final outcome. In addition, the WHO 1998 TEFs were derived from a range of REPs using different biological models or endpoints and were therefore estimates with an order of magnitude uncertainty (Van den Berg *et al.*, 1998).

PROCESS USED TO DETERMINE TEF VALUES: POINT ESTIMATES, EXPERT JUDGMENT, AND PROBABILISTIC DISTRIBUTION

Both the WHO 1993 and 1997 TEF reevaluations used point estimates derived by expert judgment from a wide range of REPs (Ahlborg *et al.*, 1994; Van den Berg *et al.*, 1998). In the 2005 TEF reevaluation, it was decided by the expert panel to use the REP database from Haws *et al*. (2006) for initial assessment of a TEF value. This recently published database and applied criteria were a refinement of the criteria and database that were developed to support the two previous WHO TEF reevaluations (Ahlborg *et al.*, 1994; Van den Berg *et al.*, 1998). The criteria for inclusion or exclusion of an REP in this database (Haws *et al.*, 2006) were accepted by the expert panel. These criteria can be summarized as follows:

- At least one test congener and a valid reference compound must have been included in the study or the reference compound must have been included in an identical experiment from the same laboratory, but in another study.
- The endpoint must have been an established AhR-mediated response known to be affected by both the test congener and the reference compound.
- In the REP database, *in vivo* and *in vitro* studies were separated.
- Repetitive endpoints (i.e., measures of the same biological response) were identified in all studies in the database, and the most representative REP value was retained for reevaluation of a TEF.
- Those studies that used only a single-dose level of either the test and/or reference compound were filtered out of the REP database and not used in the TEF reevaluation process.
- Results from non-peer–reviewed studies were not used in reevaluating a TEF value and consequently did not contribute to the distribution of REPs for individual congeners.
- REPs based on biological responses that were statistically significant were included in the 2005 REP database and contributed to the distribution of REPs for individual congeners used to reevaluate TEFs. However, when there was a very limited data set for an individual congener, the panel also considered biological responses that were not statistically significant as part of the overall expert judgment in reevaluating a TEF value.
- REPs based on quantitative structure-activity relationship studies were included in the REP database.

When using this database, the primary focus of the TEF reevaluation was on *in vivo* studies (Haws *et al.*, 2006). *In vitro* studies were only used for support in those situations where no or very few *in vivo* REP data were available. For *in vitro* REPs, only established AhR-mediated responses were used to assign REP values.

During the TEF reevaluation, the expert panel considered using REP distributions available from the REP database (Haws *et al.*, 2006) when reevaluating a TEF value. However, the REP distributions in this study are unweighted (Haws *et al.*, 2006), and it was decided that establishing a weighting criteria for REPs generated in different types of studies (*in vivo*, *in vitro*, chronic, acute, etc.) was not feasible at this meeting. In addition, it was concluded that REP distributions for a specific congener in this database could not be used to derive a TEF value because a fixed percentile would have to be used as a cutoff point. Such an approach would be like using a single point estimate, but with lower biological or toxicological relevance. This is because all types of *in vivo* studies (acute, subchronic, etc.) and different endpoints have been combined, and associated REP distributions are shown as a single box plot. Thus, with only unweighted distributions of REPs available, a final expert judgment in the TEF reevaluation process involving the type and quality of the study had preference over the unweighted REP distributions (Haws *et al.*, 2006). Nevertheless, it was recognized by the expert panel that in the future weighted REP distributions could be used for derivation of TEF values, but establishing consensus values for these REP weighting factors would require additional expertise.

The WHO expert panel decided that a combination of these unweighted REP distributions, expert judgment, and point estimates would be used to reevaluate a TEF. Figure 1 shows the unweighted distribution of *in vivo* and *in vitro* REPs and WHO 1998 TEF values for PCDDs, PCDFs, non-*ortho* PCBs, and mono-*ortho* PCBs (Haws *et al.*, 2006). These unweighted REP distributions were used to start the selection and decision process for a TEF reevaluation. The 75th percentile of the *in vivo* REP distribution for an individual congener was used as an initial decision point to review the WHO 1998 TEF for that congener. If the WHO 1998 TEF was below the 75th percentile of the *in vivo* REP distribution, the data driving this TEF value was extensively reevaluated. If the WHO 1998 TEF value was above the 75th percentile, a quick review was done regarding the decision made at the 1997 WHO meeting with respect to those studies that had been driving the 1998 WHO TEF value. In addition, results of new studies conducted after 1997 or old information missed at the 1997 WHO meeting were evaluated to determine if these would influence the WHO 1998 TEF value for that congener. Based on the combined information, a possible new TEF value was considered. Special attention was also given to validity of WHO 1998 TEF values that were near or higher than the 90th percentile, e.g., 1,2,3,7,8-PeCDF. Thus, the above TEF reevaluation process provided a way both to increase as well as to decrease a TEF value. Figure 2 illustrates the decision scheme used at the expert meeting for the initial reevaluation process of the TEFs. For transparency, the expert judgment process and rationale used by the expert panel for a possible newly assigned WHO 2005 TEF value is explained in the next paragraph. This is followed by subsequent paragraphs devoted exclusively to each congener reevaluated.

As in previous WHO TEF consultations, it was decided by the expert panel to use a stepwise scale for assigning TEFs values. However, instead of assigning TEFs in the increments used previously (0.01, 0.05, 0.1, etc.), it was decided to use half order of magnitude increments on a logarithmic scale at 0.03, 0.1, 0.3, etc. As a result, all (non)revised 2005 WHO TEFs were fitted on a logarithmic scale. This decision to assign TEFs as half order of magnitude estimates may be useful in describing, with statistical methods, the uncertainty of TEFs in the future. Thus, as a default, all TEF values are assumed to vary in uncertainty by at least one order of magnitude, depending on the congener and its REP distribution. Consequently, a TEF of 0.1 infers a degree of uncertainty bounded by 0.03 and 0.3. For a TEF value of 0.3, a degree of uncertainty bounded by 0.1 and 1 was used. Thus, the TEF is a central value with a degree of uncertainty assumed to be at least ± half a log, which is one order of magnitude. However, it should be realized that TEF assignments are usually within the 50th to 75th percentile of the REP distribution, with a general inclination toward the 75th percentile in order to be health protective. However, the latter approach was also influenced by the type and quality of study, e.g., single versus multiple dose, that could not been discerned from the REP distributions shown in Figure 1. This more conservative and health protective approach practically means that for a TEF value the likelihood of a half-log error too low is less than the likelihood of half a log error too high. Due to the new ‘‘spacing’’ to express TEFs on a half-log scale, it was also necessary in the final review process to evaluate each individual TEF value for those congeners for which there were no new data available.

WHO 2005 TEF VALUES

1,2,3,7,8-PeCDD

The WHO 1998 TEF was set at 1.0, which is above the 90th percentile of the REP distribution of 12 *in vivo* studies. New studies indicate an REP between 0.1 and 1.0 for this compound (Fattore *et al.*, 2000; Johnson *et al.*, 2000; Simanainen *et al.*, 2002). The vitamin A and tumor promotion studies provide REPs of 0.7 and 1 (Fattore *et al.*, 2000; Waern *et al.*, 1991). Results from acute toxicity studies result in REPs closer to 0.5, but, in general, REPs increase in subchronic toxicity studies (Haws *et al.*, 2006). Therefore, the consensus WHO 2005 TEF value remained at 1.

