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**Chapter 9. Toxic Equivalency Factors (TEF) for Dioxin  
and Related Compounds**

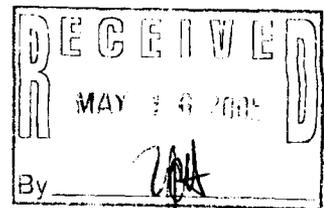
**Exposure and Human Health Reassessment  
of 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (TCDD)  
and Related Compounds**

**Part II: Health Assessment for 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin  
(TCDD) and Related Compounds**

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THIS DOCUMENT IS A PRELIMINARY DRAFT. It has not been formally released by the U.S. Environmental Protection Agency and should not at this stage be construed to represent Agency policy. It is being circulated for comment on its technical accuracy and policy implications.

National Center for Environmental Assessment  
Office of Research and Development  
U.S. Environmental Protection Agency  
Washington, DC



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## 9. TOXIC EQUIVALENCY FACTORS (TEF) FOR DIOXIN AND RELATED COMPOUNDS

### 9.1. INTRODUCTION

Previous risk assessments of dioxin and dioxin-like compounds from around the world have employed the Toxic Equivalency Factor (TEF) methodology. This method is also used throughout EPA's dioxin reassessment. This chapter has been added to the EPA's dioxin reassessment effort to address questions raised by the Agency's Science Advisory Board (SAB) in 1995. In its Report to the Administrator (U.S. EPA, 1995), the Committee said it "supports EPA's use of Toxic Equivalencies for exposure analysis..." However, the SAB suggested that, as the toxic equivalent (TEQ) approach was a critical component of risk assessment for dioxin and related compounds, the Agency should be explicit in its description of the history and application of the process and go beyond reliance on the Agency's published reference documents on the subject (U.S. EPA, 1987; 1989; 1991) to discuss issues raised in review and comment on this approach. Significant additional literature is now available on the subject, and this chapter provides the reader with a summary which is up-to-date through 2000. Future research will be needed to address uncertainties inherent in the current approach. The World Health Organization (WHO) has suggested that the TEQ scheme be reevaluated every 5 years and that TEFs and their application to risk assessment be re-analyzed to account for emerging scientific information (van den Berg et al., 1998).

### 9.2. HISTORICAL CONTEXT OF TEFs

A wide variety of polyhalogenated aromatic hydrocarbon (PHAH) compounds can be detected as complex mixtures in both abiotic and biotic samples. Because of PHAHs' known global environmental distribution and their toxicity to experimental animals (DeVito et al., 1995; DeVito and Birnbaum, 1995; Grassman et al., 1998)(see Part II, Chapters 3-6), to wildlife (Giesy and Kannan, 1998; Ross, 2000), and to humans (IARC, 1997) (see also Part II, Chapter 7), hazard characterization and risk assessment activities have tended to focus on a subset of polychlorinated dibenzo-*p*-dioxin (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs)(Figure 9-1). The subset of compounds, shown in Figure 9-1, are known as "dioxin-like" and have been assigned TEF values by WHO. In this chapter, the development of TEFs for these and other PHAHs is discussed.

#### 9.2.1. TEFs for PCDDs and PCDFs

In 1983, the Ontario Ministry of the Environment produced a Scientific Criteria Document for PCDDs and PCDFs which concluded, based on a review of available scientific information,

1 that dioxin and dibenzofurans were structurally similar compounds that shared a common  
2 cellular mechanism of action (activation of the aryl hydrocarbon receptor or AhR) and induced  
3 comparable biological and toxic responses, and that the development of environmental standards  
4 for human health concerns should be based on a “toxic equivalency” approach with 2,3,7,8-  
5 tetrachlorodibenzo-*p*-dioxin (TCDD) as the prototype (OME, 1984). The final recommendation  
6 divided all PCDD/PCDF congeners into their respective homologue groups and assigned to each  
7 group a toxicity factor relative to TCDD (Table 9-1). These numerical factors could then be  
8 applied to transform various concentrations of PCDDs and PCDFs into equivalent concentrations  
9 of 2,3,7,8-TCDD. Shortly thereafter, the first use of a TEF-like method was described by Eadon  
10 et al. (1986) as a means to estimate potential health risks associated with a PCB transformer fire  
11 in Binghamton, NY.

12 Following up on an initial risk assessment methodology designed to address the emission of  
13 dioxins and furans from waste incinerators, EPA also concluded that TEFs were the best  
14 available interim scientific policy for dealing with complex mixtures of these contaminants.  
15 With the mandate to develop active research programs that would address the limitations  
16 inherent to this risk management technique, the Agency recommended TEFs for specific  
17 congeners, rather than isomeric groups (Table 9-2; U.S. EPA, 1987). In an analogous fashion to  
18 OME's approach, concentrations of PCDDs and PCDFs would be analytically determined, the  
19 concentration of each congener would be multiplied by its respective TEF value, and all the  
20 products would be summed to give a single 2,3,7,8-TCDD equivalent. This approach has been  
21 described mathematically as:

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

23  $C_n$  equals the concentration of the individual congener in the complex mixture under analysis.  
24 TEFs were determined by inspection of the available congener-specific data and an assignment of  
25 an “order of magnitude” estimate of relative toxicity when compared to 2,3,7,8-TCDD. In vitro  
26 AhR binding and in vitro and in vivo toxicity studies were considered in setting individual TEFs.  
27 Scientific judgment and expert opinion formed the basis for these TEF values. External review  
28 of the toxicity and pharmacokinetic data utilized by EPA in setting these TEFs supported the  
29 basic approach as a “reasonable estimate” of the relative toxicity of PCDDs and PCDFs (Olson et  
30 al., 1989).

31 A 3-year study conducted by the North Atlantic Treaty Organization Committee on the  
32 Challenges of Modern Society (NATO/CCMS) also concluded that the TEF approach was the  
33 best available interim measure for PCDD/PCDF risk assessment. On the basis of examination of  
34 the available data dealing with exposure, hazard assessment, and analytical methodologies

1 related to dioxin and furans, an International Toxicity Equivalency Factor (I-TEF) scheme was  
2 presented (Table 9-2; NATO/CCMS, 1988). This review also concluded that “data strongly  
3 support the role of the AhR in mediating the biologic and toxic responses elicited by 2,3,7,8-  
4 TCDD and related PCDDs and PCDFs and provide the scientific basis for the development of  
5 TEFs for this class of compounds.” Various refinements to previous efforts included selection of  
6 TEF values based more on in vivo toxicities, assigning TEF values to octachlorodibenzo-*p*-  
7 dioxin and octachlorodibenzofuran, and removing any TEF values for all non-2,3,7,8-substituted  
8 congeners. Although it was indicated that, theoretically, it may be possible to detect nearly all of  
9 the 210 PCDD/DF isomers in the environment, seventeen 2,3,7,8-substituted congeners were  
10 known to be preferentially retained and bioaccumulated. For example, when fish or a variety of  
11 rodent species were exposed to a complex mixture of PCDDs/PCDFs from incinerator fly ash,  
12 the 2,3,7,8-substituted congeners, which were minor components of the original mixture,  
13 predominated in the analysis of their tissues (Kuehl et al., 1986; van den Berg et al., 1994). In  
14 addition, when humans were exposed to a complex mixture of more than 40 different PCDF  
15 congeners during the Oriental rice oil poisoning episodes, only the 2,3,7,8-substituted congeners  
16 were detected in subsequent blood and adipose tissue analysis (Ryan et al., 1990). EPA, which  
17 had participated in the NATO/CCMS exercise, officially adopted the revised I-TEFs in 1989,  
18 with the caveat that this risk assessment approach remains interim and continued revisions  
19 should be made (U.S. EPA, 1989; Kutz et al., 1990). The use of the TEF model for risk  
20 assessment and risk management purposes has been formally adopted by a number of countries  
21 (Canada, Germany, Italy, the Netherlands, Sweden, the United Kingdom, U.S.A.) (Yrjänheiki,  
22 1992), and as guidance by international organizations such as the International Programme on  
23 Chemical Safety, WHO.

### 24 25 **9.2.2. TEFs for PCBs**

26 During the period of TEF development for PCDDs/PCDFs, a considerable body of  
27 experimental evidence was also being generated regarding the structure-activity relationships  
28 between the different polychlorinated biphenyl homologue classes (Safe, 1990, 1994). Following  
29 the synthesis of analytical standards for all 209 theoretical PCB congeners by 1984, subsequent  
30 analysis of a variety of commercial samples was able to identify all but 26 (Jones, 1988).  
31 However, once released into the environment, PCBs are subject to a variety of photolysis and  
32 biodegradation processes, to the extent that only 50-75 congeners are routinely detected in higher  
33 trophic level species (van den Berg et al., 1995). Initial structure-activity relationship studies  
34 revealed that those congeners substituted in only the meta and para positions were approximate  
35 isostereomers of TCDD. Subsequent toxicological studies confirmed that these non-ortho-  
36 substituted, “coplanar” PCBs (e.g., PCB 77, 81, 126, 169) did induce a variety of in vitro and in

1 vivo effects similar to TCDD (Leece et al., 1985). Maximum TCDD-like activity is obtained for  
2 PCBs when there are no ortho, two or more meta, and both para positions occupied (Figure 9-1).  
3 Introduction of a single ortho substituent to the biphenyl (mono-ortho “coplanars”) results in a  
4 diminishing, but not elimination, of TCDD-like activity and toxicological responses resembling  
5 commercial mixtures of PCBs. The addition of a single ortho substituent also increases the non-  
6 dioxin-like activity of the compound. Several congeners from this group are prevalent in both  
7 commercial PCBs and a wide variety of environmental samples. Some of the more persistent  
8 mono-ortho substituted PCBs (PCBs 105, 118, 156) can be found in human serum and adipose  
9 samples at levels up to three orders of magnitude higher than the “coplanar” PCBs, PCDDs and  
10 PCDFs (Patterson et al., 1994). In limited studies a third group of PCB congeners, the di-ortho  
11 “non-coplanars,” has exhibited only minor amounts of dioxin-like activity (if any), usually 4-6  
12 orders of magnitude less potent than TCDD (Safe, 1990). Recent studies have demonstrated that  
13 some of the earlier methods of preparation of these di-ortho non-coplanar PCBs had trace  
14 contaminants of PCDFs, which may account for the weak dioxin-like activity of these  
15 compounds (van der Kolk et al., 1992). In 1991, EPA convened a workshop to consider TEFs  
16 for PCBs (Barnes et al., 1991). The consensus was that a small subset of the PCBs displayed  
17 dioxin-like activity and met the criteria for inclusion in the TEF methodology. Such proposals  
18 for the TEF methodology also seem to have utility in assessing risks to wildlife (van den Berg et  
19 al., 1998; Giesey and Kannan, 1998; Ross, 2000).

20 PCBs are often classified into two categories: “dioxin-like” and “non-dioxin-like.” The  
21 dioxin-like PCBs bind to the AhR and produce dioxin-like effects in experimental animals. All  
22 other PCBs then fall into the non-dioxin-like classification. Although the dioxin-like PCBs are  
23 generally more potent at inducing biological effects, they constitute only a minor portion of the  
24 mass of PCBs found in environmental and biological samples. The non-dioxin-like PCBs  
25 account for a majority of the mass of the PCBs found in environmental and biological samples.  
26 The use of the term non-dioxin-like PCBs is not necessarily useful. The PCBs not included in  
27 the TEF scheme (i.e., the non-dioxin-like PCBs) are not a single class of compounds and have  
28 multiple toxicities with separate structure-activity relationships (Barnes et al., 1991). Not enough  
29 congener-specific research has been performed to adequately characterize or classify these  
30 compounds. For example, the “neurotoxic” PCBs have been typically defined by structure-  
31 activity relationships for decreasing dopamine concentrations or alterations in intracellular  
32 calcium in cell culture (Shain et al., 1991; Kodavanti et al., 1996).

