TECHNICAL SUPPORT DOCUMENT FOR CANCER POTENCY FACTORS

APPENDIX C

Use of the Toxicity Equivalency Factor (TEF$_{WHO-97}$) Scheme for Estimating Toxicity of Mixtures of Dioxin-Like Chemicals

Introduction

It has been recognized for many years that 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and a group of related chlorinated compounds (often collectively referred to as "dioxins") are ubiquitous environmental contaminants, principally derived from combustion sources. They are extremely toxic, with a wide range of effects at low doses including carcinogenicity, immunotoxicity and developmental toxicity. These observations were described in a report to the Scientific Review Panel for Toxic Air Contaminants (CDHS, 1986). On this basis the California Air Resources Board in 1986 identified TCDD and other dibenzo-*p*-dioxins and dibenzofurans chlorinated in the 2,3,7 and 8 positions which contain four, five, six, or seven, chlorine atoms, as Toxic Air Contaminants (TACs) for the purposes of AB1807, the California Toxic Air Contaminants Program. However, the initial analysis by CDHS (1986) was based on the limited data available at that time, especially the bioassays of TCDD and HCDD (Hexachlorodibenzo-*p*-dioxin).

In 1993, the California Legislature amended the California Toxic Air Contaminants Program by AB 2728, which required the ARB to identify the US EPA's 189 Hazardous Air Pollutants (HAPs) as TACs. This significantly broadened the identification of chlorinated dioxins and related compounds and added the polychlorinated biphenyls (PCBs, a specific category in the HAP list). Additionally, all the halogenated dibenzo-*p*-dioxins, dibenzofurans and biphenyls are identified under the general definition of polycyclic organic matter (POM).

The original report on chlorinated dioxins and dibenzofurans (CDHS, 1986) identified carcinogenicity as the critical effect for defining risk to public health and calculated a potency (slope factor) for TCDD of 1.3×10^5 (mg/kg-day)$^{-1}$. This was based on the incidence of liver tumors in male mice in a gavage study (NTP, 1982) and was calculated to be equivalent to a unit risk of 38 (μg/m^3)$^{-1}$ for airborne exposures.

Use of TEF Methodology in Risk Assessment

At the time of the 1980 NTP study (NTP, 1980), the only other chlorinated dioxins, beside TCDD, for which there were any data suitable for potency calculation were 1,2,3,6,7,8- and 1,2,3,7,8,9-hexachlorodibenzo-*p*-dioxin (HCDD), which were tested by NTP as a binary mixture in rats and mice. However, polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (PCBs) (Figure 1) are the most commonly monitored dioxins in abiotic and biotic samples. These compounds occur in the environment as complex mixtures of a large number of different congeners with varying degrees and positions of chlorine substitution. These congeners are

believed to have different carcinogenic potencies (and effectiveness in causing other toxic effects typical of chlorinated dioxins and dibenzofurans). In order to calculate the potency of these complex mixtures the Toxicity Equivalency Factor (TEF) approach was developed to express estimates of the carcinogenic potencies of various dioxin and dibenzofuran congeners relative to that of TCDD (van den Berg *et al.*, 2000). Thus, TEF indicates an order of magnitude estimate of the toxicity of a compound relative to TCDD, and numerous toxicological end points beside carcinogenicity are considered. Careful scientific judgment based on the examination of all available scientific data are used to derive consensus TEF values. Van den Berg *et al.* (1998) were members of the expert committee panel who evaluated the scientific information used to derive TEF$_{WHO-97}$ values. Scientific publications selected for this purpose are included in a database based on specific criteria (van den Berg *et al.*, 1998) such as:

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.

Compounds included in the database for the TEF scheme meet criteria of inclusion described in the next section. These compounds are the 2,3,7,8-substituted PCDDs and PCDFs and those PCBs with established dioxin-like activity, especially the non- and mono-*ortho* PCBs.

The US EPA, (2000) report "Exposure and Human Health, Reassessment of 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (TCDD) and Related Compounds" devotes a chapter to this topic. The use of TEFs is widespread in the scientific literature, but its usage as a key component of risk assessment has not been without controversy. In response to this controversy, the World Health Organization (WHO) has suggested that the Toxicity Equivalency (TEQ) scheme be reevaluated every 5 years and that TEFs and their application to risk assessment be re-analyzed to account for new scientific information (van den Berg *et al.*, 1998).

History of TEFs development

In 1983, based on a review of the scientific information available at the time, the Ontario Ministry of Environment (OME) set the basis for the TEF methodology by introducing the concept that PCDDs and PCDFs:

1. Are structurally similar compounds that share a common cellular mechanism of action (activation of the aryl hydrocarbon receptor [*Ah*R]), and
2. Produce similar biological and toxicological responses.

They also proposed that the toxic response of these compounds could be added up using a "toxic equivalence" scheme with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) as a reference compound to assess human risk (OME, 1984).

In the original health risk assessment for PCDDs and PCDFs conducted under the California Toxic Air Contaminants program (CDHS 1986), the Department of Health Services utilized two cancer bioassays conducted by the National Toxicology Program to estimate TEFs for the 2,3,7,8-chlorinated congeners (Table 1). These California TEFs were eventually replaced when the I-TEF scheme (described below) was adopted by OEHHA, following review by the Scientific Review Panel (SRP), in 1999.

In an analogous fashion to OME's and California's approaches, US EPA developed its own TEF methodology to characterize toxicity of complex mixture of PCDDs/PCDFs from waste incinerators. Rather than dividing PCDDs and PCDFs congeners into their respective homologue groups and then assigning a TEF number to each group, as proposed by OME, US EPA recommended that each congener receive a TEF number. The concentration of each monitored PCDD and PCDF congener could then be multiplied by its respective TEF value and all the products summed to give 2,3,7,8-TCDD equivalent concentration (TEQ) (US EPA, 1987). This approach is mathematically described as:

$$\text{Total Toxicity Equivalence (TEQ)} = \sum_{n=1}^{k} C_n * TEF_n$$

Where TEF_n = toxic equivalency factor of individual congener and C_n = concentration of the individual congener in the complex mixture.

In this approach, TEFs are determined by inspecting the available congener-specific data and by assigning an "order of magnitude" estimate of relative toxicity when compared to 2,3,7,8-TCDD. Scientific data considered in this scheme included: *in vitro* binding to the *Ah* receptor and *in vitro* and *in vivo* toxicity studies.

Subsequently, the North Atlantic Treaty Organization Committee on the Challenges of Modern Society, after conducting a 3-year study (NATO/CCMS, 1988), proposed from international consensus an International Toxicity Equivalency Factor (I-TEF) scheme. The committee recognized that the data reviewed in its study strongly support the role of the *Ah* receptor as a mediator in the biological and toxic response of 2,3,7,8-TCDD. Improvement from previous efforts in the determination of TEFs included: selection of TEF values based more on *in vivo* toxicity testing, assigning TEF values to octachlorodibenzo-*p*-dioxin and octachlorodibenzofuran and removing any TEF values for all non-2,3,7,8-substituted congeners. US EPA officially adopted the revised I-TEF in 1989 as the preferred method of calculating risks from exposure to dioxin-like compounds but with the recommendation that this risk assessment approach remains interim and that continued revisions should be made. Canada, Germany, Italy, the Netherlands, Sweden, the United Kingdom and the United States formally adopted the use of I-TEF model for risk assessment and risk management purposes (Yrjänheiki, 1992). This so-called "I-TEF" scheme was also used in California air

toxics programs. It was recommended for use in the Hot Spots program (AB 2588) by OEHHA (1999), and adopted after review by the SRP.

As TEFs for PCDDs and PCDFs were developed, considerable efforts went into the study of quantitative structure activity relationships (QSAR) for polychlorinated biphenyls (PCBs). PCB congeners substituted in the para and at least 2 of the meta positions but not at any of the ortho positions can adopt structural conformations most resembling that of 2,3,7,8-TCDD, therefore have the greatest potency and exert their toxicity through the *Ah* receptor pathway.   These coplanar PCB congeners are structurally similar to 2,3,7,8-tetrachorodibenzo-*p*-dioxin and therefore are termed dioxin-like PCBs. Introduction of one chlorine in the ortho position results in a decrease in toxic potency and PCBs with more than one chlorine in the ortho positions lack some effects exerted by non- and mono-ortho PCBs. These PCB congeners show a different spectrum of toxic effects (Safe, 1994).

In 1991, US EPA considered using the TEF methodology for PCBs. They noted that only a small subset of the 209 PCB congeners elicits dioxin-like activity and meet the criteria for inclusion in the TEF methodology.

In an attempt to harmonize TEF schemes for dioxin-like compounds, the World Health Organization - European Center for Environmental Health (WHO-ECEH) and the International Program on Chemical Safety (IPCS) generated a database consisting of almost 1,200 peer-reviewed publications, representing all the available toxicological data for PCBs up to the end of 1993. From a selected number of these publications and based on four inclusion criteria, the WHO-ECEH and the IPCS proposed TEF values for 13 dioxin-like PCBs (Ahlborg *et al.*, 1994). The inclusion criteria are:

1. The compound should show structural similarity to PCDDs and PCDFs.
2. It should bind to the *Ah* receptor.
3. It should induce dioxin-specific biochemical and toxic responses.
4. It should be persistent and accumulate in the food chain.

In addition, the first WHO PCB TEF consultation (Ahlborg *et al.*, 1994) recommended expanding the current database to include all relevant information on PCDDs, PCDFs and other dioxin-like compounds that satisfied the four inclusion criteria.

Some terminologies and definitions applicable to TEFs were reviewed prior to the second WHO-ECEH consultation (van Leeuwen, 1997). The term TEF, used in the past to describe any experimental end point to be compared with TCDD was reconsidered since not all end points are "toxic" end points. For example, end points such as binding to the *Ah* receptor and induction   of   ethoxyresorufin-O-deethylase   (EROD)   are   mostly   considered biological/biochemical responses. Therefore, experimental end points, for which numerical values are compared to the response to TCDD, should be termed "Relative Potency" values (REPs). These REPs could be the result of a single laboratory experiment looking at a single end point. REPs are derived from the available data either used as reported in each publication, or calculated by comparing dose-response curves or ratios of medium effective doses (ED_{50}), median lethal dose (LD_{50}), median effective concentration (EC_{50}) etc. A

chemical's TEF is then derived from all available REPs examined for that compound. Thus, the term TEF is be restricted to describe an overall estimate of the order-of-magnitude of the toxicity of a compound relative to the toxicity of TCDD. This estimate is derived by consensus, using careful scientific judgment of all available data (van Leeuwen, 1997; van den Berg *et al.*, 1998). The derivation of TEF consensus using *Ah* receptor-specific end points gives more weight to toxic responses than to biochemical (e.g., enzyme induction) responses and it puts more weight on *in vivo* data than on *in vitro* results. In fact, the weighting order of contributing *in vivo* data was: chronic > subchronic > subacute > acute.