1,2,3,4,7,8-HxCDD

The WHO 1998 TEF was set at 0.1, which is around the 80th percentile of the REP distribution of five *in vivo* studies. One new study determined REPs ranging from 0.04 to 0.12 (Gao *et al.*, 1999), while two other recent studies observed REPs between 0.06 and 0.4 (Simanainen *et al.*, 2002; Takagi *et al.*, 2003). Very little data indicate that the TEF value should be changed to either 0.3 or 0.03. Therefore, it was decided to keep the WHO 2005 TEF value at 0.1.

1,2,3,6,7,8-HxCDD

The WHO 1998 TEF was set at 0.1. No *in vivo* studies are available for this HxCDD isomer. This TEF value is above the 75th percentile of the REP distribution of four *in vitro* studies (Haws *et al.*, 2006). A more recent *in vitro* study (Bols, 1997) supports this TEF value, and therefore, no change for the WHO 2005 TEF was decided.

1,2,3,7,8,9-HxCDD

Similar to the above previous hexa-isomers, the WHO 1998 TEF was set at 0.1. It was noted that very little *in vivo* data are

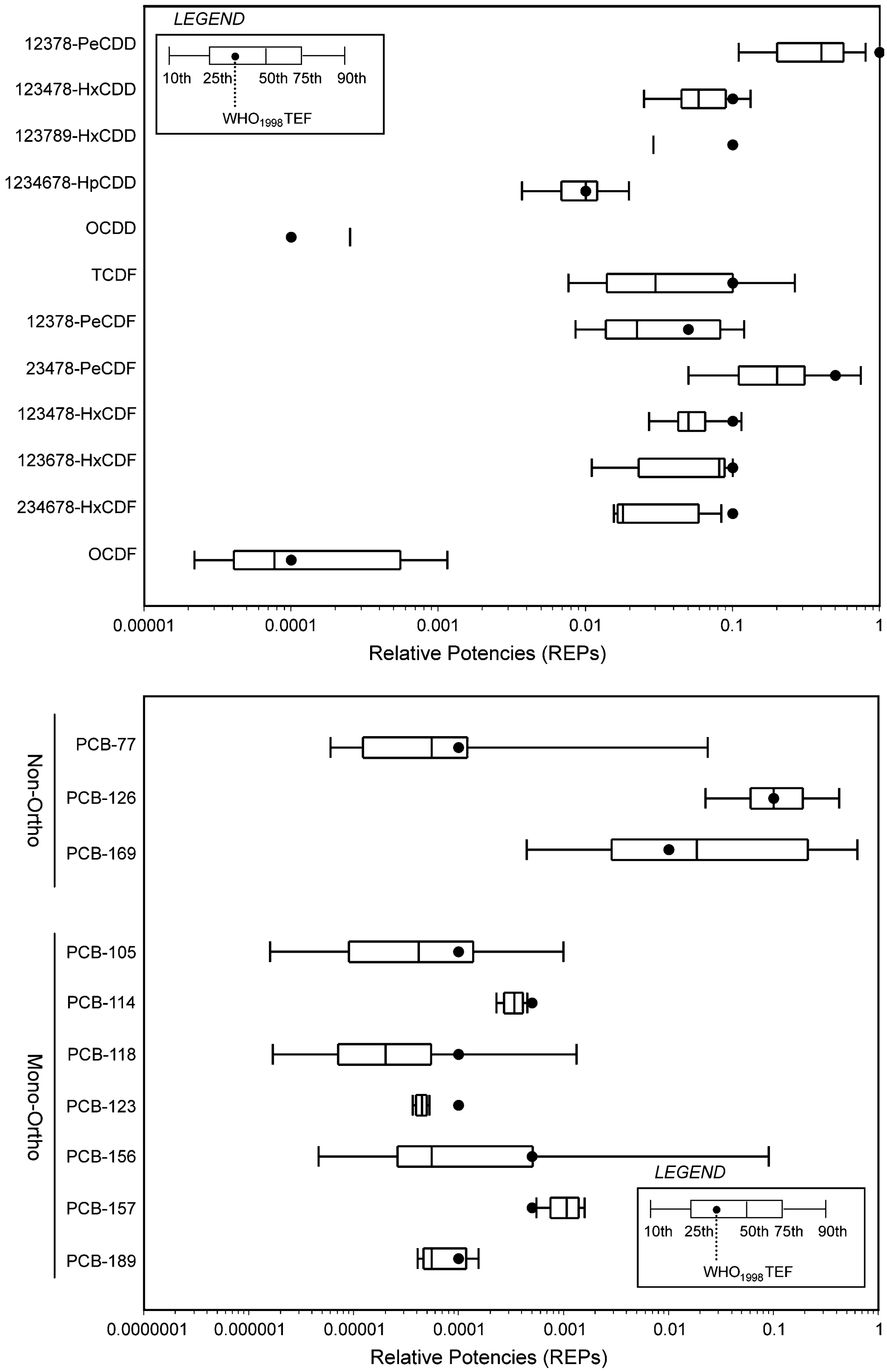

FIG. 1. Distribution of *in vivo* unweighted REP values in the REP_{2004} database. Reprinted with permission from (Haws *et al.*, 2006).

available with a recent study giving an REP of 0.029 (Takagi *et al.*, 2003). In addition, four *in vitro* studies produced REPs up to 0.07 (Lipp *et al.*, 1992; Schrenk *et al.*, 1991), which is above the 75th percentile of the distribution. The expert panel considered decreasing the TEF value to 0.03 but decided that there was not enough data to support such a change. *In vitro* data were observed to be consistent between HxCDD isomers. In view of the homology between the HxCDD isomers, it was therefore decided to retain the old value of 0.1 as the WHO 2005 TEF value.

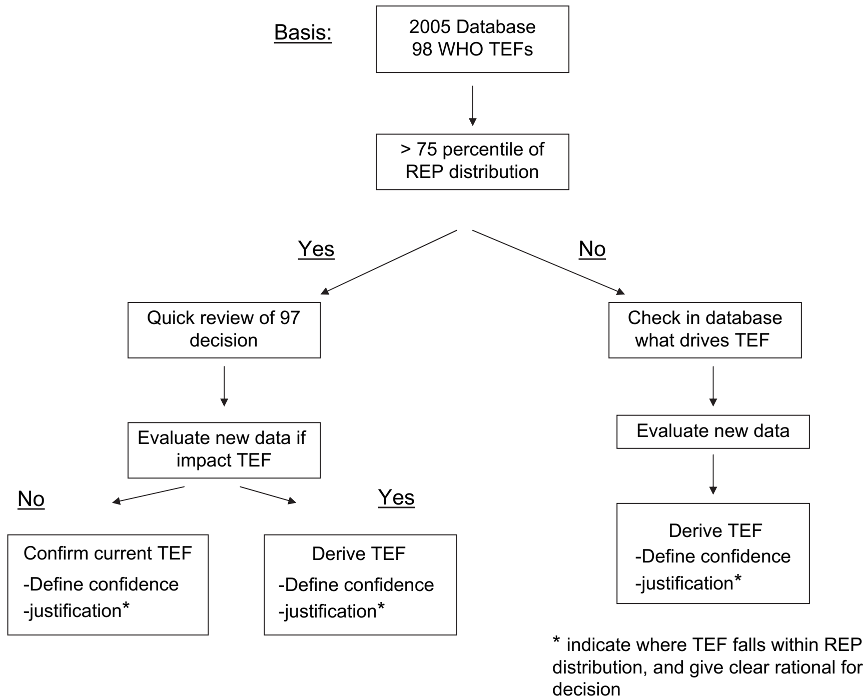

FIG. 2. Decision scheme used in the 2005 reevaluation of the 1998 WHO TEF values (Van den Berg *et al.*, 1998) assigned to individual PCDD, PCDF, and PCB congeners.