33 As part of the joint WHO European Centre for Environmental Health (WHO-ECEH) and the  
34 International Programme on Chemical Safety (IPCS) project to harmonize TEF schemes for  
35 dioxin-like compounds, a database was generated consisting of all available relevant  
36 toxicological data for PCBs up to the end of 1993. Of almost 1,200 peer-reviewed publications,

1 146 were selected and analyzed on the basis of the following criteria: at least one PCB congener  
2 was investigated; TCDD or a reference coplanar PCB (77, 126, 169) was used during the  
3 experiment or results were available from previous experiments (same author, laboratory,  
4 experimental design); and the endpoint in question was affected by both the reference compound  
5 and the PCB congener in question (i.e., dioxin-specific). TEFs were then determined from a total  
6 of 60 articles/manuscripts on the basis of the reported results for 14 different  
7 biological/toxicological parameters. Following scientific consultation by 12 experts from 8  
8 different countries, interim TEF values were recommended for 13 dioxin-like PCBs (Table 9-2),  
9 based on four inclusion criteria: (1) the compound should show structural similarity to PCDDs  
10 and PCDFs; (2) it should bind to the Ah receptor; (3) it should induce dioxin-specific  
11 biochemical and toxic responses; and (4) it should be persistent and accumulate in the food chain  
12 (Ahlborg et al., 1994). Increased consideration was given to selection of a TEF value based on  
13 repeat-dosing in vivo experiments, when available.

14 There is experimental evidence to suggest that a limited number of PCB congeners classified  
15 as weak or non-AhR agonists could effect concentration-dependent nonadditive interactions with  
16 dioxin-like compounds (Safe, 1990; 1994). Both antagonistic (Safe, 1990; Morrissey et al.,  
17 1992; Smialowicz et al., 1997b) and synergistic (Safe, 1990; van Birgelen et al., 1996a,b; van  
18 Birgelen et al., 1997) interactions between TCDD and PCBs have been observed in experimental  
19 systems. The non-additive interactions of the PCBs are thought to be mediated through non-AhR  
20 pathways (Smialowicz et al., 1997). These interactions usually occur at extremely high doses of  
21 the PCBs that are not environmentally relevant, and thus the nonadditive interactions are thought  
22 not to significantly detract from the TEF methodology (van den Berg et al., 1998; Birnbaum,  
23 1999).

### 24 25 **9.2.3. The Most Recent Evaluation of TEFs for PCDDs, PCDFs, and PCBs**

26 An additional recommendation from the first WHO PCB TEF consultation was that the  
27 current database should be expanded to include all relevant information on PCDDs, PCDFs, and  
28 other dioxin-like compounds that satisfied the four inclusion criteria. Prior to the second WHO-  
29 ECEH consultation in 1997, various terminologies or definitions applicable to TEFs were  
30 reviewed and standardized. Whereas previously the term TEF had been used to describe all  
31 scientific endpoints used in comparison with TCDD, it was noted that a variety of experimental  
32 parameters may not be considered “toxic,” but are considered as biological/biochemical  
33 responses, such as AhR binding and alkoxyresorufin O-dealkylase induction. The decision was  
34 that any experimental endpoint for which a numerical value of the relative potency compared to  
35 TCDD had been generated from a single laboratory examining a single endpoint would be known  
36 as a relative potency value, or REP. The term TEF would then be restricted to describe an order-

1 of-magnitude consensus estimate of the toxicity of a compound relative to the toxicity of TCDD  
2 that is derived using careful scientific judgment of all available data (van Leeuwen, 1997; van  
3 den Berg et al., 1998).

4 At the second WHO-ECEH consultation in 1997, relative potency factors were calculated  
5 based on the following methodology (van den Berg et al., 1998):

- 6
- 7 ● Assigned as reported in the publication/manuscript (verified from available data).
  - 8 ● Calculated from the dose-response curves using linear interpolation of log doses comparing  
9 the same effect levels with correction for different control levels.
  - 10 ● Calculated from ratios of low or no observed effect levels (LOELs, NOELs) and effect  
11 concentration/dose 10%, 25% or 50% values (ED/EC<sub>10,25,50</sub>).
  - 12 ● Calculated from ratios of tumor promotion indexes or maximal enzyme induction levels.
  - 13 ● Calculated from ratios of Ah receptor binding affinities (K<sub>d</sub>).
- 14

15 Whereas the resulting range of in vitro/in vivo REP values for a particular congener may span  
16 3-4 orders of magnitude, final selection of a TEF value gave greater weight to REPs from repeat-  
17 dose in vivo experiments (chronic > subchronic > subacute > acute). As with the PCB TEF  
18 consultation, dioxin-specific endpoints were also given higher priority. A rounding-off  
19 procedure (nearest 1 or 5) was also employed for final TEF selection (Table 9-2). It should be  
20 noted that the TEF was rounded up or down depending on the compound, the data, and scientific  
21 judgment.

22 Notable amendments to the previous NATO/WHO TEF schemes include:

- 23
- 24 ● On the basis of new REPs from in vivo tumor promotion and enzyme induction, a TEF of  
25 1.0 was recommended for 1,2,3,7,8-PeCDD.
  - 26 ● Originally the TEF for OCDD was based on body burdens of the compound following  
27 subchronic exposures; a TEF based on administered dose is reduced to 0.0001.
  - 28 ● New in vivo enzyme induction potency and structural similarity with OCDD support the  
29 TEF change to 0.0001 for OCDF.
  - 30 ● REPs from an in vivo subchronic toxicity study (enzyme induction, hepatic retinol  
31 decreases) support reducing the TEF to 0.0001 for PCB 77.
  - 32 ● A TEF value of 0.0001 was assigned for PCB 81. Even though PCB 81 was not assigned a  
33 TEF value at the 1993 WHO consultation because of lack of human residue and  
34 experimental data, more recent data demonstrate similar qualitative structural activity  
35 results compared to PCB 77.

1 ● Because of the lack of in vivo enzyme induction (CYP 1A1/A2) and reproductive toxicity  
2 with structurally similar congeners (PCB 47 and PCB 153), the previous interim TEF  
3 values for the di-ortho-substituted PCBs 170 and 180 were withdrawn.  
4

5 Although a number of uncertainties associated with the TEF concept have been identified  
6 (nonadditive interactions with non-dioxin-like PCBs, natural ligands for the Ah receptor,  
7 questionable low-dose linearity of REP responses), the 1997 WHO expert meeting decided that  
8 an additive TEF model remained the most feasible risk assessment method for complex mixtures  
9 of dioxin-like PHAHs.

10 The WHO working group acknowledged that there are a number of other classes of chemicals  
11 that bind and activate the Ah receptor. The chemicals include, but are not limited to,  
12 polyhalogenated naphthalenes, diphenyl ethers, fluorenes, biphenyl methanes, quaterphenyls, and  
13 others. In addition, a number of brominated and chloro/bromo-substituted dioxin analogues of  
14 the PCDDs and PCDFs have been demonstrated to cause dioxin-like effects. The WHO working  
15 group concluded that “at present, insufficient environmental and toxicological data are available  
16 to establish a TEF value for any of the above compounds” (van den Berg et al., 1998).

17 The development and refinement of the TEF methodology can be thought of as an iterative  
18 process. As we accumulate more data on the biological effects of dioxin-like chemicals and a  
19 better knowledge base of their mode of action, the TEF methodology is improved. The latest  
20 evaluation of the TEF methodology for use in human health risk assessment by the WHO  
21 working group provides the most accurate assessment of the TEFs for dioxin-like chemicals.  
22 The WHO<sub>98</sub> TEF values are recommended for use in human health risk assessment.

23 In January 1998, EPA and the U.S. Fish and Wildlife Service sponsored a meeting entitled  
24 “Workshop on the Application of 2,3,7,8-TCDD Toxicity Equivalency Factors to Fish and  
25 Wildlife.” The major objective of the workshop was to address uncertainties associated with the  
26 use of the TEF methodology in ecological risk assessment. Twenty-one experts from academia,  
27 government, industry, and environmental groups participated in the workshop. The consensus of  
28 the workgroup was that while there are uncertainties in the TEF methodology, the use of this  
29 method decreases the overall uncertainty in the risk assessment process. However, quantifying  
30 the decrease in the uncertainty of a risk assessment using the TEF methodology remains  
31 ambiguous, as does the exact uncertainty in the TEF methodology itself (U.S. EPA, 2001).

32 This first section has outlined the process of assessing the relative potency of chemicals and  
33 the assignment of a consensus TEF value. There are still many questions on the use of the TEF  
34 method and the validity of some of the underlying assumptions. A detailed discussion and  
35 review of the data supporting the development and use of the TEF method, as well as the data  
36 relating to the issue of additivity, is included within the specific issues section that follows.

#### 9.2.4. Illustrative Examples of the Data Used for Deriving the TEF Values

The TEF scheme includes 17 PCDDs and PCDFs and 13 PCBs. However, in human tissue samples and food products, only five of these congeners, TCDD, 1,2,3,7,8-PCDD, 1,2,3,6,7,8-HxCDD, 2,3,4,7,8-PeCDF, and PCB 126, account for over 70% of the TEQ. There is considerable data on the relative potency of these compounds in both in vitro and in vivo studies. Table 9-3 provides a summary of the REPs from in vivo data available for the compounds that account for approximately 80% of the TEQs in humans (see Part I, Volume 3, Section 4.2.). This information was obtained from the WHO database used to derive TEF values for PCDDs, PCDFs, and PCBs (Van den Berg et al., 1998). The WHO database contains duplicate recordings of studies for several of the compounds. The data in Table 9-3 does not include the duplicates. In addition, the WHO database also contains studies that used a single dose level of the test chemical, and REP values were not estimated for these studies. For example, in the WHO database for PCB 126, there are 144 in vivo endpoints. Of these 144, 50 do not have REP values associated with the entry because the study used only a single dose level. In other cases, for a given endpoint from a particular study, the REP value is presented as estimated by the authors as well as by alternative analyses by members of the WHO workgroup. In total, there are 62 data sets that have dose-response relationships sufficient enough to estimate the relative potency of PCB 126. These data sets examine enzyme induction, changes in organ and body weights, immunotoxicity, developmental toxicity, thyroid hormones, renal and hepatic retinoids, and tumor promotion. The WHO database for 1,2,3,7,8-PCDD contained studies examining enzyme induction, changes in organ and body weights, hepatic porphyria, hepatic retinoids, and tumor promotion. The WHO database for 2,3,4,7,8-PCDF contained studies examining enzyme induction, changes in organ and body weights, immunotoxicity, developmental toxicity, thyroid hormones, hepatic retinoids, hepatic porphyria, and tumor promotion. The data presented in Table 9-3 for 1,2,3,6,7,8-HxCDD is from U.S. EPA (1989) because the WHO database contained no new in vivo data for this compound. There are only three in vivo studies on the effects of 1,2,3,6,7,8-HxCDD, one of which is the NTP carcinogenicity study on a mixture of 31% 1,2,3,6,7,8-HxCDD and 67% 1,2,3,7,8,9HxCDD (NTP, 1980).