In its most recent consultation in 1997, the WHO-ECEH proposed amendments to the previous NATO/WHO I-TEF scheme (NATO/CCMS, 1989). For revision of the existing mammalian TEFs, the WHO-ECEH committee agreed that if the available information was considered insufficient to warrant a change, the existing value would remain. The suggested WHO$_{97}$ TEFs for humans and mammals, including a short description of the recommendations for each of the congeners (PCDD/PCDF and PCB), are presented in Table A. Taking advantage of new data and understanding of the underlying mechanisms of toxicity of dioxin-like compounds, the WHO-ECEH's re-evaluation and extension of the TEF concept lead to the following amendments (Table 1 and Table 3). It should be noted that no change from the I-TEF value was recommended for 1,2,3,4,6,7,8-HpCDD. The listing of a different previous value in Part II, Table 9-2 of a draft of the US EPA's dioxin document (US EPA, 2000) is incorrect.

- For 1,2,3,7,8-PeCDD, an increase in TEF value from 0.5 to 1.0 was recommended, based on new *in vivo* tumor promotion data and CYP 1A1/A2 induction potencies from subchronic studies.
- For OCDD, the TEF value was reduced from 0.001 to 0.0001 based on a recalculation of the old data in which exposure versus tissue concentrations were compared (administered dose); originally the TEF was based on body burdens of the chemical following subchronic exposures.
- For OCDF, the TEF value was changed from 0.001 to 0.0001 based on new *in vivo* EROD induction potency values (81) and an expected structural similarity with OCDD; thus, for the *in vivo* situation, a change in analogy with OCDD is recommended...
- The TEF value for PCB 77 was reduced from 0.0005 to 0.0001 based on REPs from recent *in vivo* subchronic studies with EROD induction and hepatic retinol decreases; the new TEF value is lower than the previous value based on tumor promotion; in addition, impurities and background contamination (e.g. PCB 126) are identified problems in the data on this congener.
- A TEF value of 0.0001 was recommended for PCB 81. PCB 81 was not assigned a TEF value at the 1993 WHO consultation because of lack of human residue and experimental data. However, based on recent *Ah* receptor binding, *in vitro* CYP1A induction and QSAR calculations, a value similar to PCB 77 was suggested; it should be noted that *in vitro* REPs possibly over estimated 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.

- ▪ The previous interim TEF values for the di-ortho-substituted PCBs 170 and 180 were withdrawn because the limitations of the data set, which were recognized in 1993, still exist. Additionally, subchronic results *in vivo* with relevant end points (including CYP 1A1/A2 induction and reproductive toxicity) for the structurally similar congeners PCB 153 and 47 do not confirm the weak *Ah* receptor agonist properties reported from experiments *in vitro*.

Table 1 represents some of the various iterations in the TEF scheme development, which have been referenced in earlier California risk assessment guidance. For each of these iterations, it was noted that although TEF methodology is subject to several criticisms, it is still the best available approach to assess health risks from mixtures of dioxin-like chemicals. However, the WHO-ECEH recommended that the TEF methodology be reevaluated every five years in order to account for new scientific findings in this area. In 2002, WHO designated the Institute for Risk Assessment Sciences (IRAS) in Utrecht, Netherlands, as a Collaborating Centre, and their workplan includes the updating of the database on comparative toxicity of PCDDs and PCDFs. (Dr. Maged Younes, Department of Protection of the Human Environment, WHO, 2002, personal communication). The timetable for completion of the next review process has not been defined, although no major changes are foreseen for compounds currently listed in the TEF table.

Physiological basis of the TEF Methodology: The *Ah* Receptor

Mechanism of Dioxin Toxicity

Many PCDDs, PCDFs, coplanar PCBs and other structurally related polyhalogenated aromatic hydrocarbons are believed to share a common mechanism of action intimately related to similarities in their structural configuration. Most of what is known regarding the mechanism of action of these compounds is derived from research in three areas: structure-activity relationships for receptor binding and induction of a variety of biochemical and toxicological responses; genetic studies using inbred mouse strains; and studies at the molecular level that have elucidated key events in the actions of the receptor (Pohl *et al.*, 2000).

Most mechanistic studies to date support the assumption that binding to the aryl hydrocarbon receptor (*Ah*R) is a key first step prior to dioxin-like compounds eliciting a toxic and biochemical response. There are numerous reviews available on this subject (Birnbaum, I994a; b; Okey *et al*., 1994; Pohl *et al*., 2000; Poland and Knutson, 1987; Safe, 1990). Perhaps the strongest support of the role of the *Ah* receptor in mediating the toxicity of 2,3,7,8 -TCDD and related compounds arise from genetic data. Responsiveness of certain mouse strains to 2,3,7,8-dibenzo-*p*-dioxin toxicity appears to segregate with the presence of the *Ah* receptor locus (Poland and Glover, 1980). For instance, C57BL/6J mouse have an *Ah* receptor protein with a relatively high binding affinity for inducers of AHH such as 3-methylcholanthrene, ß-naphthoflavone, 2,3,7,8 -TCDD, and other isostereomers of 2,3,7,8 -TCDD, and are sensitive to the toxic effects of these chemicals. In contrast, DBA/2 mouse have an *Ah* receptor protein that has a lower ligand affinity (Okey *et al*., 1989), and are much less sensitive to the toxic effects of these compounds. Fernandez-Salguero *et al*. (1996),

using an *Ah* receptor-deficient mouse strain, also demonstrated the role of the *Ah* receptor protein in mediating the toxicity of 2,3,7,8-TCDD and other dioxin-like compounds. Thus, the hypothesis that TCDD and related compounds toxicity are initiated by binding to the *Ah* receptor is the general basis for the TEF scheme (Safe, 1990; Okey *et al.*, 1994; Birnbaum, 1994b; Birnbaum, 1994a; Hankinson, 1995). However, the events following *Ah* receptor-ligand binding that lead to the toxic response are however, not fully established.

The *Ah*R is a cytosolic protein present across species in target tissues and organs. This protein is a member of the basic Helix-Loop-Helix-Per-Arnt-Sim (bHLH-PAS) family of transcription factors. When bound to a ligand, the *Ah*R-ligand (*Ah*R-L) complex associates with another bHLH-PAS protein, the *Ah*R nucleus translocator (ARNT) in the nucleus of cells (Holmes and Pollenz, 1997). The heterodimeric DNA-binding protein complex, *Ah*R-L/ARNT, binds to the xenobiotic response elements (also termed dioxin response elements or DREs) of the DNA located near the 5' regulatory region of genes such as the CYP1A1 gene (Fisher *et al.*, 1989). DREs are dioxin-responsive regulatory DNA domains, which have the properties of a transcriptional enhancer (Fisher *et al.*, 1990; Neuhold *et al.*, 1986). They require both receptor protein and ARNT protein for enhancer function.

Thus, the ligand-bound *Ah* receptor does not itself bind DNA (Gasiewicz *et al.*, 1991) and the ARNT protein does not bind 2,3,7,8-TCDD, nor does it bind to DNA in the absence of the liganded *Ah* receptor protein (Whitelaw *et al.*, 1993). The binding, to the DRE, of the heterodimeric DNA-binding protein complex modulates the transcription of various genes like the CYP1A1 gene (Kawajiri *et al.*, 1995). In addition to the enhancer, the DNA upstream of the CYP1A1 gene has a second control element (a transcriptional promoter), which ensures that transcription is initiated at the correct site. Neither enhancer nor promoter functions in the absence of the other (Jones and Whitlock, 1990).

2,3,7,8-TCDD and related compounds, after binding to the *Ah* receptor, have been shown to alter the transcription and/or translation of a number of genes. These include several oncogenes and genes encoding growth factors, receptors, hormones and drug metabolizing enzymes (Birnbaum, 1994b; Birnbaum, 1994a). Also affected are certain enzymes and proteins (e.g. kinases) involved in various signal transduction processes, as well as cell cycle control (Birnbaum, 1994a,b; Weib *et al.*, 1996). *Ah*R-mediated gene expression is also involved in several critical life processes (e.g. cell type-specific differentiation, cell division, apoptosis) via signal transduction mechanisms (Micka *et al.*, 1997).

The activation of genes encoding for drug-metabolizing enzymes is important in both the metabolic potentiation of substrates to genotoxic reactive intermediates and ultimate carcinogens and the detoxification of toxic or carcinogenic drugs and other environmental pollutants. The elicited induction of certain drug metabolizing enzymes such as the cytochrome P450 1 subfamily (CYP1A1, CYP1A2 and CYP1B1) which is one of the most sensitive responses observed in a variety of different animal species, including humans. However, there exists a gap between knowledge of these changes and the degree to which they are related to the biological and toxicological end points elicited by 2,3,7,8-TCDD and related compounds (Pohl *et al.*, 2000).

There is some evidence suggesting that other *Ah*-receptor-mediated pathways, not dependent on the interaction of the *Ah* receptor with nuclear elements, may exist for the alteration of gene expression. Matsumura (1994) suggested that the interaction of 2,3,7,8-TCDD with the *Ah* receptor may initiate a phosphorylation / dephosphorylation cascade, which would subsequently activate other transcription factors. An increase in protein kinase activity was observed within 1-10 min following the addition of 2,3,7,8-TCDD to nuclear-free preparations of guinea pig adipose tissue (Enan and Matsumura, 1996). As discussed by Pohl *et al.* (2000), 2,3,7,8-TCDD may modulate signal transduction processes and gene expression by at least two pathways: through the direct interaction of the *Ah* receptor and its heterodimer partners with gene regulatory elements and from the initiation of a phosphorylation / dephosphorylation cascade and the subsequent modulated activity of other nuclear transcription factors. The prominent pathway may differ for acute versus chronic responses to the dioxin-like compounds and for particular developmental periods.

Ligand binding also results in rapid depletion of *Ah*R in both cell culture and animal studies (Pollenz *et al.*, 1998; Roman *et al.*, 1998). These findings lead to the hypothesis that a decrease in *Ah*R protein may be important in the regulation of *Ah*R-mediated-signaling (Pollenz *et al.*, 1998).

Thus, regulation of the *Ah*R could have important impact on the toxicological outcome of exposure to dioxin and dioxin-like chemicals. In developing rats, *Ah*R protein levels in ventral and dorsolateral prostate decreased with age, declining approximately 70% between postnatal days 1 and 21. ARNT (*Ah*R nuclear translocator) protein levels also decreased with age in dorsolateral, but not ventral prostate (Sommer *et al.*, 1999). This decrease was associated with a decrease in *Ah*R and ARNT mRNA. It was also shown that TCDD treatment (0.2, 1, 5, or 25 µg/kg po, 24 h) in adult male rats decreased *Ah*R but not ARNT protein in ventral and dorsolateral prostate, vas deferens and epididymis (Sommer *et al.*, 1999). This study also reported in *in utero* and lactational TCDD exposure (1.0 µg/kg dam p.o., from gestation day 15) a lack of response for ARNT levels, a decrease in prostatic *Ah*R protein levels on postnatal day 7 and a delayed developmental decrease in *Ah*R protein in ventral and dorsolateral prostate. Also, pretreatment of rat pups for 24 h with TCDD (5 µg/kg ip) down-regulated prostatic *Ah*R protein on postnatal day 7, but not on postnatal day 1. The authors concluded that prostatic *Ah*R and ARNT protein and mRNA levels are regulated with age, whereas only *Ah*R protein concentration is altered by TCDD exposure.