1,2,3,4,6,7,8-HpCDD

The WHO 1998 TEF was set at 0.01, which is at the 50th percentile of the REP distribution range of four *in vivo* studies. New studies again point toward an REP of 0.01 for this congener (Simanainen *et al.*, 2002; Viluksela *et al.*, 1994, 1997a,b). An earlier tumor promotion study also indicated a similar REP (Schrenk *et al.*, 1994). It was also discussed whether or not the available information from the important studies mentioned above would be sufficient to increase the TEF to 0.03, which is well above the 90th percentile of the REP distribution. This suggestion was rejected by the expert panel. It was decided to retain the WHO 2005 TEF value as 0.01.

Octachlorodibenzo-p-*dioxin*

The WHO 1998 TEF was set at 0.0001, which is well outside the 10th percentile of the range of *in vivo* and *in vitro* REP values (Haws *et al.*, 2006). At present, the only *in vivo* REPs meeting the stringent conditions of the database (Haws *et al.*, 2006) are based on one study that was reported in two different papers using different endpoints (Fattore *et al.*, 2000; Wermelinger *et al.*, 1990). It was discussed whether or not this TEF should be increased to bring it in line with the results of the subchronic toxicity study (Fattore *et al.*, 2000; Wermelinger *et al.*, 1990). The new *in vivo* REP data from Fattore *et al.* (2000) were evaluated, and these would support a TEF greater than 0.0001. One concern that was expressed within the expert panel was that the animals used in a more recent publication (Fattore *et al.*, 2000) were the same animals used in an earlier study (Wermelinger *et al.*, 1990), and this octachlorodibenzo-*p*-dioxin (OCDD) was reported to be contaminated with other more potent 2,3,7,8–substituted congeners such as 2,3,4,7,8-PeCDF. Using the NTP data now available for 2,3,4,7,8-PeCDF (Walker *et al.*, 2005), it was calculated that the reported contamination of 2.5 ppm (pg/μg) 2,3,4,7,8-PeCDF was not of toxicological relevance for the results (Calculation of 2,3,4,7,8-PeCDF contamination in OCDD Fattore *et al.* study [2000]. 2.5 ppm = 2.5 pg/μg OCDD. Highest OCDD dose 800 ppb = 800 ng/g = 0.8 μg/g feed. At this dose level, the PeCDF dose must have been 2.5 pg PeCDF/0.8 μg OCDD/g feed, which is equivalent with 2 pg PeCDF/g feed. Assuming a rat of 200 g with 20 g feed per day, the PeCDF dose must have been 40 pg PeCDF/200 g rat, which is equivalent with 200 pg PeCDF/kg/day or 0.2 ng PeCDF/kg/day. This dose is two orders of magnitude lower than the lowest dose (20 ng PeCDF/kg/day) used in the National Toxicology Program and well below the no observed effect level (NOEL) of all endpoints that were looked at.). Overall, it was concluded that there is very limited *in vivo* information available with only one subchronic toxicity study (Fattore *et al.*, 2000; Wermelinger *et al.*, 1990). The expert panel decided that the information provided by both *in vivo* studies derived from only one experiment did not provide a solid basis to increase the TEF value for this compound to 0.001, but the combined information from *in vivo* and *in vitro* data (Haws *et al.*, 2006) did justify a raise in TEF value. Therefore, it was decided to increase the WHO 2005 TEF value to 0.0003. The expert panel is aware of the implications that the increase in this WHO TEF value for OCDD might have from a regulatory and risk management point of view. However, with respect to the high concentrations of OCDD in some environmental matrices, a number of critical remarks regarding the inappropriate use of the present WHO TEFs are made in the section on the use of TEQ for abiotic environmental matrices.

2,3,7,8-TCDF

The WHO 1998 TEF was set at 0.1. This value is at the 75th percentile of the REP distribution of nine *in vivo* studies for this compound (Haws *et al.*, 2006). Only one new study has been reported (Takagi *et al.*, 2003), and an REP of 0.07 was found for increased cleft palate formation, which is close to the TEF of 0.1. Consequently, it was decided that the WHO 2005 TEF should remain at 0.1.

1,2,3,7,8-PeCDF

The WHO 1998 TEF was set at 0.05, which is within the 50th and 75th percentile of the REP distribution of eight *in vivo* studies. A new study by Fattore *et al*. (2000) found an REP of 0.01 for effects on hepatic vitamin A reduction, but a study by Takagi *et al*. (2003) reported an REP of 0.045 for cleft palate. The majority of the *in vivo* studies report an REP value below 0.1, but many relevant studies have REPs above 0.01. Therefore, it was decided that the 2005 WHO TEF should become 0.03.

2,3,4,7,8-PeCDF

The WHO 1998 TEF was set at 0.5, which is well above the 75th percentile of the REP distribution of eight *in vivo* studies. Results from the long-term NTP study in female Sprague-Dawley rats using many different endpoints are now available to evaluate this earlier TEF value more closely. The REPs for neoplastic endpoints from the NTP study (Walker *et al.*, 2005) are around 0.2–0.3, while nonneoplastic endpoints have REPs that range from 0.7 to 1.1. An earlier subchronic study by Pluess *et al.* (1998) pointed toward an REP of 0.4. More recent studies using hepatic vitamin A reduction and immunological effects as endpoints also point toward a TEF below 0.5 (Fattore *et al.*, 2000; Johnson *et al.*, 2000). In view of this new information, it was decided by consensus of the expert panel to change the WHO 2005 TEF to 0.3.

1,2,3,4,7,8-HxCDF

The WHO 1998 TEF was set at 0.1, which is above the 75th percentile of the REP distribution of six *in vivo* studies. No new *in vivo* studies have been published since 1997, and in view of the limited data, there was no reason to change this value. Thus, the WHO 2005 TEF value remains 0.1.

1,2,3,6,7,8-HxCDF

The WHO 1998 TEF was also set at 0.1, and this value is above the 75th percentile of the distribution of three *in vivo* REPs, and when the results of *in vitro* and *in vivo* studies with the PCDF are combined, REP values lie within the 50th and 75th percentile. A new study reported an REP of 0.03 for hepatic vitamin A reduction (Fattore *et al.*, 2000). However, the animals analyzed were from an earlier study from which an REP of 0.1 for subchronic toxicity was reported (Pluess *et al.*, 1998). In view of the limited number of studies available and the fact that WHO 1998 TEFs of 0.1 for most HxCDFs were all around the 50th to 75th percentile (Haws *et al.*, 2006), the expert panel decided not to discriminate between TEF values for these congeners. As a result, the 2005 WHO TEF remains at 0.1.

1,2,3,7,8,9-HxCDF

The WHO 1998 TEF for this HxCDF was set at 0.1. There are no *in vivo* results, and only two earlier *in vitro* studies for this congener with REPs of 0.2 and 0.1 (Brown, 2001; Tysklind *et al.*, 1994) supporting the 0.1 TEF value similar to the other HxCDFs. Consequently, the 2005 WHO TEF remains as 0.1.

2,3,4,6,7,8-HxCDF

The WHO 1998 TEF value is 0.1, and it is around the 50th percentile of the REP distribution range of the combined *in vivo* and five *in vitro* studies (Haws *et al.*, 2006). Most *in vitro* studies suggest a TEF value slightly above 0.1 (Bandiera *et al.*, 1984; Brown, 2001; Mason *et al.*, 1987; Tysklind *et al.*, 1994). There is only one *in vivo* study for this hexa-isomer indicating REPs for different endpoints ranging from 0.02 to 0.1. Given this weak and limited REP database and approximate similarities in responses for this and certain other HxCDFs, there was consensus in the expert panel to retain the 2005 WHO TEF at 0.1.