The REPs for 1,2,3,7,8-PCDD in the in vivo studies vary by approximately a factor of five. A TEF value was assigned to 1,2,3,7,8-PCDD based on the REP for tumor promotion which ranged from 0.8-1.0. The REPs for 2,3,4,7,8-PCDF and PCB 126 have a greater variability, but the assigned TEF values are similar to the means of the REP values. The mean±standard deviation for all in vivo REP values for 2,3,4,7,8-PCDF is 0.4±0.7. If only subchronic studies are examined, the mean±standard deviation of the REP values is 0.2±0.13. These REP values for 2,3,4,7,8-PCDF are similar to the TEF value of 0.5. The REPs for PCB 126 range over two orders of magnitude with a mean for all in vivo responses of 0.2±0.2. The mean REP for

1 subchronic studies examining PCB 126 is  $0.13 \pm 0.13$ . The TEF for PCB 126 is 0.1, which is  
2 slightly lower than the mean of the REP values. With the exception of 1,2,3,6,7,8-HxCDD, the  
3 REPs are based on several studies from different laboratories examining different endpoints.  
4

#### 5 **9.2.5. Variability in the REPs Across Endpoint, Species, Dosing Regimen and** 6 **Laboratories.**

7 Using PCB 126 as an example, the variability of the REPs across endpoint, species,  
8 laboratory and dosing regimen will be described. PCB 126 has the most in vivo studies  
9 comparing the its relative potency to TCDD of all the chemicals in the WHO data base. Upon  
10 examining this data base, it is apparent that within an endpoint there is considerable variability  
11 (greater than an order of magnitude). For instance, the REPs for hepatic EROD induction in  
12 mice following a single exposure to PCB 126 are 0.005, 0.012, 0.38 and 0.55. These studies use  
13 similar dosing paradigms and time course for endpoint determinations so there is no clear reason  
14 why these values should range over two orders of magnitude. In some cases, interlaboratory  
15 variability appears to be a significant cause for variance in the estimates of the REPs. In order to  
16 examine REPs across endpoints and control for interlaboratory variability, two studies were  
17 examined. Hemming et al (1993) examined the REPs for tumor promotion, hepatic EROD  
18 induction, and alterations in liver, thymus and body weights in rats compared to TCDD. In this  
19 study, the REPs were 0.16, 0.3, 0.05, 0.07, and 0.1 for liver, thymus and body weight changes,  
20 hepatic EROD induction and tumor promotion, respectively. While the range of these REPs is  
21 0.05-0.3, the authors only provided point estimates of the REPs and no information was provided  
22 on the variance of these values. Thus, it is impossible to determine if the REP values are  
23 statistically different from one another. The study by Hemming et al (1993) is typical of the  
24 literature estimating the REPs for dioxin-like chemicals in that no information on the variance of  
25 these estimates are available. A recent study by DeVito et al (2000), demonstrated that the REPs  
26 for PCB 126 for hepatic and dermal ethoxyresorufin-O-deethylase (EROD) activity, a marker for  
27 CYP1A1 induction, and hepatic acetanilide 4-hydroxylase (ACOH) activity, a marker for  
28 CYP1A2 induction, were equivalent. However in this study, the REP for pulmonary EROD  
29 induction was an order of magnitude lower than the other endpoints.

30 The example described above suggests that the source of the variability in the REP values  
31 remains uncertain. Most studies do not provide estimates of the variance of the REP values.  
32 This decreases the ability to compare REP values across endpoints, species, dosing regimens and  
33 laboratories. One of the few studies that did provide estimates of the variance around the REPs  
34 examined only a single biochemical (ethoxyresorufin-O-deethylase activity ) endpoint in  
35 different tissues and it is uncertain whether the results from this study are applicable to other  
36 endpoints (DeVito et al., 2000).

1  
2 **9.2.6. Critical Considerations in the Application of the TEF Methodology.**

3 There are a number of underlying assumptions used in the development of the TEF  
4 methodology and these assumptions have significant implications in the application of this  
5 method. Some of these assumptions and there implications are listed below.

- 6
- 7 ● The Ah receptor mediates most if not all of the biologic and toxic effects of  
8 TCDD.
  - 9 ● The TEF methodology attempts to estimate the potential TCDD-like effects of a  
10 chemical. Toxic effects of a chemical induced through mechanisms other than the  
11 Ah receptor are not accounted for in this method.
  - 12 ● Even though not all the molecular mechanisms following Ah receptor binding are  
13 understood, the TEF methodology is still valid.
  - 14 ● The chemical binds to Ah receptor and is a full agonist for endpoints of concern.
  - 15 ● The relative potency of a chemical is equivalent for all endpoints of concern.
  - 16 ● The relative potency of a chemical is equivalent for all exposure scenarios.
  - 17 ● The relative potency of a chemical in rodents is predictive of its relative potency  
18 in humans.
  - 19 ● The toxicity of a mixture of dioxins is dose additive based on the relative  
20 potencies or TEFs of the individual components .
  - 21 ● The TEF methodology ignores the interactions of dioxins with other chemicals  
22 present.
  - 23 ● Naturally occurring chemicals with short half-lives and varying degrees of affinity  
24 to the Ah receptor and intrinsic activity do not interfere with the predictions of  
25 dioxin equivalents in the mixture.
  - 26 ● TEFs are not calculated. They are assigned based on the following criteria:  
27 - Greater weight is given to REPs from repeat-dose in vivo experiments  
28 (chronic > subchronic > subacute > acute).  
29 - Dioxin-specific or Ah receptor mediated effects were given also higher priority.  
30 - A rounding-off procedure (nearest 1 or 5) was also employed for final TEF  
31 selection (Table 9-2). It should be noted that the TEF was rounded up or down  
32 depending on the compound, the data, and scientific judgment.
- 33

34 Many of the assumptions are necessary because of a lack of data. For example, TCDD  
35 and a mixture of hexachlorinated dioxins are the only congeners which have been tested for  
36 carcinogenicity. Thus, in order to estimate the carcinogenic potency of a mixture of dioxins, it

1 must be assumed that the REPs for non-cancer endpoints approximate those for cancer. While  
2 these assumptions lead to uncertainties, there is a consensus that the TEF methodology decreases  
3 the overall uncertainty of a risk assessment (USEPA, 2001). More detailed discussion of these  
4 points is presented in the following section.

### 6 **9.3. SPECIFIC ISSUES**

#### 7 **9.3.1. Ah Receptor and Toxicity Factors**

8 Issues relating to the role of the Ah receptor as the common mediator of toxicity of  
9 dioxin-like compounds and the cross-species comparability of AhR structure and function  
10 frequently arise when the TEF approach is discussed. Recent data relating to each of these issues  
11 are discussed below.

#### 12 **9.3.2. The Role of the AhR in the Toxicity of Dioxin-Like Compounds**

13 The general basis for the TEF scheme is the observation that the AhR mediates most if  
14 not all of the dioxin-like biological and toxic effects induced by compounds included in the TEF  
15 scheme (Safe, 1990; Okey et al., 1994; Birnbaum, 1994; Hankinson, 1995). Binding to the  
16 receptor is necessary, but not sufficient, to generate the wide variety of toxic effects caused by  
17 dioxin-like halogenated aromatic hydrocarbons (Sewall and Lucier, 1995; De Vito and  
18 Birnbaum, 1995) (for additional review references, see Part II, Chapter 2). There are several  
19 lines of evidence that the Ah receptor is important in the toxicity of the dioxin-like compounds.  
20 A brief discussion of this evidence shall be presented in the following section. Those wishing a  
21 more detailed discussion of this issue are referred to Part II, Chapter 2.

22 Initial studies on the toxicity of PAHs demonstrated that the sensitivity to these  
23 compounds varied by strain of mice and segregated with the Ah locus. The Ah locus was then  
24 found to encode a receptor designated as the aryl hydrocarbon receptor or AhR. Sensitive strains  
25 of mice expressed receptors with high binding affinity for these compounds, while the resistant  
26 mice expressed a receptor that poorly bound the PAHs. One of the best ligands for this receptor  
27 was TCDD. Shortly after the discovery of the AhR, structure-activity relationship studies  
28 demonstrated a concordance between binding affinity to the Ah receptor and toxic potency in  
29 vivo in mice. Further support of the role of the AhR in the toxicity of dioxin-like compounds  
30 was demonstrated following the development of AhR knockout mice (Fernandez-Salguero et al.,  
31 1995; Schmidt et al., 1996; Mimura et al., 1997; Lahvis and Bradfield, 1998). The Ah receptor  
32 knockout mice are a strain of mice in which the Ah receptor has been genetically altered so that  
33 the receptor is not expressed or “knocked-out” in these mice. Administration of TCDD at doses  
34 more than 10 times the LD<sub>50</sub> of wild-type mice has not produced any significant dioxin-like  
35 effects, either biochemical or toxicological, in the AhR knockout mice (Fernandez-Salguero et  
36

1 al., 1996; Peters et al., 1999). These data as a whole demonstrate that the binding to the AhR is  
2 the initial step in the toxicity of dioxin-like compounds.

### 3 4 **9.3.3. Species Comparison of the AhR**

5 Although binding to the AhR initiates a cascade of molecular and cellular events leading  
6 to toxicity, the exact mechanism of action of dioxin-like compounds is not completely  
7 understood. One difficulty in determining the mechanism is our limited understanding of the  
8 normal physiological role of the AhR, which would aid in understanding of potential species  
9 differences in response to dioxin-like chemicals. The available data indicate that the AhR does  
10 play an important role in normal processes and that there are a number of similarities in the  
11 action of the AhR between species. These data strengthen our confidence in species  
12 extrapolations with these chemicals.

13 There are several lines of evidence suggesting that the AhR is an important factor in  
14 developmental and homeostatic processes. The AhR is a ligand-activated transcription factor  
15 that is a member of the basic-helix-loop-helix-Per-Arnt-Sim (bHLH-PAS) superfamily. The  
16 bHLH-PAS superfamily consists of a growing list of at least 32 proteins found in diverse  
17 organisms such as *Drosophila*, *C. elegans*, and humans. Many of these proteins are transcription  
18 factors that require either hetero- or homodimerization for functionality. These proteins regulate  
19 circadian rhythms (per and clock) and steroid receptor signaling (SRC-1, TIF2, RAC3) and are  
20 involved in sensing oxygen tension (Hif-1, EPAS-1/HLF) (Hahn, 1998). The AhR is also a  
21 highly conserved protein that is present in all vertebrate classes examined, including modern  
22 representatives of early vertebrates such as cartilaginous and jawless fish (Hahn, 1998). In  
23 addition, an AhR homologue has been identified in *C. elegans* (Powell-Coffman et al., 1998).  
24 The classification of the AhR as part of the bHLH-PAS superfamily and its evolutionary  
25 conservation imply that this protein may play an important role in normal physiological function.  
26 It has been proposed that understanding the function of the bHLH-PAS family of proteins and the  
27 phylogenetic evolution of the AhR may lead to an understanding of the role of this protein in  
28 normal processes (Hahn, 1998).

29 The process of development is a complex phenomenon that involves the specific  
30 expression of numerous genes in a spatial and temporal pattern. The importance of a particular  
31 gene in developmental biology is often inferred by its spatial and temporal expression during  
32 development. The AhR is expressed in a tissue, cell, and temporal pattern during development  
33 (Abbott et al., 1995). It is highly expressed in the neural epithelium, which forms the neural crest  
34 (Abbott et al., 1995). The expression of the AhR at critical periods during development suggests  
35 that this protein has important physiological functions.

1 Further evidence of the role of the AhR in developmental processes is provided by the  
2 development and study of AhR knockout mice. Three strains of AhR knockout mice have been  
3 produced using a targeted disruption of the AhR locus (Fernandez-Salguero et al., 1995; Schmidt  
4 et al., 1996; Mimura et al., 1998; Lahvis and Bradfield, 1998). The AhR  $-/-$  mice develop  
5 numerous lesions with age (Fernandez-Salguero et al., 1995). Mortality begins to increase at  
6 about 20 weeks, and by 13 months almost half of the mice either die or become moribund.  
7 Cardiovascular alterations consisting of cardiomyopathy with hypertrophy and focal fibrosis,  
8 hepatic vascular hypertrophy and mild fibrosis, gastric hyperplasia, T-cell deficiency in the  
9 spleen, and dermal lesions are apparent in these mice and the incidence and severity increases  
10 with age (Fernandez-Salguero et al., 1995). Although male and female AhR  $-/-$  mice are fertile,  
11 the females have difficulty maintaining conceptus during pregnancy, surviving pregnancy and  
12 lactation, and rearing pups to weaning (Abbott et al., 1999). It should be noted that the AhR  
13 knockout mice are resistant to the toxic effects of TCDD.