Polymorphism of the *Ah* Receptor

There is a great variability in the response of individuals following exposure to dioxins. In mice, a polymorphism based on a single nucleotide difference in the ligand binding domain of the *Ah* receptor is sufficient to reduce the affinity for ligands by more than 10-fold in non-responsive strains (Fernandez-Salguero *et al.*, 1996; Wong *et al.*, 1997). In humans, Needham *et al.* (1998) reported, in a follow-up study of the Seveso incident, that the mean serum lipid TCDD concentration of children with chloracne was 18,700 ppt with a range of 1,680 to 56,000 ppt. Yet, other individuals with as high or higher serum dioxin levels did not develop chloracne. The individual variability in sensitivity to TCDD was proposed to be due to polymorphism in the *Ah*R. In addition, Needham *et al.* (1998) also reported longer half-

life in serum TCDD for women and a biphasic half-life with an initial rapid phase in children, when compared to adult men (average half-life for TCDD = 7.8 years). Smart and Daly (2000) reported similar gender effect by measuring significantly lower levels of EROD activity in women compared to men (means = 4.50 and 9.01, respectively). Smart and Daly (2000) also showed an apparent, but not significant, difference between men and women (means 17246 U and 10520 U, respectively) for induced CYP1A1 protein levels.

As in experimental animals, human populations exhibit a greater than 20-fold range in the CYP1A1 inducibility/*Ah*R affinity phenotype. (Micka *et al.*, 1997). Determination of binding affinity toward TCDD in 86 human placenta samples showed a greater than twenty fold range in binding affinity. This range encompasses binding affinities similar to those observed in sensitive and resistant mice (Okey *et al.*, 1997).

Furthermore, Smart and Daly (2000), by screening both the CYPIAI gene and the *Ah* receptor gene (*Ah*R) for polymorphisms, reported interindividual variation of the order of 103-fold for the inducibility of CYPlA1 activity in humans. To measure CYP1A1 inducibility, the authors incubated lymphocytes from 30 volunteers (20 women and 10 men, age 25 – 37) with 3-methylcholantrene for 96-h and then monitored ethoxyresorufin-O-deethylase (EROD) activity in the cell suspension. However, this study group did not show any association between induced CYP1A1 activity and the presence of the novel alleles identified by single-strand conformational polymorphism (SSCP) analysis. Four genetic polymorphisms within the CYPIAI gene have been described and those alleles were termed: CYP1A1*2A, CYP1A1*2B, CYP1A1*3 and CYP1A1*4. The polymorphism coded by the allele CYP1A1*2A has been associated with increased lung cancer in a Japanese population. However, this finding was found to be not significant in a Caucasian population probably due to a lower frequency for this polymorphism in the Caucasian population. The allele CYP1A1*3 is specific to African American population and was not clearly associated with lung cancer susceptibility. As for the recently identified polymorphism CYP1A1*4, its function has not yet been elucidated. The SSCP methodology used in this study has been estimated to detect at least 80% of point mutations.

Polymorphism in the *Ah* receptor could also result in alteration in CYP1A1 inducibility. Screening for the 11 exons of the *Ah*R gene by SSCP analysis confirmed the existence of the previously described G1721A (guanine at position 1721 replaced by adenine) polymorphism in a Caucasian population and found a novel G1768A polymorphism. Individuals with at least one copy of the G1721A *Ah*R variant allele showed a significantly higher inducibility of CYP1A1 activity compared with individuals without the occurrence of the polymorphism (*p* = 0.0001) (Smart and Daly, 2000). Similar findings were obtained for induced CYP1A1 protein levels as determined by immunoblotting. It appears that genotype for the *Ah*R G1721A polymorphism shows a better correlation with induced CYP1A1 levels than several other factors including the genotype for several previously described CYP1A1 alleles. In the same line, Wong *et al.* (2001) reported two polymorphisms in *Ah*R that show apparent linkage disequilibrium with the codon 554 polymorphism: 1) a previously described polymorphism, V570I; 2) a novel human *Ah*R polymorphism, P571S. In addition, the authors noted that none of these variants showed abnormal ligand binding or DNA binding activities in an *in vitro* assay. However, the combined Ile(570) + Lys(554) variant *Ah*R form

failed to support induction of CYP1A1 in cells treated with TCDD (Wong *et al.*, 2001). This rare combination of variant genotypes appears to be restricted to individuals of African descent. The authors postulated that this genotype might result in reduced susceptibility to the carcinogenic effects of polycyclic aromatic hydrocarbons.

Gender-specific polymorphism for the *Ah*R was also found. Women showed significantly lower inducibility of CYP1A1 activity when compared to men. The gender difference for the *Ah*R was also demonstrated in rodents and in nonhuman primates. Furthermore, *in vitro* glucose uptake by adipose tissue was decreased in male guinea pigs treated with TCDD, but not in females. A similar pattern of response was observed in macaques (Enan *et al.*, 1996). Enan *et al.* (1996) also reported that TCDD induced lipid peroxidation in the adipose tissues of male guinea pigs, while it had no effect in females. Moreover, TCDD binding affinity studies in adipose explant tissues showed that tissues from male guinea pigs and monkeys had a higher binding capacity for TCDD than that from female tissues.

Smart and Daly (2000) observed that gender as well as genotypes for the G1721A *Ah*R polymorphism could be determinants of the level of induced CYP1A1 activity. Thus, the authors proposed that inter-individual variation in levels of induced CYP1A1 activity could be associated more with regulatory factors than polymorphism in the CYP1A1 gene. Good correlations have been found between structural polymorphisms in the gene and functional variants in various genetic strains of mice. In humans, however, work on polymorphisms and their possible role in gene function and cancer susceptibility is just beginning (Garte and Sogawa, 1999).

Ligands for the *Ah* Receptor

*Ah*R ligands include 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, "dioxin"), the most toxic member of the polyhalogenated aryl hydrocarbon (PHAH) family. The binding of PHAHs to the *Ah*R protein is an essential step in eliciting dioxin-like effects. Several other PHAHs such as 2,3,7,8-substituted polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and non-ortho substituted polychlorinated biphenyls (PCBs) also bind to the *Ah* receptor and induce toxic responses similar to those observed with TCDD. PCDDs, PCDFs and PCBs are industrial compounds or by-products that have been widely identified as environmental contaminants. Traces of these chemicals have been detected in fish, wildlife and humans. Moreover, for these PHAHs there is a rank order correlation between their structure-*Ah* receptor binding affinities and their structure-toxicity relationships. This supports a role for the *Ah*R in mediating these responses. (Safe, 1998).

One of the criteria for the inclusion of anthropogenic chemicals in the TEF methodology is their persistence and bioaccumulation in wildlife and humans. There are other anthropogenic and naturally occurring chemicals capable of binding to the *Ah*R. However, these chemicals are not included in the TEF scheme since they are either ligands with weak affinity for the *Ah*R, elicit toxic responses that are mostly mediated through a pathway other than that mediated by *Ah*R, or have a short half-life. Anthropogenic chemicals with affinity for the *Ah*R include industrial chemicals (polyhalogenated biphenyls, halogenated naphthalenes, chlorinated paraffins, etc.), pesticides (hexachlorobenzene) and contaminants

(polyhalogenated dioxins and furans) associated with various manufacturing, production, combustion and waste disposal processes (US EPA, 2000).   In addition, unsubstituted polycyclic aromatic hydrocarbons (PAHs) can also bind with moderate to high affinity to the *Ah*R (Poland and Knutson, 1987; Nebert, 1989; Chaloupka *et al.*, 1993).

Chemicals such as hexachlorobenzene are only weakly dioxin-like, and have significant toxicological effects that are not mediated by the *Ah* receptor. For classes of chemicals such as the chlorinated napthalenes, environmental concentrations and human exposure are uncertain. PAHs are not included either in the TEF scheme because of their short half-lives and relatively weak *Ah*R activity.   Brominated dioxins, benzofurans, biphenyls and naphthalene also induce dioxin-like effects in experimental animals (Miller and Birnbaum, 1986; Birnbaum *et al.*, 1991; Hornung *et al.*, 1996; DeVito *et al.*, 1997; Weber and Greim, 1997). The potency of brominated compounds in comparison to their chlorinated homologue depends on the specific congener (Birnbaum *et al.*, 1991; DeVito *et al.*, 1997). In general, exposure data for these chemicals are limited and exposure of the general population is unknown. However, there are currently concerns regarding the steadily increasing level of polybrominated diphenyl ethers (PBDEs) in the environment, wildlife and human tissue (de Wit, 2002; Hardy, 2002; Darnerud *et al.*, 2001; She *et al.*, 2002).   Thus, future TEF evaluation should investigate these chemicals. The next TEF evaluation by the WHO might consider these brominated chemicals if there are sufficient data to justify their inclusion in the TEF methodology.

In contrast to anthropogenic ligands for the *Ah*R, naturally occurring *Ah*R ligands have short half-lives, but nevertheless have frequently been cited in criticism of the TEF methodology. Naturally occurring *Ah*R-ligands include: indole derivatives (indole-3-carbinol (I-3-C), 3,3'-diindolylmethane (DIM), indolocarbazoles (ICZs) etc.), heterocyclic aromatic amines (HAAs) and oxidized essential amino acids.

Indole chemicals such as I-3-C and DIM are present in a variety of cruciferous vegetables. These two major secondary metabolites are capable of inducing phase I and II metabolic enzymes (CYP1A-dependent glutathione and glucuronyl transferases, oxidoreductases) in experimental animals (Bradfield and Bjeldanes, 1984; 1987), human cell lines (Bjeldanes et al., 1991; Kleman et al., 1994) and humans (Michnovicz and Bradlow, 1990; 1991). However, these compounds have a low binding affinity for the *Ah* receptor (Gillner et al., 1985). In contrast, indolo[3,2b]carbazole exhibits high binding affinity for the rodent *Ah*R (approximately equipotent to 2,3,7,8-tetrachlorodibenzofuran) and can induce CYP1A1 activity in cultured cells (Bjeldanes et al., 1991; Gillner et al., 1993; Chen et al., 1995). The ICZ family, of which indolo[3,2b]carbazole is a member, are byproducts of DIM - acid condensation reactions, or formed by bacterial metabolism of the common dietary amino acid tryptophan.

Other dietary *Ah*R ligands appear to be formed in cooked meat. Experimental animals and humans fed thermally treated meat protein (cooked meat) exhibited CYP1A2 induction (Degawa et al., 1989). The CYP1A2 induction was associated with the formation of a number of heterocyclic aromatic amines (HAAs) in human volunteers (Sinha et al., 1994). Oxidized essential amino acids, such as UV-oxidized tryptophan, were also shown to induce

CYP1A1 activity in mouse hepatoma cells through an *Ah*R-dependent mechanism and induced hepatic and pulmonary CYP1A1 activity in rats exposed *in vivo* (Sindhu et al., 1996).