1,2,3,4,6,7,8- and 1,2,3,4,7,8,9-HpCDFs

The WHO 1998 TEFs for both HpCDFs were set at 0.01. Since 1997, there are no new *in vivo* studies published. Only two *in vitro* studies have been published (Brown, 2001; Tysklind *et al.*, 1994) reporting REPs, respectively, of 0.02 and 0.3 for 1,2,3,4,6,7,8-HpCDF and 0.04 and 0.02 for 1,2,3,4,7,8,9-HpCDF. Although these *in vitro* results do suggest a slightly higher TEF than 0.01, the expert panel thought that there was too much uncertainty in this limited database to raise the TEF. In addition, it was expected that *in vivo*, there would be low absorption of these HpCDFs from the gastrointestinal tract, thereby reducing their relative potency below that of the *in vitro* REPs. Based on these arguments, it was decided that the WHO 2005 TEFs would remain the same for both isomers, 0.01.

Octachlorodibenzofuran

The WHO 1998 TEF value of 0.0001 is within the 50th and 75th percentile of the REP distribution range of three *in vivo* studies, but when these data are combined with *in vitro* results, it falls below the 50th percentile (Haws *et al.*, 2006). The recent study by Fattore *et al.* (2000) using the same animals as Wermelinger *et al.* (1990) indicate an REP for octachlorodibenzofuran (OCDF) greater than 0.0001 based on hepatic vitamin A reductions. Some earlier *in vivo* studies also indicated an REP higher than the WHO 1998 TEF (DeVito *et al.*, 1998; van Birgelen *et al.*, 1996a; Waern, 1995). As with OCDD, there was originally concern among the expert panel about impurities with 2,3,7,8-chlorine–substituted congeners (Fattore *et al.*, 2000; Wermelinger *et al.*, 1990), but calculations indicated that the reported contamination with 1,2,3,4,6,7,8-HpCDF was of no toxicological concern. When the limited number of *in vivo* and *in vitro* REPs (< 10) are reviewed, REPs range from 4×10^{-6} to 0.0028 with a 50th percentile of 0.0007 (Haws *et al.*, 2006). As with OCDD, the expert panel decided that the limited *in vivo* information available would not warrant a factor of 10 increase of the WHO 1998 TEF value, but increasing the WHO 2005 TEF value to 0.0003 is appropriate in view of some of the higher *in vivo* REPs reported. This would also be in line with comparable REP values obtained in a recent study including both OCDD and OCDF (Fattore *et al.*, 2000).

PCB 77

The WHO 1998 TEF value of 0.0001 is just below the 75th percentile in a very nonhomogenous distribution of six *in vivo* REPs. The available subchronic toxicity studies are all around the 75th percentile (Chu *et al.*, 1995; Hakansson *et al.*, 1994). Immunotoxicological studies with mice were given less weight (Harper *et al.*, 1995; Mayura *et al.*, 1993) because these were acute studies involving the ip route of exposure, and no information on purity was provided. It was decided by the expert panel that the subchronic study was still the most representative (Chu *et al.*, 1995). As a consequence, the WHO 2005 TEF value remained at 0.0001.

PCB 81

The WHO 1998 TEF value was 0.0001. PCB 81 has been observed in wildlife and human milk (Kumar *et al.*, 2001), confirming the validity of inclusion of this PCB in the TEF scheme. There are no new *in vivo* data for this PCB congener. Older *in vivo* data were excluded because these involved single-dose studies from which the expert panel believed that no reliable REP value could be determined. Various *in vitro* studies with human hepatoma HepG2 cells and monkey hepatocytes indicate that PCB 81 is more potent than PCB 77 (Brown, 2001; Pang *et al.*, 1999; van der Burght *et al.*, 1999; Zeiger *et al.*, 2001). Based on the *in vitro* REP distribution, it is noticeable that the WHO 1998 TEF is located at the very low end of the REP distribution range (Haws *et al.*, 2006). Thus, based on the information that PCB 81 is more potent *in vitro* and more persistent than PCB 77, the expert panel decided to raise the WHO 2005 TEF value to 0.0003. However, the expert panel expressed its low confidence in the PCB 81 REP database because it lacks *in vivo* REP data.

PCB 126

The WHO 1998 TEF was set at 0.1, which is at the median of the REP distribution range of 20 *in vivo* studies. This 1998 TEF value was mainly driven by the tumor promotion study with this compound (Hemming *et al.*, 1995). New *in vivo* studies from the NTP covering many endpoints (Johnson *et al.*, 2000; Walker *et al.*, 2005) support the TEF of 0.1. In rat studies, the expert panel recognized the tight range of REPs for this congener (Haws *et al.*, 2006), which supports the use of PCB 126 as reference compound with a TEF of 0.1 when comparing rat studies. Information from mice studies and some human *in vitro* systems (especially for enzyme induction) suggest that the REP for PCB 126 might be lower than 0.1 (Birnbaum and DeVito, 1995; DeVito *et al.*, 2000; Harper *et al.*, 1993; van Birgelen *et al.*, 1996a; van Duursen *et al.*, 2003; Zeiger *et al.*, 2001). Clearly, more information is necessary regarding this issue. Although concern was expressed about interspecies variability in REPs, the expert panel considered the present information too limited to make a decision other than to retain 0.1 as the WHO 2005 TEF.

PCB 169

The WHO 1998 TEF was set at 0.01, which is below the median in the REP distribution range of seven *in vivo* studies. The 1998 TEF was mainly driven by a 4-week repeated dose mouse study measuring enzyme induction and generating an REP of less than 0.001 (DeVito *et al.*, 1998). On the other hand, REPs from several other *in vivo* studies ranged from less than 0.01 to 0.7 (Harper *et al.*, 1993; Parkinson *et al.*, 1981; Yoshimura *et al.*, 1979). Thus, large differences in REPs have been observed for PCB 169 between both species and endpoints. In view of the fact that the WHO 1998 TEF was also below the median of the *in vivo* REP distribution (Haws *et al.*, 2006), the expert panel decided that it was appropriate to raise the TEF between the 50th and 75th percentile (see Figure 1). Nevertheless, many single-dose studies were observed to have significantly higher REPs (around 0.1) than those observed in a 13-week study. In view of these significant differences between single- and multiple-dose studies, the expert panel judged that the WHO 2005 TEF for PCB 169 of 0.03 would be more appropriate than a potentially overly conservative REP of 0.1.