14 Comparisons between the AhR of experimental animals (primarily rodents) and the  
15 human AhR have revealed a number of similarities in terms of ligand and DNA binding  
16 characteristics and biochemical functions. Tissue-specific patterns of expression of AhR mRNA  
17 are similar in rats, mice, and humans, with highest levels generally detected in lung, liver,  
18 placenta, and thymus (Dolwick et al., 1993; Döhr et al., 1996). Nuclear AhR complexes isolated  
19 from human and mouse hepatoma cells (Hep G2 and Hepa 1c1c7, respectively) have similar  
20 molecular weights. Although the human AhR appears more resistant to proteolytic digestion by  
21 trypsin or chymotrypsin, the major breakdown products were similar between the two species,  
22 and photolabeling analysis with TCDD suggested common features in the ligand binding portion  
23 of the receptors (Wang et al., 1992).

24 Limited analysis has suggested the average human AhR exhibits a lower binding affinity  
25 for various HAHs than “responsive” rodent strains. However, similar to a variety of  
26 experimental animals, human populations demonstrate a wide variability in AhR binding affinity  
27 (Micka et al., 1997). Recent determination of AhR binding affinity ( $K_d$ ) toward TCDD in 86  
28 human placenta samples showed a greater than twenty-fold range in the binding affinity, and this  
29 range encompasses binding affinities similar to those observed in sensitive and resistant mice  
30 (Okey et al., 1997). Whereas the concentration of various ligands required to activate a human  
31 AhR reporter gene construct was higher than required with rodent cell cultures, the actual rank  
32 order of binding affinities was in agreement (Rowlands and Gustafsson, 1995). Although  
33 comparisons have been made of the TCDD binding affinity to the AhR of different species,  
34 caution should be used when attempting to predict species sensitivity to TCDD and related  
35 compounds. For mice, the sensitivity to the biochemical and toxicological effects of TCDD and  
36 related compounds is associated with the relative binding affinity of TCDD to the AhR in the

1 different strains (Birnbaum et al., 1990; Poland and Glover, 1990). However, the relative  
2 binding affinity of TCDD to the AhR across species does not aid in the understanding of  
3 interspecies differences in the response or sensitivity to TCDD (DeVito and Birnbaum, 1995).

4 The human AhR also demonstrates other slight differences when compared to the AhR  
5 from experimental animal species. The molecular mass of the human AhR ligand-binding  
6 subunit appears to be greater than the AhR subunit from certain TCDD “responsive” mouse  
7 strains but similar to the receptor molecular mass for rats (Poland and Glover, 1987). Currently  
8 there has been no association established between differences in the molecular mass of the AhR  
9 and sensitivity to a particular biochemical or toxicological response across species (Okey et al.,  
10 1994). The non-liganded human AhR appears thermally more stable compared to AhR from  
11 various rodent species, whereas the reverse situation exists with the liganded human AhR (Nakai  
12 and Bunce, 1995). Transformation of the ligand-bound human AhR receptor (isolated from  
13 colon adenocarcinoma cells) to the DNA-binding state, unlike rodent hepatic AhR, is  
14 temperature dependent (Harper et al., 1992). The importance of these species differences in  
15 transformation and stability of the AhR in the species sensitivity to TCDD remain uncertain.  
16 However, in critical areas of receptor function such as ligand recognition, transformation, and  
17 interaction with genomic response elements, the human AhR is comparable to the AhR isolated  
18 from experimental animals.

19 Ligand-bound or transformed AhR from a variety of mammalian species, including  
20 humans, bind to a specific DNA sequence or “dioxin response element” with similar affinities  
21 (Bank et al., 1992; Swanson and Bradfield, 1993). The bHLH structure of receptor proteins such  
22 as AhR ensures appropriate contact and binding with DNA recognition sites. Amino acid  
23 sequence analysis between mouse and human AhR shows an overall sequence homology of  
24 72.5%, whereas the bHLH domain shows 100% amino acid concordance (Fujii-Kuriyama et al.,  
25 1995). In comparison, the deduced amino acid composition of the AhR from killifish was 78%-  
26 80%, similar to the amino acid sequence of rodent and human AhR (Hahn and Karchner, 1995).  
27 These studies demonstrate a concordance between the structure of the receptor and its function  
28 across species.

29 The majority of scientific evidence to date supports the theory that binding to the AhR is  
30 a necessary first step prior to dioxin-like compounds eliciting a response, as discussed in Part II,  
31 Chapter 2. Current research has identified the AhR in a variety of human tissues and cells that  
32 appear to function in a similar manner to the AhR from experimental animals, including fish,  
33 birds, and mammals. When multiple endpoints are compared across several species, there exists  
34 a high degree of homogeneity in response and sensitivity to TCDD and related compounds  
35 (DeVito et al., 1995). Therefore, these data provide adequate support for the development of the  
36 TEF methodology. However, these data also reflect the true complexity of intra- and interspecies

1 comparisons of biochemical and toxicological properties. Continued research into the variety of  
2 additional cytoplasmic and nuclear proteins capable of interacting with the AhR signaling  
3 pathway will ultimately lead to a better understanding of the observed species and strain  
4 variability in the response to dioxin-like chemicals and may be useful in further refining TEFs.  
5

#### 6 **9.3.4. Mode of Action and Implications for the TEF Methodology**

7 Many of the toxic effects of dioxins are mediated by disruption of normal growth and  
8 differentiation processes. For example, TCDD alone is capable of producing cancer in  
9 experimental animals. However, its genotoxicity is limited. From an operational point of view,  
10 TCDD is a tumor promoter (See Part II, Chapter 6). Tumor promoters act by disrupting the  
11 natural balance between cell replication and cell death. Similarly, many of the non-cancer  
12 effects, such as immunotoxicity and developmental toxicities, are due to TCDD-induced  
13 alterations in cell growth and differentiation. While these events are initiated by the activation of  
14 the Ah receptor, the exact molecular and cellular alterations beyond receptor binding remain  
15 uncertain. One criticism of the TEF methodology is that the exact molecular mechanisms for the  
16 toxic effects of these chemicals is uncertain and thus one cannot apply this method to mixtures  
17 with certainty. The uncertainties in understanding the exact molecular mechanism of dioxin  
18 action is not unique and does not detract significantly from the utility of the TEF methodology.  
19 The exact molecular mechanisms of the biochemical and physiological effects of estrogens are  
20 also uncertain. This does not decrease our confidence that if a chemical binds to the estrogen  
21 receptor and induces uterine growth in vivo that the chemical is estrogenic and that it can be  
22 useful to describe its potency relative to estradiol. Similarly, if a chemical binds to the Ah  
23 receptor and induces dioxin-like effects, we can classify the chemical as dioxin-like and describe  
24 its relative potency to TCDD without understanding every molecular event leading to the  
25 biological effect. For many of the chemicals assigned TEF values, there are in vitro Ah receptor  
26 binding data and a number of in vivo studies estimating the REP of these chemicals for toxic and  
27 biochemical effects.  
28

#### 29 **9.3.5. Ah Receptor Ligands**

30 A wide variety of structurally diverse anthropogenic and natural chemicals are capable of  
31 interacting with the AhR. These chemicals also have a broad range of potencies at inducing  
32 dioxin-like effects in experimental systems. One of the major differences between the  
33 anthropogenic chemicals included in the TEF methodology and the natural AhR ligands is their  
34 pharmacokinetics. The anthropogenic chemicals included in the TEF methodology are persistent  
35 and bioaccumulate in wildlife and humans. In contrast, most if not all of the natural AhR ligands  
36 are rapidly metabolized and eliminated from biological systems. The following section will

1 examine the differences between the chemicals included in the TEF methodology and remaining  
2 AhR ligands not included in this approach.

### 3 4 **9.3.5.1. Industrial/Synthetic AhR Ligands**

5 The synthetic compounds that bind to AhR include a number of different classes of  
6 chemicals, most notably the PCDDs, PCDFs, and PCBs. Other synthetic AhR ligands include  
7 industrial chemicals (polybrominated biphenyls, polychlorinated naphthalenes, chlorinated  
8 paraffins, etc.), pesticides (hexachlorobenzene), and contaminants (polybrominated dioxins,  
9 dibenzofurans, and naphthalenes) associated with various manufacturing, production, combustion,  
10 and waste disposal processes. In addition, pyrolysis of organic material can produce a number of  
11 non-halogenated polycyclic aromatic hydrocarbons (PAHs) with moderate to high affinity for  
12 AhR (Poland and Knudson, 1982; Nebert, 1989; Chaloupka et al., 1993).

13 Not all of the anthropogenic sources of dioxin-like compounds are included in the TEF  
14 methodology. Many of these chemicals, such as hexachlorobenzene and the brominated diphenyl  
15 ethers, are only weakly dioxin-like and have significant toxicological effects that are not  
16 mediated by the AhR. For these chemicals, it is not clear that adding them to the TEF  
17 methodology would decrease the uncertainty in the risk assessment process. For other classes of  
18 chemicals, such as the chlorinated naphthalenes, environmental concentrations and human  
19 exposures are largely uncertain.

20 The PAHs are one class of anthropogenic chemicals not included in the TEF scheme  
21 despite evidence for AhR binding. The PAHs are not included in the TEF methodology because  
22 of their short half-lives and relatively weak AhR activity. In addition, the role of the Ah receptor  
23 in the toxicity of the PAHs is uncertain. For example, both benzo[a]pyrene and chrysene induce  
24 CYP1A1 activity through an AhR-mediated mechanism (Silkworth et al., 1995). However,  
25 while the Ah receptor also plays a role in the immune suppressive effects of benzo[a]pyrene it  
26 does not appear to be involved in the immune suppression induced by chrysene (Silkworth et al.,  
27 1995). Furthermore, PAHs are DNA reactive and mutagenic and these mechanisms play a large  
28 role in both the carcinogenicity and immunotoxicity of the PAHs (Ross and Nesnow, 1999). In  
29 contrast, TCDD and other dioxin-like compounds are not DNA reactive. While there are PAHs  
30 that bind to the AhR, the role of AhR or other competing pathways in the toxicity of these  
31 compounds has not been clearly defined.

32 Brominated dioxins, dibenzofurans, biphenyls, and naphthalenes also induce dioxin-like  
33 effects in experimental animals (Miller and Birnbaum, 1986; Zacherewski et al., 1988;  
34 Birnbaum et al., 1991; Hornung et al., 1996; DeVito et al., 1997; Weber and Greim, 1997). The  
35 brominated dioxins and dibenzofurans may be more or less potent than their chlorinated  
36 orthologues, depending on the congener (Birnbaum et al., 1991; DeVito et al., 1997). The

1 sources of the brominated dioxin-like compounds are not well characterized. Some of the  
2 chemicals, such as the brominated biphenyls and their contaminants the brominated  
3 naphthalenes, were synthesized and sold as commercial flame retardants. The manufacture and  
4 use of polybrominated biphenyls has been prohibited. Brominated dibenzofurans are produced  
5 as byproducts of synthesis and pyrolysis of some brominated flame retardants. There is some  
6 evidence of human exposure to brominated dioxins and dibenzofurans from extruder operators  
7 (Ott and Zober, 1996). Polybrominated, polychlorinated, and mixed bromo- and chloro- dioxins  
8 and dibenzofurans have been found in soot from textile processing plants (Sedlak et al., 1998).  
9 Although these chemicals have been found in humans, these studies are limited to a small  
10 population and exposure to the general population remains undetermined. Future examinations  
11 of the TEF methodology should include a more detailed discussion of the brominated dioxins and  
12 dibenzofurans.