The discovery of these naturally occurring *Ah*R ligands gave rise to vigorous criticism of the TEF methodology. It was proposed that calculation of the TEQ for dioxin-like contaminants could be skewed by the (dietary) intake of relatively high level of naturally occurring *Ah*R agonists (Safe, 1997) and thus lessen the suitability of the TEQ calculation. This criticism was, however rejected in the US EPA's assessment of dioxin health effects (US EPA, 2000). Although, it was proposed that more than 90% of the TEQ is derived from natural or dietary compounds (Safe, 1995), these naturally occurring *Ah*R ligands have short half-lives and generally do not bioaccumulate. In contrast, the PCDDs, PCDFs and PCBs included in the TEF methodology clearly bioaccumulate and have long biological half-lives, typically in years. Therefore, if contributions to the total TEQ are estimated on steady-state body burdens of these chemicals instead of daily intake, then TCDD and other PCDDs/PCDFs and PCBs contribute more than 90% of the total TEQ compared to the "natural" ligands (DeVito and Birnbaum, 1996; US EPA, 2000). This difference was further characterized by comparing indolo[3,2b]carbazole potency to TCDD. After 4 hours of exposure of Hepa-1 cells to these chemicals, the relative potency of indolo[3,2b]carbazole was 0.1 that of TCDD (Chen et al., 1995). However, after 24 hours of exposure to the same compounds, the relative potency of indolo[3,2b]carbazole compared to TCDD was 0.0001 (Chen et al., 1995). Moreover, it has been argued that the characteristic dioxin-like toxicity is only manifested by highly persistent chemicals which cause stimulation of the *Ah* pathway over long periods of time; short-term stimulation may result only in the induction of the cytochrome P450 and Phase II enzymes which are effective in clearing most *Ah*R-binding chemicals from the body. OEHHA agrees with the US EPA view.

These results illustrate the importance of considering pharmacokinetic factors in the inclusion of compounds in the TEF methodology. Although some chemicals, including chemicals that occur naturally, bind to the *Ah*R and some may elicit dioxin-like activity, it is clearly not sufficient to be considered in the TEQ calculation. Other toxicological factors, such as biological half-life, exposure and toxicity data *in vivo* should be considered.

Basis of TEF and TEQ Calculation: the Assumption of Additivity

The TEF/TEQ methodology is based on the scientific assumption that the *Ah*R mediates the biochemical and toxicological actions of dioxin-like chemicals. Another essential assumption in the development of the TEF methodology is the one of additive interactions. Although there are numerous scientific reports on the synergistic or antagonistic interaction of mixture of dioxin-like and/or non-dioxin-like chemicals with TCDD, reports on the additive effects of dioxin-like chemicals predominate. Several published studies aimed to validate the concept of the TEF methodology as a tool to predict the risk of exposure to dioxin-like chemical mixture.

Group of 20 male and 20 female rats were gavaged with five doses of a mixture of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), 1,2,3,7,8-pentaCDD, (PeCDD) 1,2,3,4,7,8-hexaCDD

(HxCDD) and 1,2,3,4,6,7,8-heptaCDD (HpCDD) divided into four daily loading doses and six biweekly maintenance doses (Viluksela *et al.*, 1998a). Positive control groups were treated with PeCDD and HxCDD. The animals were dosed for 13 weeks and then divided into two groups; half of the rats were killed and the rest were provided with a 13-week off-dose period. Viluksela *et al.* (1998a) reported a dose-dependent increase in liver ethoxyresorufin-O-deethylase (EROD) activity, an enzyme associated with CYP1A1 induction and a dose-dependent decrease in liver phosphoenolpyruvate carboxykinase in rats dosed with the mixture. There was also a dose-dependent decrease of serum thyroxin (T_4) in the mixture-, PeCDD- and HxCDD-treated groups. Other effects elicited by exposure to the mixture included: decreased liver tryptophan 2,3-dioxygenase (TdO) activity, increased serum tryptophan concentrations and decreased concentration of serum glucose. Positive control group responses followed a pattern similar to that of the mixture-treated groups.

In a follow-up experiment using the same experimental conditions, Viluksela *et al.* (1998b) reported a dose-dependent weight gain reduction in mixture-treated rats. The authors concluded that TEFs derived from acute studies could be used to predict the toxicity of mixtures of polychlorinated dibenzodioxins regardless of whether they are administered as single compounds or as a mixture. Their results confirmed the notion of additive toxicity for the polychlorinated dibenzodioxins and validated the TEF methodology (Viluksela *et al.*, 1998a; 1998b).

Gao *et al.* (2000) exposed gonadotropin-primed immature female rats (23-day of age) to individual congeners: 2,3,4,7,8-pentachlorodibenzofuran (PeCDF), 3,3',4,4',5-pentachloro-biphenyl (PeCB) and 2,2',5,5'-tetrachlorobiphenyl (TCB), or a mixture of polychlorinated dibenzo-*p*-dioxins (PCDDs). The PCDD mixture included : 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), 1,2,3,7,8-pentachlorodibenzo-p-dioxin (PeCDD) and 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin (HxCDD) in addition to PeCDF and PeCB. Equine chorionic gonadotropin (eCG; 5 IU) was injected 24 h after dosing to induce follicular development. At the day of expected ovulation, 72 h after eCG injection, treatment with the individual congener PeCDF, PeCB and/or their mixture with PCDDs generated parallel dose-response curves for the inhibition of the eCG-induced ovulation. Serum concentrations of 17-β-estradiol (E2) were increased by PeCDF, PeCB and the mixture. In contrast, serum progesterone (P4) and follicle stimulating hormones (FSH) were decreased at that same time point. Ovarian histology revealed ova in large preovulatory follicles and a lack, or a reduced number, of corpora lutea for rats treated with PeCDF, PeCB and the mixture. These histological effects were very similar to those observed in PCDDs-treated rats. The authors concluded that these findings and the similarity in the slope of the dose-response relationships for the individual congeners (PeCDF and PeCB) and their equipotent mixture with PCDDs support the concept of toxic equivalency (TEQ) for the inhibition of ovulation (Gao *et al.*, 2000).

van der Plas *et al.* (2001) exposed female Sprague Dawley rats to a complex mixture of dioxin-like polyhalogenated aromatic hydrocarbons (PHAHs) covering more than 90% of the total toxic equivalents (TEQs) contaminants present in Baltic herring. The severe decrease in hepatic retinoid level observed in the rats treated with the dioxin-like PHAH mixture was similar to the effect of a TEQ equivalent dose of 1 µg 2,3,7,8-TCDD/kg body weight/week.

However, plasma retinol decrease could not be predicted using the TEF concept. Treatment with the dioxin-like PHAH mixture decreased plasma retinol by 21 % whereas an increase of 21 % was observed in TCDD-treated rats. Total plasma thyroid hormone exhibited a more severe decrease in the PHAH mixture-treated group when compared to the TCDD-treated group (60 and 38 %, respectively). The authors noted that the discrepancy between the observed and the predicted effects for plasma retinol and thyroid hormone levels could be attributed to the additional effect of hydroxylated PCBs formed by metabolism of PHAHs present in the mixture.

Additionally, van der Plas *et al.* (1999) exposed female Sprague Dawley rats to a laboratory derived mixture of PHAHs in a medium term two-stage initiation/promotion bioassay. The PHAH mixture containing 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (PeCDD), 2,3,4,7,8-pentachlorodibenzofuran (PeCDF), 3,3',4,4',5-pentachlorobiphenyl (PCB 126), 2,3',4,4',5-pentachlorobiphenyl (PCB 118), 2,3,3',4,4',5-hexachlorobiphenyl (PCB 156), 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153) represented more than 90% of the total toxic equivalents (TEQ) contaminants present in Baltic herring. Diethylnitrosamine injection (30 mg/kg body wt i.p.) 24 h after a partial 2/3 hepatectomy was used as an initiator. Starting six week after initiation, rats were given subcutaneous injections of the PHAH mixtures, alone or in combination with PCB 153, a di-ortho substituted PCB, weekly for 20 weeks. PHAH treatment caused liver enlargement and increased activity in hepatic cytochrome P450 1A1, 1A2, 2B1 and 2B2. In addition, rats dosed with 1 µg TEQ PHAH mixture/kg body weight/week exhibited a significantly lower volume fraction of liver occupied by foci in comparison to the TEQ equivalent TCDD-dosed group (3.8 and 8.7%, respectively). When the PHAH mixture was administered in combination with PCB 153, the volume fraction of liver with foci was significantly increased by 0.5, 1 and 2 µg TEQ/kg body wt/week (4.5, 5.2 and 6.6%, respectively) compared to the control group (2% of liver volume containing foci). However, this increase was less than the increase expected based on the TEQ doses. The authors concluded that the TEQ-based administered dose overestimated the observed tumor-promoting effects of the PHAH mixture by a factor of two. Nevertheless, the authors noted that their results support the assumption of additivity for the TEF methodology (van der Plas *et al.*, 1999).

Gao *et al.* (1999) orally exposed immature female rats (23-day old) to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (PeCDD) and 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin (HxCDD) as single congener or as a mixture of the three congeners. Rats were primed 24 h later with gonadotropin to induce follicular maturation. A dose-dependent decrease in the number of ova per ovary and a reduction of ovarian weight gain induced by gonadotropin were observed. The parallelism of the dose-response curves generated by individual congeners and/or their mixture indicates a common mechanism of action. In addition, treatment with polychlorinated dibenzo-*p*-dioxins enhanced gonadotropin-induced increase in serum 17-ß-estradiol (E2) in rats on the day of expected ovulation. Serum progesterone (P4) and follicle stimulating hormone (FSH) were decreased at that same time point. The authors concluded that the impacts of the tested polychlorinated dibenzo-*p*-dioxins on ovulation and on the reproductive hormones (e.g., LH, FSH, P4 and E2) result from the same mechanism of action (Gao *et al.*, 1999). Furthermore,

the parallelism of the dose-response curves for the individual congeners and their equipotent mixture represents a validation of the TEQ concept for the inhibition of ovulation.

Groups of five adult female Sprague Dawley rats were gavaged with 0, 2.5, 25, 250 or 1000 ng TCDD/kg body weight/day or TCDD in combination with a mixture of PCB congeners (PCBs) at 2 or 20 μg/kg bw/day for a period of 28 days (Wong *et al.*, 1997). Rats treated with either 1000 ng TCDD alone, or the mixture of 1000 ng TCDD + 2 μg PCBs showed growth suppression, increased absolute and relative liver weights and decreased thymic weight. Of these three TCDD-induced responses, only growth suppression appears to be altered by co-administration of TCDD and PCBs. Growth suppression appeared to be more pronounced in the group receiving 1000 ng TCDD + 2 μg PCBs than the one receiving TCDD alone. Hepatic microsomal methoxy resorufin-O-demethylase (MROD) and ethoxy resorufin-O-deethylase (EROD) activities were significantly increased in 250 and 1000 ng TCDD-treated rats, but co-administration of PCBs antagonized these responses. Similarly, co-administration of 20 μg PCBs and 250 ng TCDD elicited statistically significant antagonistic effects on serum cholesterol and on liver UDP glucuronyl transferase activity and ascorbic acid. On the other hand, co-administration of 1000 ng TCDD with PCBs did not affect the following responses: increased serum albumin, decreased liver vitamin A and increased kidney vitamin A and liver microsomal glutathione-S-transferase activity. Based on these results, the authors concluded that the effects of the PCB mixture and TCDD may be additive or antagonistic depending on the dose level and end points measured. Knowledge of mechanisms of actions and toxicokinetics is therefore required to predict toxicity for mixtures containing PCBs (Wong *et al.*, 1997). The discrepancy in the effects may be partly explained by the fact that the typical PCB mixture contains a substantial proportion of the ortho-substituted PCBs that are inactive in terms of dioxin-like toxicity, but are well known to have other significant effects, including an anti-thyroid hormone action. It is worth noting that PCB commercial mixture may vary greatly, from lot to lot, in the proportion of ortho-substituted PCBs (Burgin *et al.*, 2001; Kodavanti *et al.*, 2001).