*Mono-*Ortho*–Substituted PCBs 105, 114, 118, 123, 156, 157, 167, and 189*

The WHO 1998 TEF values for the mono-*ortho* PCBs ranged from 0.00001 to 0.0005. A major issue with the REP values for the different mono-*ortho* PCBs is that they span four to five orders of magnitude, depending on the congener. In Figure 3, this wide variation in REPs is illustrated. Even if only *in vivo* studies are considered, the 90% distribution range is extremely large (see Figure 1). This great variation in REP values was of serious concern to the expert panel. The panel considers possible, inconsistent, and low level contamination of the mono-*ortho* PCBs with more potent dioxin-like compounds to play, at least in part, a role in causing this large variation. De Vito (2003) found that less than 1% contamination of PCB 77 by PCB 126 significantly impacted the apparent REP of PCB 77. Shortly before the WHO 2005 TEF reevaluation meeting, two laboratories of panel members performed a number of *in vitro* experiments in an attempt to elucidate the possible impact of impurities on REPs for the mono-*ortho* PCBs (Peters *et al.*, 2006). This study showed that after being purified on charcoal, the mono-*ortho* PCBs 105, 118, 156, and 167 did not cause AhR-mediated activation and CYP1A1 induction in two genetically modified rodent hepatoma CA-FLUX cell lines at concentrations that would generally justify an REP larger than 0.0001. Based on the combined information, the expert panel expressed low confidence in the higher REP values for certain mono-*ortho* PCBs. It was concluded that the unusually wide variability of REP values for mono-*ortho* PCBs can probably be explained by the occurrence of impurities with 2,3,7,8-chlorine–substituted PCDDs and PCDFs or PCB 126. As the occurrence of these impurities clearly depends on the route of synthesis and the degree of cleanup, it was not possible to make a general statement about how it occurs in all cases. It was concluded that for future studies with mono-*ortho* PCBs or any other weak AhR agonists, a purity of > 99% is clearly not sufficient to establish a reliable REP. The expert panel compiled Figure 3 to make a decision on the TEF values of the mono-*ortho* PCBs, acknowledging the impurity issue and that the earlier decision scheme with ≥ 75th percentile (Fig. 2) was not appropriate. In this case, the most environmentally relevant mono-*ortho* PCBs are 105, 118, and 156, and it was decided to use the medians of the REP distribution range of these PCB congeners as a guide. This resulted in a recommended TEF of 0.00003 for these three mono-*ortho* PCBs. A differentiation for all other remaining mono-*ortho* PCBs was considered not feasible by the expert panel due to the lack of sufficient experimental data. Consequently, the recommended WHO 2005 TEF for all mono-*ortho* PCBs is 0.00003.

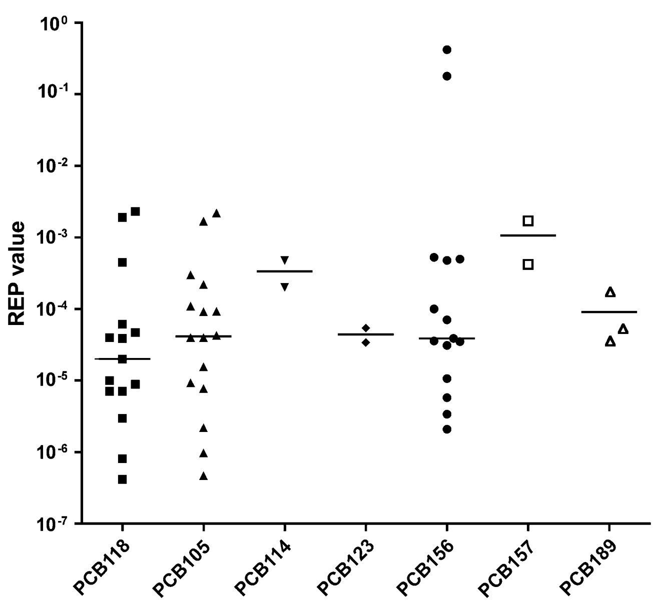

FIG. 3. Distribution of REP values for the different mono-*ortho* PCBs based on AhR-mediated effects.

OTHER COMPOUNDS DISCUSSED FOR POSSIBLE INCLUSION IN THE TEF SCHEME

PCB 37

PCB 37 is commonly found in the environment (Hansen, 1998). It has also been detected in edible fish species at levels comparable with PCB 77 and 81 (Sapozhnikova *et al.*, 2004). In seals, it has been measured in relatively high levels, indicating possible bioaccumulative properties in the food chain (Addison *et al.*, 1999). It has also been found in human milk (Hansen, 1998). In an *in vitro* study with the human MCF-7 breast carcinoma and HepG2 hepatoma cell lines, no induction of CYP1A1 or 1B1 could be found. However, PCB 37 was found to be a significant catalytic inhibitor of both CYP activities (Pang *et al.*, 1999). In view of the above information, there is a clear need for more *in vivo* and *in vitro* information to decide if this PCB needs to be included in the TEF scheme.

*Polybrominated Dibenzo-*p*-Dioxins and Polybrominated Dibenzofurans*

Both *in vitro* and *in vivo* studies have shown that polybrominated dibenzo-*p*-dioxins (PBDDs) and polybrominated dibenzofurans (PBDFs) have AhR agonist properties and cause dioxin-like effects (Birnbaum *et al.*, 2003; Mason *et al.*, 1987). Emerging data from Japan and the Baltic Sea indicate that PBDDs and PBDFs can be found in sediment, mussels, and higher trophic species like the cormorant (Choi *et al.*, 2003; Malmvarn *et al.*, 2005; Takigami *et al.*, 2005; Watanabe *et al.*, 2004). In addition, there is limited recent information showing that these compounds are found in human milk and adipose tissue at levels that can contribute significantly to the total amount of TEQ (Choi *et al.*, 2003; Kotz *et al.*, 2005; Ohta *et al.*, 2005). It appears that environmental levels might be significantly lower than those of the PCDDs, PCDFs, and PCBs already in the TEF scheme. However, a better exposure assessment especially with regard to humans is needed. If the presence of PBDDs and PBDFs in human food as well as in people is more extensively demonstrated, there would be a clear need for assigning TEFs to these compounds. At present, it is unclear to what extent the ongoing use of brominated flame retardants, especially polybrominated diphenylethers (PBDEs), could lead to an increase in human and environmental exposure to PBDDs and PBDFs. Therefore, it is recommended by the expert panel to perform a more thorough exposure analysis for humans. In addition, it was concluded that among all compounds proposed in this paragraph for development of WHO TEFs, the PBDDs and PBDFs should be given high priority. More REP studies on PBDDs and PBDFs are urgently needed.

*Mixed Halogenated Dibenzo-*p*-Dioxins and Mixed Halogenated Dibenzofurans*

Due to the extremely high number of congeners, analysis of mixed halogenated dibenzo-*p*-dioxins (PXCDDs) and mixed halogenated dibenzofurans (PXCDFs) is still a major problem. Very little is known about the possible relevance of these compounds for human exposure (Birnbaum *et al.*, 2003). If the mixed halogenated (bromine- and chlorine-substituted) dioxins and dibenzofurans are indeed detected in humans and their food, these should definitely be considered for inclusion in the TEF scheme. Early *in vitro* studies suggest that these compounds follow the same structure-activity rules as the PCDDs and PCDFs (Behnisch *et al.*, 2001; Mason *et al.*, 1987; Weber and Greim, 1997).

Hexachlorobenzene

It has been suggested that hexachlorobenzene (HCB) fulfills the criteria for inclusion in the TEF concept (van Birgelen, 1998), although arguments for doing so have been criticized (Pohl *et al.*, 2001; Schwab, 1999; Vos, 2000). HCB has mixed inducer properties in analogy with the mono-*ortho* PCBs. Before inclusion in the TEF concept is considered, it should be confirmed that highly purified HCB has indeed AhR agonistic properties, as contamination of HCB with PCDDs and PCDFs has been reported (Goldstein, 1979) (Analysis of HCB done for the U.K. Medical Research Council indicated levels of 16,000 ng OCDD/g, 6000 ng OCDF/g, 1000 ng HpCDF/g, and 88 ng TCDD/g in HCB of high chemical quality [M. Rose, personal communication].). Thus, results from earlier HCB studies could have an impurity problem similar to that observed for the mono-*ortho* PCBs. Priority should thus be given to confirm the compound's dioxin-like properties using highly purified HCB with measured absence of 2,3,7,8-chlorine–substituted dioxins and dibenzofurans or dioxin-like PCBs.