#### 14 **9.3.5.2. Naturally Occurring AhR Ligands**

15 The evolutionary conservation of AhR and its biological function following activation by  
16 dioxin-like compounds have led to the hypothesis that there must be an endogenous or  
17 physiological ligand(s) for this receptor. Presently, the endogenous ligand remains  
18 undetermined. However, efforts to discover the natural ligand have led to the discovery of a  
19 number of naturally occurring AhR ligands. A number of naturally occurring chemicals present  
20 in the diet are capable of binding to AhR and inducing some dioxin-like effects in experimental  
21 animals (Bradfield and Bjeldanes, 1984; 1987) and humans (Michnovicz and Bradlow, 1991;  
22 Sinha et al., 1994). The question of how the interaction of these chemicals relates to the toxicity  
23 of those chemicals designated as dioxin-like has become the subject of much debate.

24 One class of naturally occurring chemicals that activate the AhR is the indole derivatives.  
25 Indole derivatives, naturally present in a variety of cruciferous vegetables, are capable of  
26 modulating the carcinogenicity of PAHs (Wattenberg and Loub, 1978). Indole-3-carbinol (I-3-C)  
27 and 3,3'-diindolylmethane (DIM) are major secondary metabolites found in cruciferous  
28 vegetables and induce both phase I and II metabolic enzymes (CYP1A-dependent glutathione and  
29 glucuronyl transferases, oxidoreductases) in experimental animals (Bradfield and Bjeldanes,  
30 1984, 1987), human cell lines (Bjeldanes et al., 1991; Kleman et al., 1994), and humans  
31 (Michnovich and Bradlow, 1990, 1991). Although both compounds induce CYP450 enzymes  
32 under AhR transcriptional control, they exhibit relatively low binding affinity for the Ah receptor  
33 (Gillner et al., 1985). Further investigation revealed that I-3-C is relatively unstable in the acidic  
34 environment of the digestive tract and readily forms DIM. In turn, DIM can participate in acid  
35 condensation reactions to form indolocarbazoles (ICZs) (Chen et al., 1995). ICZs are also  
36 produced by bacterial metabolism of the common dietary amino acid tryptophan. ICZs, in

1 particular indolo[3,2b]carbazole, exhibit high binding affinity for the rodent AhR, approximately  
2 equipotent to 2,3,7,8-tetrachlorodibenzofuran, and can induce CYP1A1 activity in cultured cells  
3 (Bjeldanes et al., 1991; Gillner et al., 1993; Chen et al., 1995). ICZ and a methylated derivative,  
4 5,11-dimethylindolo[3,2b]carbazole (MICZ), are also capable of binding to and activating the  
5 AhR in human hepatoma cells (HepG2) (Kleman et al., 1994). With considerably lower efficacy,  
6 I-3-C and DIM can partially displace TCDD from the AhR from human breast cancer cells  
7 (T47D) (Chen et al., 1996). These results would suggest that this group of compounds may  
8 represent a class of physiologically active AhR ligands derived from natural sources, which could  
9 either mimic dioxin-like compounds in their action or act as competitors for AhR binding.

10 In addition to the plant-derived indoles, experimental animals consuming thermally  
11 treated meat protein as well as humans fed cooked meat can exhibit induced CYP1A2 activity  
12 (Degawa et al., 1989). High-temperature cooking (250°C, 22 minutes) of ground beef resulted in  
13 the formation of a number of heterocyclic aromatic amines (HAAs) in part-per-billion levels,  
14 which were thought to be responsible for the observed CYP1A2 induction in human volunteers  
15 (Sinha et al., 1994). Mechanistic analysis of one particular HAA, 2-amino-3,8-  
16 dimethylimidazo[4,5-f]quinoxaline (MeIQx), has shown that it is capable of both interacting with  
17 the AhR and inducing CYP1A1/A2 activity in rats (Kleman and Gustafsson, 1996). These data  
18 should be viewed cautiously because recent data indicate that CYP1A2 can be induced through  
19 non-AhR mechanisms (Ryu et al., 1996). Because there are multiple pathways to induce  
20 CYP1A2, the increase in CYP1A2 activity following exposure to complex mixtures, such as  
21 cooked meat, does not necessarily indicate the presence of dioxin-like compounds.

22 Other diet-derived chemicals that can interact with the AhR include oxidized essential  
23 amino acids. UV-oxidized tryptophan is capable of inducing CYP1A1 activity in mouse  
24 hepatoma cells through an AhR-dependent mechanism (Sindhu et al., 1996). Rats exposed to  
25 UV-oxidized tryptophan in vivo also exhibited induction of hepatic and pulmonary CYP1A1  
26 activity. Both in vitro and in vivo enzyme induction were transient, with the oxidized tryptophan  
27 possibly being metabolized by CYP1A1 (Sindhu et al., 1996). Tryptanthrins, biosynthetic  
28 compounds produced from the metabolism of tryptophan and anthranilic acid by yeast commonly  
29 found in food, are agonists for the rat AhR (Schrenk et al., 1997). Various tryptanthrins also  
30 induce CYP1A1-related enzyme activity in mouse hepatoma cells with the approximate efficacy  
31 of indolo[3,2b]carbazole.

32 Recent studies have demonstrated that physiological chemicals can bind to the AhR.  
33 Bilirubin was recently found to transform the AhR from mouse hepatoma cells into its DNA-  
34 binding state, resulting in CYP1A1 induction. Hemin and biliverdin can also be metabolically  
35 converted to bilirubin, resulting in AhR-dependent gene activation (Sinal and Bend, 1997).  
36 Despite these results, there is no clear evidence that these are the physiological ligands for the

1 AhR, nor is there evidence that these compounds can modulate the activity of dioxin-like  
2 compounds or lead to dioxin-like toxic effects in humans or animals.

### 3 4 **9.3.5.3. Industrial vs. Natural AhR Ligands**

5 There are a number of structurally diverse chemicals that bind to the Ah receptor. Some  
6 of these chemicals are industrial chemicals produced intentionally (PCBs, PBBs, etc.). Others  
7 are by-products of industrial processes (PCDDs and PCDFs). There are also a number of  
8 “natural” AhR ligands that are either plant derived (i.e. I-3-C) or are synthesized endogenously,  
9 such as bilirubin. It has been postulated that the natural ligands could be the major contributors  
10 to the daily dose of TEQs, because of their higher estimated intakes (Safe, 1995). The natural  
11 ligands tend to have short half-lives and do not accumulate. The PCDDs/PCDFs and PCBs  
12 included in the TEF methodology clearly bioaccumulate. If contributions to the total TEQ are  
13 estimated on steady-state body burdens of these chemicals instead of daily intake, then TCDD  
14 and other PCDDs/PCDFs and PCBs contribute more than 90% of the total TEQ compared to the  
15 natural ligands (DeVito and Birnbaum, 1996). The difference in the results of these analyses  
16 demonstrates our uncertainty of the relative potencies, exposures and dose metrics used in the  
17 comparisons of the synthetic dioxins vs. the natural AhR ligands.

18 When a comparison is attempted between the perceived relative risk from natural vs.  
19 anthropogenic AhR agonists, a number of factors should be taken into consideration. The  
20 potency of AhR ligands depends on several factors, including AhR binding affinity and  
21 pharmacokinetic properties. When estimating the relative potency of a chemical compared to  
22 TCDD, the larger the difference in pharmacokinetic properties, the greater the effect that study  
23 design has on the relative potency. Initial studies comparing the potency of  
24 indolo[3,2b]carbazole to TCDD demonstrate the importance of the pharmacokinetic differences  
25 between these chemicals. In Hepa-1 cells exposed for 4 hours, the relative potency for induction  
26 of CYP1A1 mRNA of indolo[3,2b]carbazole compared to TCDD is 0.1 (Chen et al., 1995). If  
27 the relative potency is determined after 24 hours of exposure, the potency of  
28 indolo[3,2b]carbazole drops 1,000-fold to 0.0001 (Chen et al., 1995). In addition, the dioxin-like  
29 effects of low doses of indolo[3,2b]carbazole in Hepa-1 cells are transient. Similar transient  
30 effects of other dietary-derived AhR ligands have also been reported (Xu and Bresnick, 1990;  
31 Berghard et al., 1992; Riddick et al., 1994). These data demonstrate that the relative potencies of  
32 these chemicals compared to TCDD are dependent upon the pharmacokinetic properties of the  
33 chemicals and the experimental design used in the comparisons. In addition, these data also  
34 demonstrate that for rapidly metabolizable AhR ligands, the effects are transitory and not  
35 persistent like TCDD. It appears that the transient nature of the effect is due to the transient

1 concentrations of these chemicals in these experimental systems. These data also demonstrate  
2 our uncertainty of the relative potency of the dietary-derived AhR ligands.

3 The chemicals included in the TEF scheme are those that not only bind to AhR but also  
4 bioaccumulate and have long biological half-lives in humans, typically on the order of years. In  
5 contrast, the pharmacokinetics of the endogenous or natural group are not well studied, but these  
6 chemicals tend to be short-lived, with half-lives on the order of minutes to hours. Although both  
7 PAHs and the halogenated aromatics bind to AhR and induce cytochrome P450-related enzyme  
8 activities, only the latter group produces the additional dioxin-like spectrum of toxicological  
9 responses. These toxicities are thought to be due to the persistent exposures attributable to the  
10 long half-lives of these chemicals (Riddick et al., 1994).

11 One caution when comparing the relative exposures to dietary AhR ligands and the  
12 anthropogenic AhR ligands is that few in vivo studies have examined the relative potency of the  
13 dietary or natural AhR ligands for toxic responses. Using the criteria of the WHO workgroup for  
14 PCDDs, PCDFs, and PCBs results in only two in vivo studies of I-3-C which compared the REP  
15 to TCDD (Wilker et al., 1996; Bjeldanes et al., 1991). In an in vivo study of the developmental  
16 effects of I-3-C, in utero exposure of rats to I-3-C resulted in a number of reproduction-related  
17 abnormalities in male offspring, only some of which resemble those induced by TCDD (Wilker  
18 et al., 1996). Because of the different spectrum of effects of I-3-C compared to TCDD in these  
19 developmental studies, it is likely that mechanisms other than AhR activation are involved in  
20 these effects. I-3-C and some of its acid catalyzed oligomerization products alter androgen and  
21 estrogen metabolism (Wilson et al., 1999; Telang et al., 1997), which may contribute to the  
22 developmental effects of I-3-C. While a number of in vitro studies have demonstrated dioxin-  
23 like enzyme induction of the indole derivatives, in order to have REP values that adequately  
24 describe the in vivo potency of these chemicals, future in vivo studies examining toxic responses  
25 are required.

#### 27 **9.3.5.4. *Limitations in Comparing the Quantitative Interactions between Industrial/Synthetic*** 28 ***and Natural AhR Ligands***

29 Although there are limited data on the in vivo biochemical and toxicological effects of  
30 these ligands, the effects of mixtures of anthropogenic and natural AhR ligands is altogether  
31 lacking. There is one study examining the interactions of I-3-C and DIM on the effects of  
32 TCDD in cell culture systems. However, it is uncertain how to extrapolate these in vitro  
33 concentrations to present human in vivo exposures. The limited data available do not adequately  
34 address the interactions between these chemicals. Future in vivo studies are required in order to  
35 better understand the potential interactions between these classes of AhR ligands.

1 Another limitation in comparing the natural AhR ligands to the dioxins is the multiple  
2 effects induced by the natural AhR ligands. In vivo and in vitro studies of I-3-C indicate that it  
3 induces a number of biochemical alterations that are not mediated through the AhR (Broadbent  
4 and Broadbent, 1998). The activation of these additional pathways creates difficulties in making  
5 direct comparisons with TCDD and related chemicals. Similarly, the PAHs also have non-AhR-  
6 mediated biochemical and toxicological effects that also complicate direct comparisons with  
7 TCDD and related dioxins. For example, co-exposure to TCDD and PAHs have demonstrated  
8 both synergistic and antagonistic interactions in mice depending upon the endpoint examined  
9 (Silkworth et al., 1993).