Körner *et al.* (2001) compared the inductive potency of PCDD and PCDF mixtures to that of TCDD in Wistar rats treated 16 times (every 3rd day) subcutaneously with a defined mixture. Each single dose of the PCDD mixture and PCDF mixture was calculated to contain 57 or 39 ng I-TEQ/kg body weight, respectively. Both mixtures contained a large excess of non-2,3,7,8-substituted congeners. Based on EROD induction in rat hepatic microsomes, the authors reported a 3 to 4-fold overestimation of the I-TEQ factors for the concentration range tested in this study. Based on liver concentration of the mixture, the concentration-response curves for both the PCDD and PCDF mixtures run parallel to the curve for 2,3,7,8-TCDD. Körner *et al.* (2001) explained the discrepancy in potency between the mixtures and TCDD by the differential kinetic, tissue distribution and elimination half-life of the various congeners. Moreover, the authors noted that a 3 to 4 fold discrepancy from TCDD TEQ is well below the 10-fold safety factor normally used in risk assessment. Thus, these results support the additivity concept.

DeVito *et al.* (2000) dosed mice, by gavage, with either 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), 2,3,3',4,4'-pentachlorobiphenyl (PCB 105), 2,3',4,4',5-pentachlorobiphenyl (PCB 118), 3,3',4,4',5-pentachlorobiphenyl (PCB 126), or 2,3,3',4,4',5-hexachlorobiphenyl (PCB

156), five days/week for 13 weeks. Based on the ethoxyresorufin-O-deethylase activity in liver, lung and skin and the acetanilide-4-hydroxylase activity in liver, the relative potency for these congeners varied across end points by a factor of less than 4 when calculated based on an administered dose. In general, for every chemical, the relative potency for at least one response differed by an order of magnitude or more from the other responses. The authors concluded that the relative potencies may be dose-dependent (DeVito *et al.*, 2000).

Harper *et al.* (1995) investigated the immunosuppressive effect of several polychlorinated biphenyl (PCB) mixtures and congeners on the splenic plaque-forming cell (PFC) response and serum IgM units to the antigen, trinitrophenyl-lipopolysaccharide in female B3C3F1 mice. The ED50 values for Aroclor 1260-, 1254-, 1248- and 1242-induced immunotoxicity varied by less than twofold from 355 to 699 mg/kg. The immunotoxicity-derived toxic equivalency factors (TEFs) for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), and some chlorobiphenyl (CB) compounds: 3,3',4,4'-tetraCB, 3,3',4,4',5-pentaCB, 3,3',4,4',5,5'-hexaCB, 2,3,3',4,4'-pentaCB, 2,3',4,4',5-pentaCB, 2,3,3',4,4',5-hexaCB, 2,3,3',4,4',5,5'-heptaCB, 2,2',3,3',4,4',5-heptaCB, and 2,2',3,4,4',5,5'-heptaCB could be calculated from the ratios ED50(TCDD)/ED50(congener). The TEF values were within the range of those previously determined for other aryl hydrocarbon receptor-mediated responses. Based on the known concentrations of these congeners in the PCB mixtures, TCDD or toxic equivalents (TEQs) in the mixture were calculated [i.e., TEQ = Σ (PCB congener x TEF)] using the immunotoxicity-derived TEFs (plaque-forming cells/10^6 viable cells). TEQ values for Aroclors1260, 1254, 1248 and 1242 were 16.0, 54.4, 260.4 and 197 ppm, respectively. Based on the ED50 value for the immunosuppressive activity of TCDD (4.8 µg/kg), the calculated ED50 values for immune suppression by Aroclors 1260, 1254, 1248 and 1242 were 300, 88, 18 and 24 mg/kg, respectively. The ED50 (observed)/ED50 (calculated) ratios were 1.2, 5.9, 21 and 22.0 for Aroclors 1260, 1254, 1248 and 1242, respectively. The authors concluded that for Aroclors 1254, 1248 and 1242, the high ED50 (observed)/ED50 (calculated) ratios (i.e., 5.9 to 22.0) indicate that the TEF approach overestimates the toxicity of these mixtures due to non-additive (antagonistic) interactions of the PCBs. In contrast, the TEF approach was useful in determining the immunotoxicity of the Aroclor 1260 mixture (Harper *et al.*, 1995).

Dioxin-like PCBs, *i.e.* the non-ortho- and mono-ortho-substituted congeners, elicit *Ah*R-mediated responses. Studies with mixtures of PCB 126 or PCB 156 and TCDD found little deviation from additivity. The additivity assumption was further confirmed by fish early life stage studies with binary mixtures of PCDDs, PCDFs and PCBs congeners or complex mixtures of dioxin-like and non-dioxin-like PCDDs, PCDFs and PCBs at environmentally relevant dose ratios and dose levels (Walker and Peterson, 1991; Walker *et al.*, 1996; Zabel *et al.*, 1995). The validity of the additivity assumption was assessed by determining the significance of interactions between pairs of polychlorinated dibenzo-*p*-dioxin (PCDD), dibenzofuran (PCDF) and biphenyl (PCB) congeners when injected into newly fertilized rainbow trout eggs in ratios relevant to those found in feral lake trout eggs from the Great Lakes (Zabel *et al.*, 1995). Most of the congener pairs tested acted additively in causing rainbow trout early life stage mortality: 1,2,3,7,8-PCDD/TCDD; 2,3,4,7,8-PCDF/1,2,3,7,8-PCDD; 2,3,4,7,8-PCDF/TCDD; 2,3,7,8-tetrachlorodibenzofuran/2,3,4,7,8-PCDF; 3,3',4,4'-tetrachlorobiphenyl (PCB 77)/3,3',4,4',5-pentachlorobiphenyl (PCB 126); 2,3,3',4,4'-

pentachlorobiphenyl (PCB 105)/TCDD; 2,2',4,4',5,5'-hexachlorobiphenyl/TCDD; PCB 105/ PCB 126; and 2,3',4,4',5-pentachlorobiphenyl/PCB 126. However, TCDD/PCB 77 and TCDD/PCB 126 showed evidence of a statistically significant interaction that deviated from additivity. From these data, Zabel *et al.* (1995) concluded that the use of fish-specific TEFs might not exactly predict the mortality risk posed to fish early life stages by the mixture of TCDD-like congeners in the eggs. Nevertheless, the relatively small deviation (1- to four-fold) from additive interaction in this study warrants the additivity assumption in assessing the risk to fish early life stage mortality posed by TCDD and related compounds in eggs. In general, a safety factor of 10-fold is used for non-carcinogenic end points.

Walker *et al.* (1996) examined the toxicity equivalency (TEQ) additive properties of 11 TCDD-like congeners and three non-TCDD-like congeners combined at ratios typically found in Lake Michigan lake trout. Early life stage mortality elicited by the mixture or TCDD alone exhibited parallel dose-response curves in lake trout and rainbow trout. Based on LD50 values, the dose-response curves for the mixtures were significantly shifted to the right of the TCDD dose-response curves by 1.3- and 1.8-fold for lake trout and rainbow trout, respectively. The data suggest that TCDD-like congeners, although acting through a common mechanism of toxicity in early life development in fish, may not act strictly additively when combined in a mixture of TCDD- and non-TCDD-like congeners at ratios found in Great Lakes fish. However, Walker *et al.* (1996) concluded that the slight deviation from additivity was below the safety factor of 10-fold commonly used in ecological risk assessments. Therefore, the toxicological equivalency additive model could be used for assessing risk posed by complex mixtures of PCDDs, PCDFs and PCBs to fish (Walker *et al.*, 1996).

Thus, the predominance of additive interactions between dioxin-like PCDDs, PCDFs and PCBs supports the concept of TEF. This is true for various species of fish, birds and mammals exposed to congener dose ratios and congener levels at environmentally relevant doses (Safe, 1990; 1994; Cook *et al.*, 1997; van den Berg *et al.*,1998).

Uncertainties Associated with the Use of the TEF Methodology

Quantifying uncertainty surrounding the TEF estimate is difficult. TEF estimates are generated from several sources of experimental data and for some congeners can vary by several orders of magnitude. This apparent variability has been attributed to different exposure regimens, test species, or purity of the test compound (US EPA, 2000). For tests involving exposure to commercial mixtures of PCBs such as Aroclor 1254, significant differences in the composition profile of PCB congeners (Kodavanti *et al.*, 2001) and toxicological responses (Kodavanti *et al.*, 2001; Burgin *et al.*, 2001) were reported to exist between lots.

It is worth noting that TEF estimates are point estimates. They are derived from scientific judgment based on examination of relative exposure potency (REP) for various end points. However, these semi-quantitative judgments are made in the context of risk assessment, and provide valuable insight in the estimation of TEQs.

Variability in estimated REPs for individual congeners may not significantly impact risk estimates. For example, using WHO TEF values (van den Berg *et al.*, 1998) to look at background exposure from a typical U.S. diet, it is clear that only a limited number of congeners significantly contribute to the total TEQ. More than 60% of the TEQ$_{WHO-97}$ associated with background dietary exposure (1 pg/kg/d) is attributable to only four congeners: 2,3,7,8-TCDD (8%), 1,2,3,7,8-PeCDD (21.5%), 2,3,4,7,8-PeCDF (10.7%) and PCB 126 (21%) (US EPA, 2000). The variability in the REP values reported in the literature for these congeners is much lower than for other congeners that contribute minimally to background TEQ. Since the TEFs for the major congener constituents of background exposure (or other exposure with a similar congener profile) have consistently been determined empirically to be within a factor of 2-3, it is therefore unlikely that the estimated TEQ overestimates the "true" TEQ by more than a factor of five (US EPA, 2000). Moreover, non-dioxin-like PCBs at background level are unlikely to significantly affect the uncertainty of TEQ estimates as discussed later in this section.

The assumption of additivity is essential in the TEF approach. Although antagonistic and/or synergistic interactions are seen at some dosage ratios and doses of congeners for specific toxicity end points, these types of interaction are seldom observed. Rather, additivity appears to be the most common interaction reported in the scientific literature describing *Ah* receptor-mediated chemical toxicity.

Criticisms concerning the TEF approach mainly focus on four areas (van den Berg *et al.*,, 2000).
1. Non-additive interaction of dioxin-like congeners when there is co-exposure to non-dioxin-like congeners, particularly PCB 153.
2. Differences in species responsiveness.
3. Differences in the shape of the dose-response curves between individual *Ah* receptor agonists
4. Mono-ortho PCBs in the TEF concept

Non-additive interactions

Non-additive interactions in mixtures containing both PCDDs/Fs and specific ortho-substituted PCBs such as PCB 153 (a di-ortho substituted PCB representing a major environmental contaminant) have been observed in laboratory studies (Safe, 1997). Several "nondioxin-like" PCB, including 2,2',4,4',5,5'-hexachlorobiphenyl (hexaCB) and commercial PCBs exhibit "anti-dioxin" or *Ah* receptor antagonist activity (Biegel *et al.*, 1989; Davis and Safe, 1989, 1990).