Polychlorinated Naphthalenes and Polybrominated Naphthalenes

Based on recent published data, there was agreement by the expert panel that these compounds definitely should be considered for inclusion in the TEF concept as polychlorinated naphthalenes (PCNs) are actually reported in food and humans (Domingo *et al.*, 2003; Falandysz, 2003; Hayward, 1998; Lunden and Noren, 1998; Weistrand and Noren, 1998; Williams *et al.*, 1993). Earlier *in vivo* studies demonstrated that PCNs and polybrominated naphthalenes (PBNs) were able to induce dioxin-like effects, such as cleft palate and hydronephrosis (Birnbaum *et al.*, 1983; McKinney and McConnell, 1982; Miller and Birnbaum, 1986). Further arguments for inclusion would be that multiple PCN and PBN congeners have distinct *in vitro* AhR activities that show analogy with PCDDs and PCDFs but are less potent (Behnisch *et al.*, 2003; Blankenship *et al.*, 2000; Darnerud, 2003; Robertson *et al.*, 1982, 1984; Villeneuve *et al.*, 2000). However, as with mono-*ortho* PCBs and HCBs, the possible impurity issue should be addressed thoroughly before inclusion in the TEF concept is decided.

Polybrominated Biphenyls

Certain polybrominated biphenyls (PBBs) have been reported to be AhR active in both *in vitro* and *in vivo* experiments (Darnerud, 2003; Robertson *et al.*, 1982, 1984). It was noted by the expert panel that some human background exposure to PBBs is still occurring. However, human exposure data outside the "Michigan episode" are surprisingly scarce. Recently, PBB exposure has been reported in bird species at the top of the food chain from Japan and Norway (Herzke *et al.*, 2005; Lindberg *et al.*, 2004; Watanabe *et al.*, 2004). This occurrence in top predator wildlife species also stresses the need to further identify present human background exposure to PBBs. Thus, based on the AhR mechanism of action, inclusion of certain PBB congeners in the TEF scheme is appropriate, but further human exposure analysis should identify the possible relevance of PBBs to the total TEQ.

Polybrominated Diphenylethers

The expert panel accepted that PBDEs by themselves do not have AhR agonist properties and should not be included in the TEF concept (Chen and Bunce, 2003; Peters *et al.*, 2004; Sanders *et al.*, 2005). However, commercial mixtures of PBDEs can contain PBDDs and PBDFs that express significant AhR-mediated activities, such as CYP1A1 induction (Birnbaum *et al.*, 2003; Hakk and Letcher, 2003). The expert panel had concerns about earlier results in the literature, indicating that PBDEs cause AhR-mediated effects because of the possible impurity issue similar to that described for the mono-*ortho* PCBs. In addition, it was also recognized that photochemical and combustion processes of PBDEs can also produce PBDDs and PBDFs. In conclusion, it was recommended that TEFs should not be assigned for PBDEs.

"NON-DIOXIN–LIKE" AHR LIGANDS AND THE TEF CONCEPT

The AhR can bind and be activated by a structurally diverse range of synthetic and naturally occurring chemicals (Denison and Heath-Pagliuso, 1998; Heath-Pagliuso *et al.*, 1998; Jeuken *et al.*, 2003; Nagy *et al.*, 2002). These chemicals are widely distributed in dietary vegetables, fruits, teas, and dietary herbal supplements sometimes at relatively high concentrations (Amakura *et al.*, 2002; Berhow *et al.*, 1998; Formica and Regelson, 1995; Herzog *et al.*, 1993a,b; Jeuken *et al.*, 2003). The ability of metabolically labile phytochemicals to induce or inhibit induction of CYP1A1-dependent activities by 2,3,7,8-TCDD in cell culture model systems have been reported by numerous laboratories (Amakura *et al.*, 2002; Jeuken *et al.*, 2003; Williams *et al.*, 1993; Zhang *et al.*, 2003). However, the majority of toxicity studies demonstrated that these naturally occurring AhR agonists fail to produce AhR-dependent toxicity (Leibelt *et al.*, 2003; Pohjanvirta *et al.*, 2002), although some developmental dioxin-like effects have been reported for indole-3-carbinol (I3C) (Wilker *et al.*, 1996). In addition, naturally occurring AhR ligands, such as I3C and diindolymethane, have been reported to inhibit 2,3,7,8-TCDD–dependent *in vivo* induction of CYP1A1 and immunotoxicity (Chen *et al.*, 1995, 1996).

The ability of some non-dioxin–like PCBs and PCDFs to inhibit 2,3,7,8-TCDD–induced CYP1A1 activity and immunotoxicity in C57BL/6J mice has also been reported (Bannister and Safe, 1987; Biegel *et al.*, 1989; Chen and Bunce, 2004; Crofton *et al.*, 2005; Davis and Safe, 1988; Loeffler and Peterson, 1999; Morrissey *et al.*, 1992; Smialowicz *et al.*, 1997), whereas other studies have shown synergistic effects on dioxin toxicity of non-dioxin–like compounds, e.g., thyroid hormones, porphyrins, reproductive toxicity, and immunotoxicity (Bannister and Safe, 1987; Birnbaum *et al.*, 1986; Crofton *et al.*, 2005; Loeffler and Peterson, 1999; van Birgelen *et al.*, 1996b).

The above studies provide evidence that non-dioxin–like compounds that are weak AhR agonists can modulate the overall toxic potency of 2,3,7,8-TCDD and related compounds. If occurring under natural background situations, these interactions might impact the magnitude and overall toxic effects produced by a defined amount of TEQ (i.e., from intake or present in the body) but not impact the determination of individual REP or TEF values for dioxin-like chemicals. The potential impact of these non-dioxin–like natural compounds on the risk of toxicity posed by exposure to a particular level of TEQs should be further investigated.

THE USE OF TEQ FOR ABIOTIC ENVIRONMENTAL MATRICES

Concurrent with the development of the TEF and TEQ approach has been its application to environmental matrices such as soil, sediment, industrial wastes, soot, fly ash from municipal incinerators, waste water effluents, etc. As such, the TEQ approach has been and continues to be used to give a single value to complex environmental matrices (Barnes, 1991; Barnes *et al.*, 1991), usually without taking into consideration whether this is actually a risk-based number. The expert panel emphasized that correct application of the present TEF scheme (see Table 1) and TEQ methodology in human risk assessment is only intended for estimating exposure to dioxin-like chemicals from consumption of food products, breast milk, etc. This limitation is derived from the fact that those REP studies that have been considered most relevant for the determination of the present TEFs are largely based on oral intake studies, often through the diet. In fact, experimental toxicological studies using abiotic matrices with dioxin-like compounds that would allow for the determination of environmental matrice-based REPs (e.g., soil or sediment) are almost nonexistent. Furthermore, the issue of matrix-specific bioavailability of these chemicals from abiotic environmental samples leads to a high degree of uncertainty for risk assessment as this is largely dependent upon the organic carbon content and age of the particles. For example, direct