10 Presently, there are several limitations in our understanding of the importance of naturally  
11 occurring dioxin-like compounds vs. the dioxin-like compounds included in the TEF  
12 methodology. First is the limited data available on the dioxin-like toxicities of the natural  
13 ligands. In addition, there is a lack of data on the interactions between these classes of  
14 chemicals. Few if any mixtures of natural AhR ligands and PCDDs or PCDFs examining a toxic  
15 response have been published. Many of the natural AhR ligands have multiple mechanisms of  
16 action that presently cannot be accounted for in the TEF methodology. For example, I-3-C has  
17 anticarcinogenic properties in tumor promotion studies, and these effects may or may not be  
18 mediated through AhR mechanisms (Manson et al., 1998). The lack of data and the role of non-  
19 AhR mechanisms in the biological effects of these chemicals prohibit a definitive conclusion on  
20 the role of natural vs. anthropogenic dioxins in human health risk assessment. Though it is  
21 important to address these issues, the available data do not lend themselves to an appropriate  
22 quantitative assessment of these issues.

23 One of the most significant differences between the industrial Ah receptor ligands (i.e.  
24 dioxins) and the natural Ah receptor ligands is the persistence of the dioxins in biological  
25 systems. Because of their long half-lives, dioxins provide a persistent activation of the Ah  
26 receptor. In contrast, the natural ligands are rapidly metabolized and the activation of the Ah  
27 receptor is short-lived. Determining the relative potency of the natural ligands compared to  
28 TCDD is not necessarily a trivial matter. The relative potency of these chemicals is due to their  
29 ability to bind and activate the Ah receptor and the persistence of this signal. Most of the studies  
30 examining the relative potency of the natural ligands are based on in vitro or short-term in vivo  
31 studies. The estimates of the relative potencies of these chemicals is greatly exaggerated in these  
32 short-term assays because of the bioaccumulative nature of TCDD. Studies comparing the  
33 relative potency of TCDD to TCDF have demonstrated that due to the differences in the half-  
34 lives of TCDF and TCDD, short-term studies overestimate the relative potency of TCDF  
35 compared to the relative potency observed in longer-term studies (DeVito and Birnbaum, 1995).  
36 The relative potencies of the natural ligands would best be estimated following long term

1 exposures. These data are unavailable and thus the estimates of the relative potencies of these  
2 chemicals is unreliable.

3 Although Safe has suggested that exposure to natural AhR ligands is 100 times that of  
4 TCDD and other dioxin-like compounds (Safe, 1995), the impact of the natural AhR ligands  
5 remains uncertain. Epidemiological studies suggest that human exposures to TCDD and related  
6 chemicals are associated with adverse effects, such as developmental impacts and cancer. In  
7 many of these studies, the exposed populations have approximately 100 times more TCDD  
8 exposure than background populations (see Part II, Chapter 7). If the exposure to natural AhR  
9 ligands is included in these comparisons, then the exposed populations should have  
10 approximately double the total TEQ exposures than the background population. It seems  
11 unlikely that epidemiological studies could discriminate between such exposures. These data  
12 suggest that the estimates of the contribution of the natural AhR ligands to the total TEQ  
13 exposure are overestimated. In addition, regardless of the background human exposure to  
14 "natural" AhR ligands, the margin of exposure to TCDD and related chemicals between the  
15 background population and populations where effects are observed remains a concern.  
16

#### 17 **9.4. TOTAL TEQ AND THE ADDITIVITY CONCEPT**

18 The issue of the scientific defensibility of additivity in determining total TEQ has been  
19 raised since the onset of the use of TEFs. Arguments regarding this approach include the  
20 presence of competing agonists or antagonists in various complex mixtures from environmental  
21 sources, interactions based on non-dioxin-like activities (inhibition or synergy), and the fact that  
22 dose-response curves for various effects may not be parallel for all congeners assigned TEFs.  
23 Although comparative pharmacokinetics have also been raised as an issue, this has generally  
24 been accounted for by the heavier weight accorded to in vivo studies in the assignment of TEFs.  
25 Despite these concerns, empirical data support the use of the additivity concept, recognizing the  
26 imprecise nature of the TEFs per se. A substantial effort has been made to test the assumptions  
27 of additivity and the ability of the TEF methodology to predict the effects of mixtures of dioxin-  
28 like compounds. These efforts have focused on environmental, commercial, and laboratory-  
29 derived mixtures. In addition, endpoints examined ranged from biochemical alterations, such as  
30 enzyme induction, to toxic responses such as tumor promotion, teratogenicity, and  
31 immunotoxicity. A brief summary of some of the more important work is given and discussed in  
32 the following section.

33 The TEF methodology has been examined by testing mixtures of chemicals containing  
34 dioxins and sometimes other chemicals. These mixtures have either been combined and  
35 produced in the laboratory or were actual environmental samples. Researchers have also used  
36 different approaches in estimating the TCDD equivalents of the mixtures. Some researchers

1 have determined the REP of the components of the mixture in the same system in which the  
2 mixture was tested and have used these REPs to estimate TCDD equivalents. These studies can  
3 provide insight into the validity of the assumption of additivity of the TEF methodology. Other  
4 researchers have used consensus TEF values to estimate the TCDD equivalents of the mixture. It  
5 is not clear if these studies can be considered true tests of the additivity assumption. The  
6 consensus TEF values have been described as conservative estimates of the relative potency of a  
7 chemical in order to protect humans and wildlife. If the consensus TEF values are conservative  
8 and protective, then they should overestimate the potency of mixtures tested in an experimental  
9 system. In essence, using the consensus TEF values should generally over predict the potency of  
10 a mixture (and therefore under predict the response) when compared to the equivalent  
11 concentrations of TCDD in an experimental system. In the following discussion of the studies  
12 examining the assumption of additivity, these differences in study design and their implications  
13 for interpretation of the data must be considered.

#### 14 15 **9.4.1. Examination of Laboratory Mixtures of PCDDs and PCDFs**

16 Bock and colleagues evaluated the TEF methodology in several systems using both  
17 individual congeners as well as laboratory-derived mixtures (Lipp et al., 1992; Schrenk et al.,  
18 1991, 1994). REPs or toxic equivalents or “TEs” (as designated by the authors) were determined  
19 for 2,3,7,8-substituted PCDDs based on enzyme induction in human HepG2 cells, rat H4IIE  
20 cells, and primary rat hepatocytes. Three laboratory-defined mixtures (M1, M2, and M3) were  
21 prepared and then examined in these same cell culture systems. TCDD contributed between  
22 6%-8% of the TEQs for M1 and M2, but was not present in M3. In M1, 1,2,3,4,6,7,8-HpCDD  
23 contributes approximately 60% of the TEQ, and 1,2,3,7,8-PCDD and 1,2,3,4,7,8-HxCDD  
24 contribute 10% each. In M2, 1,2,3,4,6,7,8-HpCDD contributes 45%, 1,2,3,7,8-PCDD and  
25 1,2,3,4,7,8-HxCDD contribute 15% each; and TCDD, 1,2,3,6,7,8-HxCDD, and 1,2,3,7,8,9-  
26 HxCDD contribute less than 10% to the total TEQ. The TEQs in M3 are derived predominately  
27 from 1,2,3,4,7,8-HxCDD (50%); 1,2,3,4,7,8-HxCDD (20%); and 1,2,3,6,7,8-HxCDD (18%).  
28 These mixtures also contain up to 49 chlorinated dibenzo-*p*-dioxins. The TCDD equivalents of  
29 the mixtures were determined on the basis of the assumption of additivity using the TEF  
30 methodology and the laboratory derived REPs or TEs as well as experimentally by comparing the  
31 EC<sub>50</sub>s of the mixtures with that of TCDD. According to the authors, in all three systems the data  
32 demonstrated that the components of the mixture act in an additive manner (Lipp, 1991; Schrenk  
33 et al., 1991). For example, in the human HepG2 cells the EC<sub>50</sub> of a mixture of 49 different  
34 PCDDs was determined experimentally at 0.034 pg TEQ/plate, compared to the calculated or  
35 predicted EC<sub>50</sub> of 0.028 pg TEQ/plate. Interestingly, the TEF methodology accurately predicted

1 the effects of M3, a mixture containing predominately OCDD, some heptaCDDs and hexaCDDs,  
2 and no pentaCDDs or TCDD (Schrenck et al., 1991).

3 Bock and colleagues also tested a mixture of 49 PCDDs in a rat liver tumor promotion  
4 study. The mixture, designated as M2, was the same mixture used in the cell culture studies  
5 described above and TCDD contributed approximately 8% of the TEQs of this mixture. In  
6 these studies, rats received an estimated 2-200 ng TCDD/kg/d or 200-20,000 ng mixture/kg/d.  
7 The doses of the mixture were equivalent to the TCDD doses using a TE of the mixture of 0.01  
8 based on enzyme induction in rat hepatocytes (Schrenk et al., 1991). A comparison of the  
9 relative potency of the mixture was based on liver concentrations of the chemicals followed by  
10 TEQ calculations using the I-TEFs (NATO/CCMS, 1988). According to the authors, in the low-  
11 dose region (2-20 ng TCDD/kg/d) the I-TEFs accurately predict the enzyme-inducing activity of  
12 the mixture but tend to overestimate the potency of the mixture at the higher doses (20-200  
13 ng/kg/d). Also, according to the authors, the I-TEFs provide a rough estimate of the tumor-  
14 promoting potency of the mixture but overestimate the mixture's potency. However, the authors  
15 did not quantify or qualify the magnitude of the overestimation.

16 In the studies by Schrenk and colleagues, the TEQs were based on tissue dose, not  
17 administered dose. Recent studies by DeVito et al. (1997b, 2000) indicate that the REP for  
18 dioxin-like compounds can differ when determined based on administered or tissue dose. The  
19 higher chlorinated dioxins tend to accumulate in hepatic tissue to a greater extent than does  
20 TCDD, and their REPs tend to decrease when estimated based on tissue dose (DeVito et al.,  
21 1997b, 2000). Because the I-TEFs are based on an administered dose, they may not predict the  
22 response when the TEQ dose is expressed as liver concentration. If the TEQ dose in the data by  
23 Schrenk et al. (1994) is compared on an administered dose, then the dose-response relationship  
24 for increases in relative volume of preneoplastic ATPase-deficient hepatic foci (% of liver) are  
25 comparable between TCDD and the mixture, indicating that additive TEFs provided an  
26 approximation of the tumor-promoting ability of a complex mixture of PCDDs (Schrenck et al.,  
27 1994). In addition, because TCDD contributed less than 10% of the total TEQ in these mixtures,  
28 these data indicate that the assumption of additivity reasonably predicts the response of complex  
29 mixtures of dioxins.

30 In responsive mouse strains, induction of cleft palate and hydronephrosis by TCDD  
31 occurs at doses between 3 and 90  $\mu\text{g}$  TCDD/kg (Nagao et al., 1993; Weber et al., 1985;  
32 Birnbaum et al., 1985, 1987, 1991). Several groups have examined the assumption of additivity  
33 using teratogenic effects of dioxins as an endpoint. Birnbaum and colleagues examined TEF  
34 methodology using mouse teratogenicity as an endpoint (Weber et al., 1985; Birnbaum et al.,  
35 1985, 1987, 1991). REPs were derived for 2,3,7,8-TCDF, 2,3,4,7,8-PeCDF, 1,2,3,7,8-PeCDF,  
36 and 1,2,3,4,7,8-HxCDF (Weber et al., 1984, 1985; Birnbaum et al., 1987). Analysis of the dose-

1 response for these chemicals, based on administered dose, demonstrated parallel slopes.  
2 According to the authors, dose-response analysis of two mixtures containing either TCDD and  
3 2,3,7,8-TCDF or 2,3,4,7,8-PeCDF and 1,2,3,4,7,8-HxCDF demonstrated strict additivity  
4 (Birnbaum et al., 1987; Weber et al., 1985).