Commercial PCB mixtures such as Aroclor 1254 and 2,2',4,4',5,5'- hexaCB inhibit TCDD-induced immunotoxicity and fetal cleft palate (teratogenicity) in C57BL/6J mice, an *Ah*-responsive strain (Biegel *et al.*, 1989). 2,2',4,4',5,5'-Hexachlorobiphenyl at doses as high as 750 to 1000 μmol/kg did not cause fetal cleft palate, suppress the splenic plaque-forming cell response to sheep red blood cells, or induce hepatic microsomal ethoxyresorufin O-deethylase (EROD) in C57BL/6J mice. However, cotreatment of TCDD and 2,2',4,4',5,5'-hexachlorobiphenyl or Arochlor 1254 in mice partially antagonized these *Ah* receptor-

mediated responses to TCDD (Biegel *et al.*, 1989).   The authors observed a competitive binding of the antagonists for the *Ah* receptor.

Moreover, Davis and Safe (1989) have demonstrated a significant antagonistic interaction in mice treated with a 25 mg/kg dose of commercial polychlorinated biphenyl (PCB) mixtures Aroclors 1260, 1254, 1248, 1242, 1016 and with a 50 mg/kg dose of a reconstituted PCB mixture (resembling a PCB extract from human milk) with TCDD (3.7 nmol/kg).   The interaction significantly antagonized the TCDD-mediated inhibition of the splenic plaque-forming cell response in C57BL/6J mice.

Zhao *et al.* (1997) reported similar results.   They compared the toxicity of 3,3',4,4',5-pentachlorobiphenyl to that of cotreatment to 3,3',4,4',5-pentachlorobiphenyl and 2,2',4,4',5,5'-hexaCB for the following end points: the induction of fetal cleft palate in offspring from C57BL/6 mice, the inhibition of splenic plaque-forming cell (PFC) response and the decrease serum IgM levels. Zhao *et al.* (1997) reported an antagonistic interaction between 2,2',4,4',5,5'-hexaCB and 3,3',4,4',5-pentachlorobiphenyl.

Nonadditive interactions between 2,2',4,4',5,5'-hexaCB and PCBs such as 3,3',4,4',5-pentaCB and 3,3',4,4',5,5'-hexaCB were also reported by Harper *et al.* (1995) in female B3C3F1 mice for the inhibition of the splenic plaque-forming cell (PFC) response and serum IgM units to the antigen, trinitrophenyl-lipopolysaccharide.

Wolfle (1998) used *in vitro* assays of transformation of carcinogen-initiated C3H/M2 mouse fibroblasts to study interactions of mixtures. They reported an additive promoting effect of a defined mixture of PCB126 and TCDD but PCB 153 antagonized the TCDD-mediated promotion. The authors concluded that the TEF-approach may be insufficient to estimate the tumor-promoting activities of PCDDs, PCDFs and PCBs in mammalian tissues in which di-ortho substituted PCBs are greatly accumulated.

Thus, nonadditive interactions between different classes of PCBs present in environmental samples, suggest that the TEF approach may overestimate the effective TEQ for some responses in animal models (Safe, 1998). Safe (1997) noted in his review that the deviations from additive interaction reported in the literature were associated with a lack of data on the actual tissue concentration or body burdens of the congeners. The deviation from additivity could also be associated with exposure to ortho-substituted PCBs, particularly with PCB 153 in combination with dioxin-like PCDDs and PCDFs.

With respect to the non-additive interaction between TCDD and PCB 153 on CYP1A1 induction, it was observed that at lower dose levels the interaction appears to be synergistic, while at the higher dose levels antagonistic effects were observed (van den Berg *et al.*, 1994). Mechanistically, the antagonistic interaction was explained by the competition for binding to the *Ah* receptor of the compound (less potent) with a low binding affinity for the *Ah*R (Astroff *et al.*, 1988; Biegel *et al.*, 1989). Similarly, additive interaction with a mixture of dioxin-like PCBs is hindered when increasing concentration of non-dioxin-like PCBs are added to the mixture (Schmitz *et al.*, 1995). For liver tumor promotion in rodents, prediction

of this effect is complicated by the dose-dependent accumulation of dioxin-like PCDDs, PCDFs and PCBs in the liver (Tritscher *et al.*, 1991; Mills and Andersen, 1993). Pharmacokinetic interaction between PCBs has also been reported. Lee *et al.* (2002) observed an increased PCB 153 retention in the liver and a decreased PCB 153 accumulation in the fat of nonpregnant C57BL/6 mice coadministered with PCB 153 (20 mg/kg) and PCB 126 (0.2 mg/kg). However, little or no significant pharmacokinetic interactions were observed in lactating mice and suckling pups. The shift of the accumulated PCB 153 from fat to liver could possibly be linked to the induction of CYP1A2 protein by PCB 126. TCDD and 2,3,4,7,8-PeCDF (a dioxin-like dibenzofuran) was shown to be sequestrated mostly in the liver in mice expressing CYP1A2 gene (Diliberto *et al.*, 1999). This disposition pattern of TCDD and 2,3,4,7,8-PeCDF was in contrast with the one observed in knockout mice lacking CYP1A2 expression.

Differences in species responsiveness

Species differences in the functional responses to TCDD and related dioxin-like compounds could be important (Peterson *et al.*, 1993). Van den Berg et al, (2000) proposed several factors to explain species differences in response to *Ah* receptor agonists. These factors include toxicokinetics, receptor distribution and affinity, agonistic action on receptor upon binding etc. However, most biological effects caused by dioxin-like compounds occur at levels of dioxin-like compounds that differ by less than one order of magnitude between species (DeVito *et al.*, 1995). There is a large difference between species in the pharmacokinetics of TCDD and related compounds. In addition, liver/adipose tissue distribution can vary significantly between species and dose levels used. Highly potent congeners such as 2,3,4,7,8-PeCDF, 2,3,7,8-TCDD and PCB 126 accumulate in the liver because of their tight binding to the CYP1A2 enzyme (DeVito *et al.*, 1995; DeVito and Birnbaum, 1995; DeVito *et al.*, 1995; Diliberto *et al.*, 1997). This feature is more pronounced in rodents than humans, with monkeys being in the intermediate position (van den Berg *et al.*, 2000). The non-linear hepatic accumulation disappears at lower dose levels (10 ng TCDD/kg) (Abraham *et al.*, 1988). Similar findings were observed in humans in the case of the Yusho incident. Highly exposed individuals had a liver/adipose tissue ratio 2 to 3 orders of magnitude higher than that of individuals exposed to background levels of 2,3,4,7,8-PeCDF. Thus, it seems that the non-linear hepatic accumulation of PCDDs/PCDFs observed in rodent studies only occurs at dose levels used to determine relative potencies of PCDDs and PCDFs. Such nonlinear hepatic accumulation of PCDDs/PCDFs is unlikely to occur in humans exposed to these chemicals at background levels (van den Berg *et al.*, 2000). Differences in tissue distribution can significantly influence TEF values, when they are based on tissue concentrations (DeVito and Birnbaum, 1995).

Several authors showed, from studies in various human cell types and tissues, that human *Ah* receptors have a range of binding affinities for TCDD generally below but overlapping that observed in rodent strains (Harper *et al.*, 1986; 1988; Roberts *et al.*, 1986; Lorenzen and Okey, 1991). However, CYP1A1 inducing activities of TCDD and PCDD congeners were found to be 10 fold less potent in human primary hepatocytes and HepG2 cells than in the respective rat model (Lipp *et al.*, 1992; Schmitz *et al.*, 1995). On the other hand, the effectiveness of inducers of CYP1Al activity in human and rat hepatoma cells differed only

moderately (Lipp *et al.*, 1992), and human and rat thymus transplanted into severe combined imunodeficient (SCID) mice showed similar sensitivity to TCDD immunotoxicity (Vos *et al.*, 1998).

In general, the binding affinity data of different *Ah*R ligands has limited usefulness as a predictor of agonist activity. Induction potency of CYP1A1 in cell culture for a number of *Ah*R ligands was poorly correlated with *Ah*R binding affinity (Santostefano *et al.*, 1992). Rather, the DNA binding form of the *Ah*R seems to be a better predictor of *Ah*R agonist potency.

Differences in the shape of the dose-response curves for individual *Ah* receptor agonists

Different agonists for the *Ah*R exhibit different dose-response curve shapes. For instance, the maximal efficacy of OCDD as an inducer of CYP1A1 is much lower than that of TCDD in rat primary hepatocytes (Schrenk *et al.*, 1991). Taking into account the numerous *Ah*R independent factors involved in *Ah*R-mediated toxicity, different slopes of the dose-response curves for dioxin-like compounds are to be expected (van den Berg *et al.*, 2000). However, for tests *in vitro* where toxicity end points are linked to *Ah*R activation in a relatively simple fashion, dose-response slopes for potent PCDDs and PCDFs are generally reported to be similar. Induction of CYP1A1 activity in hepatocytes by dioxin-like PCBs generates dose-response curves with similar slopes. However, such is not the case for PCB 77, since this congener is readily metabolized. Also, relatively high amounts of non-dioxin-like PCBs lead to alterations in slope of the concentration-response curve of the mixture (Schmitz *et al.*, 1995).

Mono-ortho PCBs in the TEF concept

Mono-ortho PCBs represent a particular case since these congeners can elicit end points such as carcinogenicity, porphyrin accumulation, and alterations in circulating thyroid hormone concentrations (Khan *et al.*, 2002). Moreover, neurotoxicity in mammalian species could arise by both *Ah* receptor-mediated and non-*Ah* receptor-mediated mechanisms (van den Berg *et al.*, 2000). Di-, tri- and tetra-ortho PCBs can also share the non *Ah*R-mediated pathway, which introduces more uncertainty in the risk assessment process determination especially when considering end points common to both of these pathways (van den Berg *et al.*, 2000). Thus, end points with clearly recognized *Ah*R-mediated mechanisms should be selected for the determination of TEF values when mono-ortho PCBs are involved.

Implication of the new TEF methodology

The TEF methodology is the best available tool for the health risk assessment of complex mixture of dioxin-like chemicals. Assuming dose-additivity of the various components of a chemical mixture, it uses the toxicological equivalent mass of TCDD to evaluate risk. Clearly, to consider each component of a chemical mixture as having the toxicological potency of TCDD would be overestimating the potential health risk of the mixture. The use of the TEF method allows for a more accurate estimate of the health risks. However, in using the TEF methodology, one must bear in mind that although the various congeners of a

mixture have relative equivalent toxicity to TCDD, these congeners do not necessarily share the same environmental fate as TCDD. Consequently, the profile of chemical constituents in a mixture could change as the released mixture moves away from its source and as it ages over time. Also, other chemicals such as di-ortho PCBs, not eliciting toxicological effects through the *Ah*R-mediated pathway and endogenous dioxin-like compounds not included in the TEF methodology might bias the risk assessment estimate obtained from the TEF methodology. Thus, improvements to the TEF methodology should include risk assessment methods considering not only *Ah*R-mediated toxicological responses but also those mediated by other toxicological pathways.