TABLE 1
Summary of WHO 1998 and WHO 2005 TEF Values

Compound	WHO 1998 TEF	WHO 2005 TEF
Chlorinated dibenzo-*p*-dioxins		
2,3,7,8-TCDD	1	1
1,2,3,7,8-PeCDD	1	1
1,2,3,4,7,8-HxCDD	0.1	0.1
1,2,3,6,7,8-HxCDD	0.1	0.1
1,2,3,7,8,9-HxCDD	0.1	0.1
1,2,3,4,6,7,8-HpCDD	0.01	0.01
OCDD	0.0001	**0.0003**
Chlorinated dibenzofurans		
2,3,7,8-TCDF	0.1	0.1
1,2,3,7,8-PeCDF	0.05	**0.03**
2,3,4,7,8-PeCDF	0.5	**0.3**
1,2,3,4,7,8-HxCDF	0.1	0.1
1,2,3,6,7,8-HxCDF	0.1	0.1
1,2,3,7,8,9-HxCDF	0.1	0.1
2,3,4,6,7,8-HxCDF	0.1	0.1
1,2,3,4,6,7,8-HpCDF	0.01	0.01
1,2,3,4,7,8,9-HpCDF	0.01	0.01
OCDF	0.0001	**0.0003**
Non-*ortho*–substituted PCBs		
3,3′,4,4′-tetraCB (PCB 77)	0.0001	0.0001
3,4,4′,5-tetraCB (PCB 81)	0.0001	**0.0003**
3,3′,4,4′,5-pentaCB (PCB 126)	0.1	0.1
3,3′,4,4′,5,5′-hexaCB (PCB 169)	0.01	**0.03**
Mono-*ortho*–substituted PCBs		
2,3,3′,4,4′-pentaCB (PCB 105)	0.0001	**0.00003**
2,3,4,4′,5-pentaCB (PCB 114)	0.0005	**0.00003**
2,3′,4,4′,5-pentaCB (PCB 118)	0.0001	**0.00003**
2′,3,4,4′,5-pentaCB (PCB 123)	0.0001	**0.00003**
2,3,3′,4,4′,5-hexaCB (PCB 156)	0.0005	**0.00003**
2,3,3′,4,4′,5′-hexaCB (PCB 157)	0.0005	**0.00003**
2,3′,4,4′,5,5′-hexaCB (PCB 167)	0.00001	**0.00003**
2,3,3′,4,4′,5,5′-heptaCB (PCB 189)	0.0001	**0.00003**

Bold values indicate a change in TEF value.

application of these WHO TEFs for assessment of OCDD or OCDF present in soil, sediment, or fly ash would lead to inaccurate assessment of the potential toxic potency of the matrix. This derives primarily from the fact that the highly hydrophobic PCDDs and PCDFs bind strongly to particles thereby significantly reducing their bioavailability for living organisms (Van den Berg *et al.*, 1994). As a result, application of these WHO TEFs for calculating the TEQ, e.g., OCDD and OCDF, in abiotic environmental matrices has limited toxicological relevance and use for risk assessment unless the aspect of reduced bioavailability is taken into consideration. Nevertheless, the expert panel recognized that it is now common practice to use the TEQ and associated TEFs directly to characterize and compare contamination by dioxin-like chemicals of abiotic environmental samples and is even codified in national and international legislation, e.g., the Stockholm Convention on Persistent Organic Pollutants.

In relation to this use of the TEQ, it should be emphasized that while these values by themselves do not have any toxicological implications or direct use in risk assessment, they can be a useful tool to compare concentrations within similar abiotic matrices and serve a prioritization function. Accordingly, it is recommended that when a human risk assessment is to be done from abiotic matrices, factors such as fate, transport, and bioavailibility from each matrix be specifically considered before a final estimate of the toxicological relevant TEQ is made. If a human risk assessment is done for abiotic matrices, the expert panel recognized that it would be preferable to use congener-specific equations throughout the whole model rather than base it on total TEQ in an abiotic matrix.

FUTURE RECOMMENDATIONS FOR DETERMINATION OF TEF VALUES

Previous WHO TEF reevaluations have used expert judgment and point estimates to establish congener-specific TEF values. In addition, the 1997 expert meeting indicated that TEF values were order of magnitude estimates (Van den Berg *et al.*, 1998). This statement was given irrespective of the type of congener, even though large differences are present in the REP studies of individual compounds (Haws *et al.*, 2006). When using point estimates and expert judgment, an advantage is that selection of a TEF can be made from those studies which are most relevant for human exposure (e.g., *in vivo* long term or subchronic). A disadvantage is that such an approach does not describe the range of REPs and may reflect a bias in judgment within the expert panel.

Recently, several authors have published papers in which they advocated the use of a probabilistic approach to determine TEFs (Finley *et al.*, 2003; Haws *et al.*, 2006). In using such an approach, there is a clear advantage because it will better describe the level of uncertainty present in a TEF value. The distribution of REPs can be expressed in terms of minimum and maximum values combined with percentiles at different levels (e.g., 25th and 75th percentiles). A disadvantage could be that such an approach lumps all data together and gives similar weight to all types of studies. In part, this problem could be avoided by separating *in vitro* from *in vivo* REPs (Haws *et al.*, 2006).

However, if probabilistic approaches for setting a future TEF are used, it is essential that weighting factors be applied to REPs that are determined from different types of studies. These weighted REP values could then be used to determine weighted REP distributions in the risk assessment process. Clearly, unweighted REP distribution ranges that bracket the TEFs incorporate biological and toxicological uncertainty. For this reason, in the WHO 2005 TEF reevaluation, unweighted REP distribution ranges, expert judgment, and point estimates were used in combination to assign TEFs. The sole use of a probabilistic approach to determine TEF values also includes other decision points, such as establishing a range instead of a point estimate for the TEF value. However, the use of a TEF range might cause problems for regulatory authorities and international harmonization of TEF values because one or more TEF values could then be selected for risk assessment calculations. This might easily lead to different TEFs being used by different countries depending on the level of conservatism used in the risk management process by national authorities. In this respect, the choice, e.g., of a 50th, 75th, or 95th percentile of the REP distribution range to assign a TEF is a risk management decision.

Similar to the use of WHO 2005 TEFs and TEQ with abiotic matrices, the application of these values to human tissue samples must be carried out with caution. This is because the present WHO TEF concept is, by default, primarily designed for intake situations. There is emerging evidence suggesting that the REP of certain dioxin-like compounds may differ when the REP is determined based on administered dose versus tissue concentration (Chen *et al.*, 2001; DeVito *et al.*, 2000; Hamm *et al.*, 2003). As a result, the use of systemic TEFs and TEQ has been suggested as an additional approach to the present WHO TEFs. From a biological and toxicological point of view, the development and use of systemic TEFs is recommended, but the expert panel was of the opinion that at present there is insufficient data to allow the development of systemic TEFs. If systemic TEFs would be developed in the future, TEF values based on blood lipid concentration might be the preferred choice. However, the use of intake TEFs from food is a valid approach for estimation of human body burdens since many of the concerns with issues of fate and transport when dealing with abiotic matrices do not exist and many of the pharmacokinetic issues are already (partially) dealt with during bioaccumulation and biomagnification up the food chain.

With respect to the use of systemic TEFs, it would also be useful to determine if *in vitro*–derived TEFs can potentially be used as surrogates for systemic TEFs derived from *in vivo* studies. If such a relationship does exist, this would allow a better use of the vast amount of *in vitro* data that has been obtained for dioxin-like compounds over the last few decades. In view of their direct biological relevance to humans, the expert panel proposed that systemic or body burden TEFs for humans should be developed in the near future. These body burden/systemic TEFs would allow a more accurate quantitative human dose-response assessment. However, it was also concluded by the expert panel that such systemic TEFs should be used in the future along with the 2005 WHO TEFs derived for ingestion situations as both types of TEFs have different valid applications. The TEQ based on intake TEFs can be used to monitor intervention programs, while systemic or body burden TEFs would be more applicable for biomonitoring systemic levels of dioxin-like chemicals in humans. In addition, body burden TEFs can also be used as the dose metric for interspecies extrapolation. At present, the WHO 2005 TEFs that are based on intake can be applied for characterization of exposure to dioxin-like chemicals in human blood or tissues and comparisons across populations, but these derived TEQ values have certain caveats from a risk assessment point of view.