5 Nagao et al. (1993) also examined the TEF methodology using teratogenicity in mice as  
6 an endpoint. Mice were exposed to a single dose of TCDD (5-90  $\mu\text{g}/\text{kg}$ ) or a mixture of PCDDs,  
7 or one of two different mixtures of PCDFs. The mixtures contained no detectable TCDD. The I-  
8 TEFs were used to determine the TEQ of the mixtures. According to the authors, the I-TEFs  
9 predicted the potency of the PCDD mixture, and the dose-response relationship was consistent  
10 with the assumption of additivity. The I-TEFs overestimated the potency of the PCDF mixtures  
11 by two- or fourfold. All three mixtures contained significant concentrations of non 2,3,7,8-  
12 chloro-substituted PCDDs and PCDFs in addition to the dioxin-like compounds present. In the  
13 studies by Birnbaum and colleagues (Weber et al., 1985; Birnbaum et al., 1985, 1987, 1991) and  
14 Nagao et al. (1993) examining the assumption of additivity using teratogenicity as an endpoint,  
15 the TEF methodology proves useful in estimating the effects of these mixtures.

16 Rozman and colleagues have examined the assumption of additivity of PCDDs in both  
17 acute and subchronic studies. In acute studies, TCDD (20-60  $\mu\text{g}/\text{kg}$ ), 1,2,3,7,8-PCDD (100-300  
18  $\mu\text{g}/\text{kg}$ ), 1,2,3,4,7,8-HxCDD (700-1,400  $\mu\text{g}/\text{kg}$ ), and 1,2,3,4,6,7,8-HpCDD (3,000-8,000  $\mu\text{g}/\text{kg}$ )  
19 were administered to male rats, and REP values were determined for lethality. A mixture of all  
20 four chemicals at equally potent concentrations was then prepared and dose-response studies  
21 were performed with the mixture at doses that would produce 20%, 50%, and 80% mortality.  
22 The mixture studies demonstrated strict additivity of these four chemicals for biochemical and  
23 toxicological effects (Stahl et al., 1992; Weber et al, 1992a,b). Following the acute studies,  
24 Viluksela et al. (1998a,b) prepared a mixture of these chemicals and estimated the TEQ based on  
25 the REPs from the acute studies. A loading/maintenance dose regimen was used for 90 days and  
26 the animals were followed for an additional 90 days. According to the authors, the assumption of  
27 additivity predicted the response of the mixture for lethality, wasting, hemorrhage, and anemia,  
28 as well as numerous biochemical alterations such as induction of hepatic EROD activity and  
29 decreases in hepatic phosphoenolpyruvate carboxykinase and hepatic tryptophan 2,3-dioxygenase  
30 (Viluksela et al., 1997; 1998). Increases in serum tryptophan concentrations and decreases in  
31 serum thyroxine concentrations were also predicted by the TEF methodology (Viluksela et al.,  
32 1998a).

33 Rozman and colleagues followed up these initial studies by examining the assumption of  
34 additivity of the effects of PCDDs as endocrine disruptors (Gao et al., 1999). Ovulation is a  
35 complex physiological phenomenon that requires the coordinated interaction of numerous  
36 endocrine hormones. In a rat model, ovulation can be inhibited by TCDD at doses between 2 to

1 32  $\mu\text{g}/\text{kg}$  (Gao et al., 1999). Dose-response analysis of TCDD, 1,2,3,7,8-PeCDD, and  
2 1,2,3,4,7,8-HxCDD demonstrate that the slopes are parallel and the REPs are 0.2 and 0.04,  
3 respectively. According to the authors, the dose response for a mixture of these chemicals, in  
4 which the components were at equally potent concentrations, further demonstrated the response  
5 additivity of mixtures of PCDDs and the predictive ability of the TEF methodology (Gao et al.,  
6 1999).

7 The research on the interactions between mixtures of PCDDs and PCDFs has taken two  
8 approaches. The first is to derive REP values in the same system in which the mixtures shall be  
9 tested. These studies confirm that the assumption of additivity can predict the response of  
10 mixtures of PCDDs and PCDFs. A second approach is to use the I-TEFs to assess the potency of  
11 a mixture. These studies tend to indicate that the I-TEFs overestimate the potency of a mixture  
12 by factors of two to four. Recently, the WHO TEFs have been described as “order of magnitude”  
13 estimates of the potency of dioxin-like compounds. However, the studies using consensus TEFs  
14 demonstrate that for mixtures of PCDDs and PCDFs, the TEF methodology will predict within a  
15 half-order of magnitude or less (Schrenck et al., 1994; Nagao et al., 1993). In either case, the  
16 TEF methodology accurately predicts the responses of experimentally defined mixtures of  
17 PCDDs and PCDFs. Furthermore, several of these studies described the effects of mixtures  
18 containing either no TCDD or with TCDD contributing less than 10% of the TEQ in the presence  
19 of significant concentrations of non-2378- CDDs and CDFs. These studies strongly support the  
20 use of the TEF methodology.

#### 21 22 **9.4.2. Examination of Commercial or Laboratory-Derived Mixtures of PCDDs, PCDFs, 23 and PCBs**

24 Commercial mixtures of PCBs elicit a broad spectrum of biological and toxicological  
25 responses in both experimental animals and humans. Some of the observed effects resemble  
26 those induced by dioxin and furans (enzyme induction, immunotoxicity, teratogenicity, endocrine  
27 alterations, etc.). Attempts to expand the TEF approach to risk assessment of PCBs have  
28 investigated the ability of both commercial PCBs and individual congeners, selected on the basis  
29 of structure-activity relationships, to induce dioxin-like effects and to interact with TCDD. One  
30 of the first studies to examine the interactions of individual PCB congeners with TCDD used  
31 mouse teratogenicity as an endpoint (Birnbaum et al., 1985, 1987). A mono-ortho PCB  
32 (2,3,4,5,3',4'-HxCPCB or PCB 156) at doses of 20 mg/kg or higher (Birnbaum, 1991) induced  
33 hydronephrosis and cleft palate in mice. When mice were co-exposed to PCB 156 and 3.0  $\mu\text{g}$   
34 TCDD/kg the interactions resulted in strict additivity.

35 The interaction of TCDD with dioxin-like PCBs has been examined by van Birgelen et al.  
36 (1994a,b) in subchronic rat feeding studies. Concentrations of PCB 126 in the diet between 7

1 and 180 ppb induced several dioxin-like effects, including CYP1A1 induction, thymic atrophy,  
2 liver enlargement, and decreases in hepatic retinol concentrations, body weight gains, and plasma  
3 thyroxine concentrations. The REP for PCB 126 was estimated by the authors at between 0.01  
4 and 0.1 (van Birgelen et al., 1994a). Co-exposure to PCB 126 and TCDD (0.4 or 5.0 ppb) in the  
5 diet demonstrated additivity for all responses except induction of CYP1A2 and decreases in  
6 hepatic retinol, where antagonism occurred at the highest doses of PCB 126 and TCDD.  
7 These nonadditive interactions were not observed at more environmentally relevant exposures,  
8 according to the author. In a similar study design, PCB 156 also induced dioxin-like effects with  
9 a REP estimated between 0.00004 and 0.001 (van Birgelen et al., 1994b). Similar to the  
10 interactions between PCB 126 and TCDD, additive interactions were observed in animals  
11 receiving mixtures of PCB 156 and TCDD in the low-dose region for all responses examined.  
12 However, at the highest exposures of PCB 156 and TCDD, the authors reported slight  
13 antagonistic interactions for decreases in hepatic retinol (van Birgelen et al., 1994b). For both  
14 PCB 126 and PCB 156, antagonistic interactions were observed with TCDD only at exposures  
15 that produced maximal CYP1A1 induction. The authors concluded that the antagonistic  
16 interactions are unlikely to occur at relevant human exposures.

17 In a series of studies examining the TEF methodology, TCDD (1.5-150 ng/kg/d),  
18 1,2,3,7,8-PeCDD; 2,3,7,8-TCDF; 1,2,3,7,8-PeCDF; 2,3,4,7,8-PeCDF; OCDF; the coplanar PCBs  
19 77, 126, and 169; and the mono-ortho substituted PCBs 105, 118, and 156 were administered to  
20 mice 5 days/week for 13 weeks. REPs were determined for EROD induction, a marker for  
21 CYP1A1, in liver, lung, and skin; ACOH activity, a marker for CYP1A2, in liver; and hepatic  
22 porphyrins (DeVito et al., 1997a; 2000; van Birgelen et al., 1996c). These data demonstrate that  
23 the dose-response curves for the PCDDs and PCDFs were parallel (DeVito et al., 1997a). Dose-  
24 response curves for some of the enzyme induction data for the individual PCBs displayed  
25 evidence of non-parallelism in the high-dose region (DeVito et al., 2000). A laboratory-derived  
26 mixture of these chemicals with congener mass ratios resembling those in food was administered  
27 to mice and rats, and indicated that despite the evidence of non- parallelism for the PCBs at high  
28 doses, the assumption of additivity predicted the potency of the mixture for enzyme induction,  
29 immunotoxicity, and decreases in hepatic retinoids (Birnbaum and DeVito, 1995; van Birgelen et  
30 al., 1996; 1997; DeVito et al., 1997; Smialowicz et al., 1996). In addition, the REPs estimated in  
31 mice also predicted the response of the mixture in rats for enzyme induction and decreases in  
32 hepatic retinyl palmitate concentrations (van Birgelen et al., 1997d; Ross et al., 1997; DeVito et  
33 al., 1997b). These studies indicate that not only do the REPs for enzyme induction in mice  
34 predict other responses, such as immunotoxicity and decreases in hepatic retinyl palmitate, they  
35 also can be used to predict responses of mixtures in another species.

1           The commercial PCB mixtures induce a variety of dioxin-like effects. Rats exposed to  
2 commercial Aroclors and observed for 2 weeks exhibited dose-dependent induction of hepatic  
3 CYP1A activity (EROD) but no thymic atrophy (Harris et al., 1993). Using REP values derived  
4 for EROD induction in rats, the TEF methodology provided good agreement with experimental  
5 estimates of the ED50 for enzyme induction. However, use of the conservative TEF values of  
6 Safe (1990) overestimated the potency of the Aroclor mixtures (Harris et al., 1993). In contrast,  
7 similar studies examining immunotoxicity as an endpoint demonstrate that both experimentally  
8 derived REP values and the conservative TEF values of Safe (1990) overestimate the potency of  
9 the Aroclor mixtures by a factor of 1.2 - 22 (Harper et al., 1995). These data demonstrate that  
10 there are nonadditive interactions between dioxin-like compounds and the non-dioxin-like PCBs  
11 and that these interactions are response specific and most likely are not due to AhR antagonism.

12           In *in vitro* systems, using H4IIE cells and rat hepatocytes, Schmitz et al. (1995, 1996)  
13 examined the assumption of additivity for individual congeners as well as commercial mixtures.  
14 After deriving REP values for enzyme induction, the authors concluded that a laboratory mixture  
15 of PCBs 77, 105, 118, 126, 156, and 169 demonstrated perfect additive behavior in these cell line  
16 systems (Schmitz et al., 1995). However, when the mixture was combined with a tenfold surplus  
17 of a mixture containing non-dioxin-like PCBs (PCB 28, 52, 101, 138, 153 and 180), the mixture  
18 demonstrated an approximate threefold higher TEQ than predicted. The authors concluded that a  
19 moderate synergistic interaction is responsible for the increased enzyme-inducing potency of the  
20 mixture containing dioxins and non-dioxin-like PCBs. Further studies by Schmitz et al. (1996)  
21 also demonstrated a slight synergistic deviation (less than threefold) from strict additivity when  
22 the calculated TEQ based on chemical analysis of Aroclor 1254 and Clophen A50 was compared  
23 to the CYP1A-induction TEQ derived in an established rat hepatoma cell line (H4IIE) (Schmitz  
24 et al., 1996).