Many values set for regulation and assessment of PCDD/PCDF are based on toxic equivalent concentrations (TEQs). These values include emission limits for industrial plants, tolerable daily intake (TDI) and environmental quality standards. However, the use of different TEF schemes, or even the absence of TEQ calculations, in the available databases make the interpretation and comparison of data quite difficult. Thus, the change from the presently used I-TEQ in California to the more recent TEF_{WHO-97} appears appropriate. The TEF_{WHO-97} is based on more sensitive toxicological end points, and includes dioxin-like PCBs.

There may be changes to the total TEQ estimates from a variety of sources. It was suggested by Dyke and Stratford (2002) that the change from the I-TEF to the TEF_{WHO-97} could generally result in a 1-10% increase of the calculated TEQ for the emissions to air. In contrast, some sludge samples, which generally contain a predominance of highly chlorinated PCDD/PCDF congeners, showed a substantial decrease in the TEQ calculation (up to 70%) when the WHO TEF scheme rather than the I-TEF was applied. This TEQ decrease in sludge samples is explained by the lower TEF values for the highly chlorinated PCDD/PCDF proposed in the WHO TEF scheme. The change for the WHO TEFs increased calculated exposure by 10-20% when considering PCDD/PCDF levels in food and calculations of exposure. In addition, the inclusion of "dioxin-like" PCBs in the WHO TEF scheme as well as the modification of the TEF table for PCDDs/PCDFs substantially increased calculated TEQ in food and overall TEQ exposure (Dyke and Stratford, 2002). However, the findings presented by these authors can only be considered as indicative of the possible changes produced by the adoption of the WHO TEF scheme, since there were no comprehensive data used in this analysis.

California data were also considered to estimate the consequences of using the TEF_{WHO-97} values rather than the I-TEF scheme (Table 4). These results are in agreement with findings reported by Dyke and Stratford (2002). Total TEQ PCDD/F emission measured in air decreased by less than 10% when the TEF_{WHO-97} scheme was used in place of the I-TEF. However, a 500% increase in total TEQ was calculated for fish (striped bass) from the San Francisco Bay using TEF_{WHO-97} rather than the I-TEF scheme. This difference is mostly attributable to the inclusion of PCB congeners in the TEQ_{WHO-97} calculation (0.60 versus 3.45 pg/g TEQ as calculated according to the I-TEF and TEF_{WHO-97} schemes, respectively (Table 4).

Conclusion

In view of the preceding analysis of the TEF concept and the extensive discussion and refinement to which it has been subjected in the scientific literature, it appears reasonable to continue to recommend the use of this approach in assessing risks from dioxin-like chemicals in the environment. However, there have been advances in knowledge since the I-TEF table currently used by California's air toxics regulatory programs. Where congener specific concentration data are available to support the calculation of TEQs, California risk assessment programs should from now on use the revised WHO-ECEH TEF table (van Leeuwen, 1997; van den Berg *et al.*, 1998; see Table 2) in estimating cancer risk from dioxin-like compounds. In addition to reflecting the more recent advances in scientific understanding, this change is consistent with current recommendations by US EPA (2000).

Apart from a few changes in individual equivalency factors, the major difference between the new TEF table and the previous one is the inclusion of the coplanar PCB congeners as compounds with dioxin-like activity. In California risk assessment guidance, this represents an addition to the compounds previously considered by the TEF methodology, although these compounds are already identified as TACs, separately from the dibenzo-*p*-dioxins and dibenzofurans. In the wider context the inclusion of the dioxin-like PCBs is not particularly novel, having been recommended by an earlier WHO expert committee (Ahlborg *et al.*, 1994).

Where cancer risk is the critical end point in a risk assessment, and individual PCB congener concentration or dose data can be obtained, the TEF methodology should be used. The PCB associated risk is considered additive to that associated with concurrent exposures to other dioxin-like compounds. Under this methodology, it is assumed that the dioxin-like (coplanar) PCB congeners are responsible for all cancer risk attributable to exposure to a PCB mixture. Other toxicological end points, associated with the non-coplanar PCBs, would continue to be assessed using the earlier methodology (based on non-cancer toxicity values for specific mixtures) for these compounds, although in future OEHHA may propose a TEF-type methodology for these compounds based on their characteristic toxic end points, which differ from those associated with the dioxin-like compounds. The previous methodology for cancer risk assessment of PCBs, using potency estimates for particular mixtures and a measure of total PCB abundance, will necessarily continue to be used where measurement of individual congeners cannot be achieved (*e.g.*, analysis of historical data on total PCB emissions or occurrence).

Summary

The scientific basis for the TEF/TEQ methodology is mostly derived from the concept of *Ah* receptor mediated biochemical and toxicological effects of dioxin-like compounds. Since its initial development in 1983, the TEF methodology has continuously evolved taking advantage of the most recent findings in toxicology studies. Although several limitations in the TEF approach have been identified, it is yet the best available method to evaluate the health risk of complex mixture of dioxins and related compounds commonly measured in abiotic and biotic samples. Numerous countries including the United States have adopted the

TEF methodology. Studies with environmental and laboratory made mixtures have demonstrated the adequacy of the TEQ dose-additivity in the risk assessment of dioxin-like compounds. The exclusion of non dioxin-like compounds from the TEF methodology represents perhaps the most obvious limitation of the TEF approach. Thus, the TEF approach has been adopted by interested parties under the condition that the TEF methodology remains an interim method and that it should be reevaluated periodically. Clearly, the ultimate goal should aim to include non dioxin-like compounds in order to have a more accurate estimate of the health risk caused by these persistent and bioaccumulative chemicals. At this point, it is important for public health protection that the most scientifically relevant TEF methodology be used. Therefore, the TEF_{WHO-97} table of equivalency values is to be used in place of the I-TEF. The TEF_{WHO-97} is based on the latest scientific findings available, and it includes dioxin-like PCBs (coplanar PCBs) that contribute to the total TEQ concentration of abiotic and biotic samples. It also facilitates the comparison of environmental measurements to other international databases.

Table 1.   TEF values used or proposed in California.

Congener	California TEF [a]	I-TEF [b]	TEF $_{WHO/97}$ [c]
PCDDs			
2,3,7,8-TCDD	1	1	1
1,2,3,7,8-PeCDD	1	0.5	1
1,2,3,4,7,8-HxCDD	0.03	0.1	0.1
1,2,3,6,7,8-HxCDD	0.03	0.1	0.1
1,2,3,7,8,9-HxCDD	0.03	0.1	0.1
1,2,3,4,6,7,8-HpCDD	0.03	0.01	0.01
1,2,3,4,6,7,8,9-OCDD		0.001	0.0001
PCDFs			
2,3,7,8-TCDF	1	0.1	0.1
1,2,3,7,8-PeCDF	1	0.05	0.05
2,3,4,7,8-PeCDF	1	0.5	0.5
1,2,3,4,7,8-HxCDF	0.03	0.1	0.1
1,2,3,6,7,8-HxCDF	0.03	0.1	0.1
1,2,3,7,8,9-HxCDF	0.03	0.1	0.1
2,3,4,6,7,8-HxCDF	0.03	0.1	0.1
1,2,3,4,6,7,8-HpCDF	0.03	0.01	0.01
1,2,3,4,7,8,9-HpCDF	0.03	0.01	0.01
1,2,3,4,6,7,8,9-OCDF		0.001	0.0001
PCBs (IUPAC #, Structure)			
77 3,3',4,4'-TCB			0.0001
81 3,4,4',5-TCB			0.0001
105 2,3,3',4,4'-PeCB			0.0001
114 2,3,4,4',5-PeCB			0.0005
118 2,3',4,4',5-PeCB			0.0001
123 2',3,4,4',5-PeCB			0.0001
126 3,3',4,4',5-PeCB			0.1
156 2,3,3',4,4',5-HxCB			0.0005
157 2,3,3',4,4',5'-HxCB			0.0005
167 2,3',4,4',5,5'-HxCB			0.00001
169 3,3',4,4',5,5'-HxCB			0.01
170 2,2',3,3',4,4',5-HpCB			0
180 2,2',3,4,4',5,5'-HpCB			0
189 2,3,3',4,4',5,5'-HpCB			0.0001

‎   = Value introduced or changed

[a] CDHS, 1986   [b] NATO/CCMS, 1989.   [c] van Leeuwen, 1997.

Table 2. WHO/97 Toxic Equivalency Factors (TEFs) (from van Leeuwen, 1997)

Congener	TEF$_{WHO-97}$
PCDDs	
2,3,7,8-TCDD	1
1,2,3,7,8-PeCDD	1
1,2,3,4,7,8-HxCDD	0.1
1,2,3,7,8,9-HxCDD	0.1
1,2,3,6,7,8-HxCDD	0.1
1,2,3,4,6,7,8-HpCDD	0.01
1,2,3,4,6,7,8,9-OCDD	0.0001
PCDFs	
2,3,7,8-TCDF	0.1
1,2,3,7,8-PeCDF	0.05
2,3,4,7,8-PeCDF	0.5
1,2,3,4,7,8-HxCDF	0.1
1,2,3,7,8,9-HxCDF	0.1
1,2,3,6,7,8-HxCDF	0.1
2,3,4,6,7,8-HxCDF	0.1
1,2,3,4,6,7,8-HpCDF	0.01
1,2,3,4,7,8,9-HpCDF	0.01
1,2,3,4,6,7,8,9-OCDF	0.0001
PCBs (IUPAC #, Structure)	
77 3,3',4,4'-TCB	0.0001
81 3,4,4',5-TCB	0.0001
105 2,3,3',4,4'-PeCB	0.0001
114 2,3,4,4',5-PeCB	0.0005
118 2,3',4,4',5-PeCB	0.0001
123 2',3,4,4',5-PeCB	0.0001
126 3,3',4,4',5-PeCB	0.1
156 2,3,3',4,4',5-HxCB	0.0005
157 2,3,3',4,4',5'-HxCB	0.0005
167 2,3',4,4',5,5'-HxCB	0.00001
169 3,3',4,4',5,5'-HxCB	0.01
189 2,3,3',4,4',5,5'-HpCB	0.0001

Figure 1.　　　Structures of Dioxin-like Compounds (from Nevalainen and Kolehmainen, 1994).
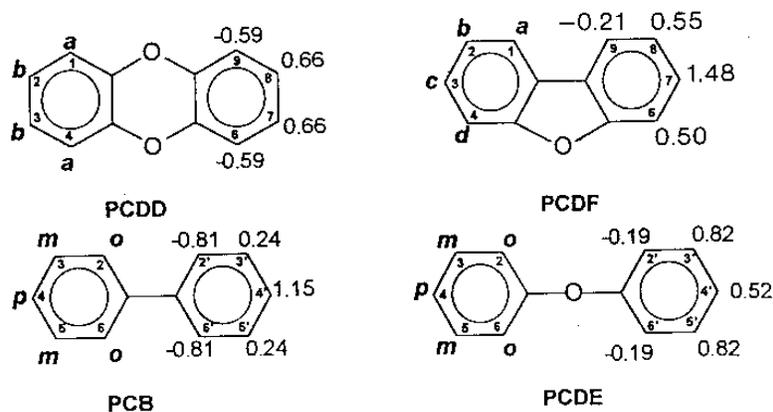
The coefficients for dioxin-like activity for chlorine substituents at different positions in the dibenzo-*p*-dioxin (PCDD), dibenzofuran (PCDF), biphenyl (PCB) and diphenyl ether rings are shown.　A positive value indicates positive activity and a negative (-) sign, negative activity.