CONCLUSIONS

Additivity, an important prerequisite of the TEF concept was found to be consistent with results from recent *in vivo* mixture studies (Fattore *et al.*, 2000; Gao *et al.*, 1999; Hamm *et al.*, 2003; Walker *et al.*, 2005). These studies showed that WHO 1998 TEF values predicted mixture toxicity within a factor two or less. Such accuracy is almost surprising in view of the fact that TEFs are derived from a range of REPs using different biological models or endpoints and are considered estimates with an order of magnitude uncertainty (Van den Berg *et al.*, 1998).

The expert panel recognized that there are studies providing evidence that non-dioxin–like AhR agonists and antagonists are able to increase or decrease the toxicity of 2,3,7,8-TCDD and related compounds. Accordingly, their possible effect on the overall accuracy of the estimated magnitude of the TEQ needs to be investigated further, but it does not impact the experimental determination of individual REPs or TEFs.

For this TEF reevaluation process, the expert panel made extensive use of the refined TEF database that was recently published by Haws *et al.* (2006). Decisions about a TEF value were based on a combination of unweighted REP distributions, expert judgment, and point estimates. The use of solely unweighted REP distributions to set a TEF value was rejected because a specific percentile would have to be used as a cutoff, which could equally well be considered as a point estimate. However, such a percentile would have a lower biological or toxicological relevance than that obtained by expert judgment.

Previous TEFs were assigned in increments of 0.01, 0.05, 0.1, etc., but for this reevaluation, it was decided to use half order of magnitude increments on a logarithmic scale at 0.03, 0.1, 0.3, etc. This should be more useful in describing, with statistical methods, the uncertainty of TEFs in the future. In Table 1, the WHO 1998 and 2005 TEF values are summarized.

Figure 4 gives some indication of the quantitative impact of the 2005 changes on WHO TEF values in some selected biotic samples. The changes are shown as the ratio between the 2005 and 1998 WHO TEF values. In general, it can be concluded that the changes in 2005 values have a limited impact on the total TEQ of these samples with an overall decrease in TEQ

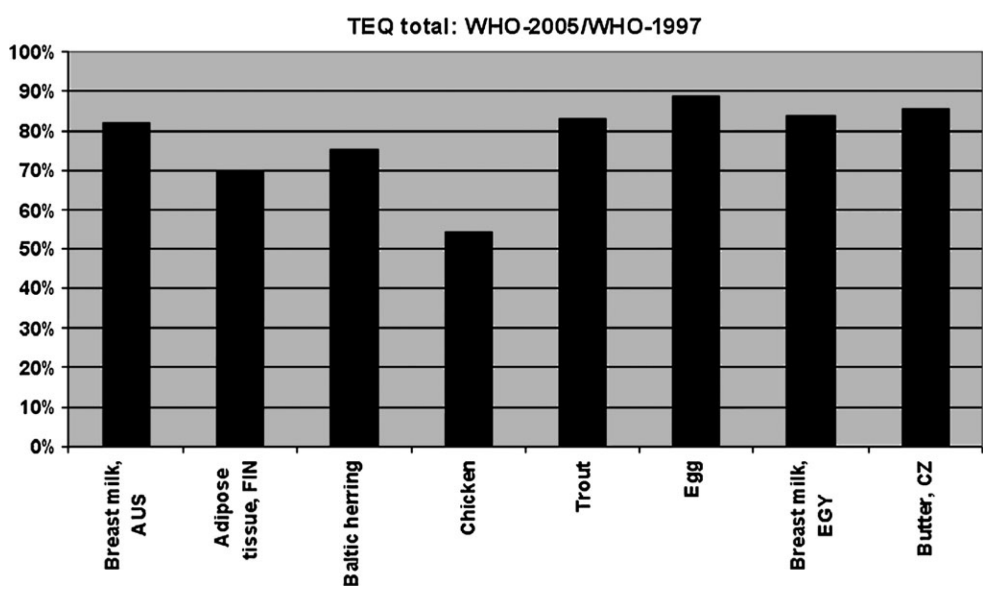

FIG. 4. Percent reduction in total TEQ levels calculated for the same biotic samples when 2005 TEFs rather than 1998 TEFs are used. For each biotic sample shown, the height of the bar is the percent that the total TEQ level determined using WHO 2005 TEFs is of the total TEQ level determined using WHO 1998 TEFs.

ranging between 10 and 25%. The exception being the chicken where the decrease of the TEF for 2,3,4,7,8-PeCDF (from 0.5 to 0.3) and of lower TEFs for the mono-*ortho* PCBs resulted in an almost 50% decrease of total TEQ. In view of this average impact of 10–25%, it should be realized that many duplicate GC-MS analyses for these compounds also have an uncertainty that can fall in the range of 10–25%.

Several groups of compounds were identified for possible future inclusion in the TEF/TEQ concept. Based on mechanistic considerations, PCB 37, PBDDs, PBDFs, PXCDDs, PXCDFs, PCNs, PBNs, and PBBs undoubtedly belong in the TEF concept. However, for most, if not all, of these compounds there is a distinct lack of human exposure data. Therefore, preliminary exposure assessments should be done in the near future to indicate if these compounds are relevant for humans with respect to TEQ dietary intake. In addition, HCB could be a possible candidate for inclusion in the TEF/TEQ concept but only if it is unequivocally shown that impurities have not been the cause of earlier dioxin-like effects observed in experimental models. With respect to PBDEs, it was concluded that there is no reason for their inclusion in the TEF/TEQ concept.

Concern is expressed about the application of the TEF/TEQ approach to abiotic environmental matrices, such as soil, sediment, etc. The present TEF scheme (see Table 1) and TEQ methodology are primarily meant for estimating exposure via dietary intake situations because present TEFs are based largely on oral uptake studies often through diet. Application of these "intake or ingestion" TEFs for calculating the TEQ in abiotic environmental matrices has limited toxicological relevance and use for risk assessment, unless the aspect of reduced bioavailability and environmental fate and transport of the various dioxin-like compounds are taken into account. If human risk assessment is done for abiotic matrices, it is recommended that congener-specific equations be used throughout the whole model, instead of using a total TEQ basis, because fate and transport properties differ widely between congeners.

A number of future approaches to determine alternative or additional TEFs were identified. The use of a probabilistic methodology to determine TEFs has the advantage that it better describes the level of uncertainty in a TEF. The disadvantage could be that this approach lumps data together and gives similar weight to all studies, a problem that can only partly be avoided by separating *in vitro* from *in vivo* REPs. In addition, the sole use of a probabilistic approach includes other decision points, e.g., establishing a range of values from which one or more TEF values could be selected for risk assessment. Clearly, such an approach might cause problems for regulatory authorities and international harmonization of TEFs. Furthermore, choosing a specific percentile (e.g., 50th, 75th, or 95th) would, in fact, not be far different from using a point estimate.

The use of the present TEF values with body burden matrices as blood and adipose tissue have certain caveats from a risk assessment point of view as they were determined from intake situations. There is emerging experimental evidence which suggests that some REPs may differ when based on administered dose versus tissue concentration. The development and use of systemic TEFs and TEQ are recommended as an additional approach to the present TEF concept, but at present, there are insufficient data to develop these systemic TEFs.

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