Table 3: Toxic Equivalency Factors (TEFs) changes for humans and mammals, and a description of the basis for recommending to keep or amend TEF values.

Congeners	Description	Old TEF	WHO-TEF
Dioxins			
1,2,3,7,8-PentaCDD	Based on new *in vivo* tumor promotion data and CYP 1A1/A2 induction potencies from subchronic sudies (79-91); 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 LD50 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 value between 0.02 and 0.03 for various end points (85,86); no revision is recommnended	0.01	0.01
OctaCDD	A recalculation of the old data in which exposure versus tissue concentrations were compared suggest that a lower value is more appropriate (87).	0.001	0.0001
Furans			
2,3,7,8-TetraCDF	Recent simichronic studies generally support a value of 0.1 (88,89); less emphasis was put on LD50 value; no revision is recommended	0.1	0.1

Congeners	Description	Old TEF	WHO-TEF
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 recommnended	0.05	0.05
2,3,4,7,8-PentaCDF	Most REPs dosing with relevant topic 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
1,2,3,4,8,9-HexaCDF	Additional *in vivo* potency values on non specific 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 recommnended	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/PCB 126) 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 persisitent *in vivo* (95). Comfirmation 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 revison 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 report a change in analogy with OctaCDD.	0.001	0.0001
Non-ortho PCBs			
3,3',4,4'-TetraCB (PCB 77)	Recent subchronic studies with EROD induction and hepatic retinol decreases suggest a value around or below 0.0001 (95-98); which is lower than the previous value based on tumor promotion (99); however, impurities 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 (PCB 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 over estimated 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 (PCB 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, 108, 109); no revision is recommended.	0.1	0.1

Congeners	Description	Old TEF	WHO-TEF
3,3',4,4',5,5'-HexaCB (PCB 169)	Recent in vivo EROD induction and thymic atrophy values support a conservative value of 0.01 for this congenier. Confirmation of recent immunotoxicity data possibly indicating a higher REP is strongly recommended (91, 106); no revision is recommended	0.01	0.01
Mono-orthoPCBs	For this group of congenrs 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 CYP 1A1/A2 induction and reproductive and teratogenic effects similar to TCDD.		
2,3,3',4,4'-PentaCB (PCB 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, 108). The immunotoxicity data suggest a TEF between 10^{-5} and 10^{-3} while acute *in vivo* values for non specific end points, e.g., liver weight gain and body weight decrease, are in the range of 10^{-8} and $\underline{10^{-4}}$, no revision is recommended	0.0001	0.0001
2,3,4,4',5-PentaCB (PCB 114)	There are no new data which suggest that the current value is not appropriate. Older Ah receptor binding and enzyme induction data need comfirmation regarding the present value (100, 101, 107); at present, no revison is recommended.	0.0005	0.0005
2,3,4,4',5-PentaCB (PCB 118)	New data supoort the original value (91, 95, 108); no revision is recommended	0.0001	0.0001
2,3,4,4',5-PentaCB (PCB 123)	No new data are available; no revision is recommended	0.0001	0.0001
2,3,3',4,4',5-HesaCB (PCB 156)	Recent data from semichronic studies suggest that the old value is appropriate (109). Higher REP measuring non specific end points, e.g., effects on thyroid hormone 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 (PCB 157)	No new relevant *in vivo* data available; no revision is recommednded	0.0005	0.0005
2,3,4,4',5,5'-HexaCB (PCB 167)	No new data are available; no revision is recommendedé	0.00001	0.00001
2,3,3',4,4',5,5'-HeptaCB (PCB 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 exist. *In vivo* subchronic assays result with relevant end points including CYP 1A1/A2 induction and reproductive toxicity, with the structurally similar congener PCB 153 (98, 104, 110-112) do not comfirm the weak Ah receptor agonist properties reported from *in vitro* experiments (11, 113, 118, as a result the previous TEF values of di-ortho has beeen withdrawed	-	-
2,2',3,3',4,4',5-HeptaCB (PCB 170)	Withdraw.	0.0001	-

Congeners	Description	Old TEF	WHO-TEF
2,2',3,4,4',5,5'-HeptaCB (PCB 180)	Withdraw.	0.00001	-

Old TEF: refers to the I-TEF for PCDDs/PCDFs and to the WHO-94 TEF for PCBs
WHO TEF: refers to the TEF_{WHO-97}
Numbers in parenthesis refer to citations of the van den Berg *et al.* (1998) paper
Source: van den Berg *et al.*, 1998

Table 4: Comparison of combustion emissions TEQ calculation by I-TEF vs. WHO/97

Dioxin samples: Congener	I-TEF	WHO TEF	West Long Beach 11/27/88 (a)			San Bernardino 11/27/88 (a)			Marion Co. incinerator (b)		
			pg/m³	I-TEQ	WHO-TEQ	pg/m³	I-TEQ	WHO-TEQ	pg/m³	I-TEQ	WHO-TEQ
2,3,7,8-TCDD	1	1	0.0143	0.0143	0.0143	0.0106	0.0106	0.0106	81	81	81
1,2,3,7,8-PeCDD	0.5	1	0.043	0.0215	0.043	0.048	0.024	0.048	9	4.5	9
1,2,3,4,7,8-HxCDD	0.1	0.1	0.043	0.0043	0.0043	0.076	0.0076	0.0076	7	0.7	0.7
1,2,3,6,7,8-HxCDD	0.1	0.1	0.019	0.0019	0.0019	0.063	0.0063	0.0063	8	0.8	0.8
1,2,3,7,8,9-HxCDD	0.1	0.1	0.019	0.0019	0.0019	0.066	0.0066	0.0066	8	0.8	0.8
1,2,3,4,6,7,8-HpCDD	0.01	0.01	0.3	0.003	0.003	0.429	0.00429	0.00429	138	1.38	1.38
OCDD	0.001	0.0001	2.05	0.00205	0.000205	0.93	0.00093	0.000093	184	0.184	0.0184
2,3,7,8-TCDF	0.1	0.1	0.019	0.0019	0.0019	0.024	0.0024	0.0024	168	16.8	16.8
1,2,3,7,8-PeCDF	0.05	0.05	0.0203	0.001015	0.001015	0.035	0.00175	0.00175	10	0.5	0.5
2,3,4,7,8-PeCDF	0.5	0.5	0.01	0.005	0.005	0.035	0.0175	0.0175	15	7.5	7.5
1,2,3,4,7,8-HxCDF	0.1	0.1	0.085	0.0085	0.0085	0.081	0.0081	0.0081	4	0.4	0.4
1,2,3,6,7,8-HxCDF	0.1	0.1	0.06	0.006	0.006	0.035	0.0035	0.0035	4	0.4	0.4
2,3,4,6,7,8-HxCDF	0.1	0.1	0.035	0.0035	0.0035	0.033	0.0033	0.0033	5	0.5	0.5
1,2,3,7,8,9-HxCDF	0.1	0.1	0.04	0.004	0.004	0.033	0.0033	0.0033	5	0.5	0.5
1,2,3,4,6,7,8-HpCDF	0.01	0.01	0.125	0.00125	0.00125	0.404	0.00404	0.00404	7	0.07	0.07
1,2,3,4,7,8,9-HpCDF	0.01	0.01	0.015	0.00015	0.00015	0.086	0.00086	0.00086	10	0.1	0.1
OCDF	0.001	0.0001	0.425	0.000425	0.0000425	0.252	0.000252	0.0000252	36	0.036	0.0036
Total TEQ (pg TCDD equiv/m³)				0.08069	0.0999625		0.105322	0.1282582		116.17	120.472

(a) Ambient dioxin data. From: letter from ENSR to Mr. R. Propper, CARB, 5/16/1989. In: CARB, 1990. Many of the entered levels are based on detection limit.

(b) Flue gas emissions, municipal waste/power generation facility, Marion Co., OR. From: Dioxin Emissions from Resource Recovery Facilities: a review of the existing data base. CAPCOA/ARB/USEPA, 1989.

Neither of these sources reported measuring any levels of PCBs. Any such additional contaminants, if present, would add to the total TEQ observed by the WHO/97 method, but not by the I-TEF method.

Table 5: Comparison of striped bass TEQ calculation by I-TEF vs. WHO/97

Dioxin/PCB Samples Congener	I-TEF	WHO TEF	Striped Bass #1 pg/g	I-TEQ	WHO-TEQ
2,3,7,8-TCDD	1	1	0.13	0.13	0.13
1,2,3,7,8-PeCDD	0.5	1	0.2	0.1	0.2
1,2,3,4,7,8-HxCDD	0.1	0.1	0.24	0.024	0.024
1,2,3,6,7,8-HxCDD	0.1	0.1	0.16	0.016	0.016
1,2,3,7,8,9-HxCDD	0.1	0.1	0.21	0.021	0.021
1,2,3,4,6,7,8-HpCDD	0.01	0.01	0.17	0.0017	0.0017
OCDD	0.001	0.0001	0.3	0.0003	0.00003
2,3,7,8-TCDF	0.1	0.1	0.74	0.074	0.074
1,2,3,7,8-PeCDF	0.05	0.05	0.14	0.007	0.007
2,3,4,7,8-PeCDF	0.5	0.5	0.31	0.155	0.155
1,2,3,4,7,8-HxCDF	0.1	0.1	0.14	0.014	0.014
1,2,3,6,7,8-HxCDF	0.1	0.1	0.14	0.014	0.014
2,3,4,6,7,8-HxCDF	0.1	0.1	0.17	0.017	0.017
1,2,3,7,8,9-HxCDF	0.1	0.1	0.18	0.018	0.018
1,2,3,4,6,7,8-HpCDF	0.01	0.01	0.28	0.0028	0.0028
1,2,3,4,7,8,9-HpCDF	0.01	0.01	0.2	0.002	0.002
OCDF	0.001	0.0001	0.29	0.00029	0.000029
PCB 77	0	0.0001	64.5	0	0.00645
PCB 81	0	0.0001	nr		
PCB 105	0	0.0001	1000	0	0.1
PCB 114	0	0.0005	nr		
PCB 118	0	0.0001	3000	0	0.3
PCB 123	0	0.0001	nr		
PCB 126	0	0.1	23.3	0	2.33
PCB 156	0	0.0005	0	0	0
PCB 157	0	0.0005	0	0	0
PCB 167	0	0.00001	nr		
PCB 169	0	0.01	2	0	0.02
PCB 189	0	0.0001	0	0	0
TEQ Dioxins (pg TCDD equiv/g)				0.59709	0.696559
TEQ PCBs (pg TCDD equiv/g)				0	2.75645
Total TEQ (pg TCDD equiv/g)				0.59709	3.453009

Notes: Adapted from: SFBRWQCB, 1995. Tissue sample from a striped bass caught in San Francisco Bay.
I-TEF values were introduced for PCBs in 1994 but these were not adopted by the California Air Toxics program.

nr = not reported

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