25           Recently, Mayes et al. (1998) compared the carcinogenicity of Aroclor 1016, 1242, 1254  
26 and 1260 in Sprague-Dawley rats. All four mixtures increased the incidence of hepatic tumors in  
27 female rats. The authors concluded that the female rats were more susceptible than the males to  
28 the hepatocarcinogenic effects of these mixtures. In the two-year bioassay of TCDD in Sprague-  
29 Dawley rats, the female rats were also more susceptible to the hepatocarcinogenic effects than the  
30 males (Kociba et al., 1978). Mayes and colleagues (1998) performed congener specific analysis  
31 of the Aroclor mixtures and calculated dioxin TEQ values for each of these mixtures. In order to  
32 compare the cancer induction potential of dioxin TEQ in PCB mixtures (Mayes et al. 1998) with  
33 that from TCDD (Kociba et al., 1978) in the same species of rat, the dose-response relationships  
34 are graphed and presented in figure 9-2. The dose-response relationship for hepatic tumors in  
35 female rats is similar between the Aroclor 1242, 1254, 1260 and TCDD dose regimen. This  
36 analysis demonstrates that the TEF methodology qualitatively and quantitatively predicts the

1 response of a complex mixture of PCBs. This is particularly important because the mass  
2 concentration of dioxin equivalents in the mixture is approximately 100,000 times less than the  
3 non-dioxin-like PCBs present in these mixtures. These data strongly support the ability of the  
4 TEF methodology to estimate the carcinogenic potency of a complex mixture of PCBs even in  
5 the presence of significant concentrations of non-dioxin-like PCBs.

6 Researchers have evaluated the applicability of the TEF methodology to mixtures  
7 containing dioxin-like PCBs by examining the interactions of binary mixtures, laboratory-derived  
8 mixtures, or commercial mixtures of PCBs. The studies examining the binary mixtures or  
9 laboratory-derived mixtures have demonstrated that the assumption of additivity provides good  
10 estimates of the potency of a mixture of PCBs and other dioxin-like compounds. In contrast,  
11 studies using commercial mixtures of PCBs suggest that the assumption of additivity may be  
12 endpoint specific, and that both synergistic and antagonistic interactions may occur for some  
13 mixtures of dioxins and PCBs for certain endpoints. A more detailed examination of these issues  
14 follows in the section on nonadditive interactions with non-dioxin-like compounds.

#### 15 16 **9.4.3. Examination of Environmental Samples Containing PCDDs, PCDFs, and/or PCBs**

17 One of the first tests of the TEF methodology examined soot from a transformer fire in  
18 Binghamton, NY (Eadon et al., 1986). Benzene extracts of soot from a PCB transformer fire  
19 which contained a complex mixture of PCDDs, PCDFs, PCBs, and polychlorinated  
20 biphenylenes were administered to guinea pigs as single oral doses, and LD<sub>50</sub> values were  
21 compared to TCDD. Relative potency values for the PCDDs and PCDFs based on guinea pig  
22 LD<sub>50</sub> values were used to estimate the TCDD equivalents of the mixture. Eadon and co-workers  
23 exposed guinea pigs to either TCDD alone or the soot and determined their LD<sub>50</sub>s. With these  
24 relative potency values, the soot extract had a TCDD equivalent concentration of 22 ppm.  
25 Comparison of the LD<sub>50</sub>s for TCDD and the soot led to a TCDD equivalent of 58 ppm for the  
26 mixture. Other endpoints examined included alterations in thymus weight, body weight, serum  
27 enzymes, and hepatotoxicity. Experimentally the TCDD equivalents of the soot varied from 2 to  
28 58 ppm. The authors concluded that because the benzene extract of the soot contained hundreds  
29 of chemicals including PCDDs, PCDFs, and PCBs, the difference between the calculated TEQ of  
30 22 ppm and the experimentally derived TEQs between 2 and 58 seems minimal. (Note: the  
31 initial analytical TEQ value of soot [22 ppm] was calculated on the basis of guinea pig LD<sub>50</sub>  
32 values of the respective components; using the current recommended TEF scheme [van den Berg  
33 et al., 1998], the “calculated” TCDD TEQ would be approximately 17 ppm.)

34 Shortly after the studies on the Binghamton transformer fire soot, investigators applied the  
35 TEF methodology to the leachate from Love Canal, NY. The organic phase of the leachate  
36 consisted of more than 100 different organic compounds including PCDDs and PCDFs. The

1 leachate did not contain PCBs or PAHs. The authors estimated the TEQ of the mixture on the  
2 basis of REP values for teratogenicity (cleft palate and hydronephrosis in mice) for the PCDDs  
3 and PCDFs present in the leachate. The authors state that the leachate contained the equivalent  
4 of 3  $\mu\text{g}$  TCDD/g and that more than 95% of the TEQ was contributed by TCDD. There were two  
5 other PCDFs present in the leachate, and their contribution to the total TEQ was approximately  
6 5% (Silkworth et al., 1989). When the TEQ of the mixture was based on dose-response analysis  
7 of the mixture compared to TCDD, the leachate was estimated to contain between 6.6 and 10.5  
8  $\mu\text{g}$  TCDD/g (Silkworth et al., 1989). The authors concluded there was a good agreement  
9 between the experimental TCDD equivalents (6.6-10.5  $\mu\text{g}$  TCDD/g) and the analytical TEQs (3  
10  $\mu\text{g}$  TCDD/g). In addition, these studies illustrate that the non-AhR components of the leachate  
11 did not interfere with receptor-mediated teratogenicity (Silkworth et al., 1989). Additional  
12 investigations have shown that the same complex mixture of non-AhR agonists slightly  
13 potentiated TCDD-induced thymic atrophy and immunosuppression (plaque-forming cells/spleen  
14 response) while decreasing the hepatic CYP1A-inducing ability of the TCDD component  
15 (Silkworth et al., 1993).

16 The assumption of additivity was also examined using a PCDD/PCDF mixture extracted  
17 from fly ash from a municipal waste incinerator (Suter-Hofmann and Schlatter, 1989). As a  
18 purification step, rabbits were fed organic extracts from the fly ash. After 10 days the livers were  
19 removed and analyzed for PCDDs and PCDFs. The rabbit livers contained predominately  
20 2,3,7,8-substituted PCDDs/PCDFs. Based on the chemical analysis of the liver, lyophilized and  
21 pulverized liver was added to the standard rat diet. This diet was fed to rats for 13 weeks and  
22 body weights and terminal thymus weights were recorded. The authors concluded that the  
23 mixture of PCDDs and PCDFs produced equivalent toxicities as TCDD, and the assumption of  
24 additivity was confirmed.

#### 25 26 **9.4.4. Nonadditive Interactions With Non-Dioxin-Like Compounds**

27 For a number of toxicological responses, there appears to be evidence for nonadditive  
28 interactions in defined dose ranges by both commercial Aroclors and major congeners with little  
29 if any AhR agonist activity (i.e., PCB 153). Both commercial Aroclors and a PCB mixture  
30 comprised of major congeners found in human breast milk were shown to antagonize the  
31 immunotoxic effects of TCDD in mice (Biegel et al., 1989; Davis and Safe, 1989; Harper et al.,  
32 1995). When immunotoxicity-derived TEF values for a variety of PCB congeners were used in  
33 an additive manner to estimate TCDD TEQs for commercial Aroclors, in comparison to the  
34 experimental TEQs, they were approximately predictive for Aroclor 1254 and 1260 (Harper et  
35 al., 1995). However, the TEF approach tended to overestimate the immunotoxicity of Aroclors  
36 1242 and 1248, suggesting some antagonism.

1 Typical responses to TCDD exposure in rodents include CYP1 enzyme induction and  
2 thymic atrophy. Rats consuming a diet containing 5 ppb TCDD for 13 weeks exhibited a 33-fold  
3 increase in hepatic CYP1A activity (EROD) and a greater than 50% reduction in relative thymus  
4 weight. Addition of PCB 153 to the diet at concentrations up to 100 ppm had no significant  
5 effect on either response (van der Kolk et al., 1992). Mice dosed simultaneously with TCDD and  
6 up to a 10<sup>6</sup>-fold molar excess of PCB 153 (1 nmol/kg vs. 1 mmol/kg) exhibited no significant  
7 dose-dependent alteration in hepatic CYP1A1/A2 protein compared to the TCDD dose group  
8 alone (De Jongh et al., 1995). There was, however, an approximate twofold increase in hepatic  
9 EROD activity in the highest combined PCB 153:TCDD dose group. Subsequent tissue analysis  
10 revealed that the increase in EROD activity appeared related to PCB 153 increasing hepatic  
11 TCDD concentrations. The same PCB congener at high doses (358 mg/kg) is able to almost  
12 completely inhibit TCDD-induced suppression of the plaque-forming cell (PFC) response toward  
13 sheep red blood cells in male C57BL/6J mice (Biegel et al., 1989; Smialowicz et al., 1997).  
14 However, as PCB 153 displays negligible AhR binding affinity, the exact mechanism(s) behind  
15 these interactions is unknown. Recently, it has been shown that PCB 153 at high doses (greater  
16 than 100 mg/kg) actually enhances the PFC response in female B6C3F1 mice, thereby raising the  
17 “control” set point. When combined doses of TCDD and PCB 153 are then compared to the  
18 elevated PCB 153 response, an apparent block of the immunosuppressive effect of TCDD is  
19 observed (Smialowicz et al., 1997). The relevance of this functional antagonism is uncertain, as  
20 the doses required to inhibit the TCDD-like effects are at least 100 mg/kg of PCB 153. These  
21 doses of PCB 153 seem unlikely to occur in human populations except under extreme conditions.

22 Commercial PCBs and various PCB congeners have been shown to potentiate or  
23 antagonize the teratogenicity of TCDD depending upon the dose ranges and response examined  
24 (Biegel et al., 1989; Morrissey et al., 1992). Treatment of developing chicken embryos with  
25 TCDD and dioxin-like PCBs induces a characteristic series of responses, including embryo  
26 lethality and a variety of embryo malformations/deformities. Combined exposure of chicken  
27 embryos to both PCB 126 and PCB 153 (2 µg/kg and 25-50 mg/kg, respectively) resulted in  
28 protection from PCB 126-induced embryo malformations, edema, and liver lesions, but not  
29 mortality (Zhao et al., 1997). In mice, doses of 125 mg PCB 153/kg or higher inhibit the  
30 induction of cleft palate by TCDD (Biegel et al., 1989; Morrissey et al., 1992). The induction of  
31 hydronephrosis by TCDD was slightly antagonized by PCB 153, but only at doses of 500 mg/kg  
32 or higher. Once again, the environmental relevance of exposures of 100 mg/kg of PCB 153 or  
33 higher remains quite speculative, and nonadditive interactions are not expected at environmental  
34 exposures.

35 Nonadditive interactions have also been observed in rodents exposed to both TCDD and  
36 mixtures of various PCB congeners for hepatic porphyrin accumulation and alterations in

1 circulating levels of thyroid hormones. A strong synergistic response was seen with hepatic  
2 porphyrin accumulation in female rats following the combined dietary exposure to TCDD and  
3 PCB 153 (van Birgelen, 1996a). The mechanism accounting for the interaction was thought to  
4 be a combination of both AhR-dependent (CYP1A2 induction) and AhR-independent  
5 ( $\delta$ -aminolevulinic acid synthetase [ALAS] induction) events. Additionally, subchronic exposure  
6 of mice to a mixture of PCDDs, PCDFs, and dioxin-like PCBs in a ratio derived from common  
7 foods also resulted in a highly synergistic response, when compared to an equivalent dose of  
8 TCDD alone, for both hepatic porphyrin accumulation and urinary porphyrin excretion (van  
9 Birgelen et al., 1996b). PCB 153, although not porphyrinogenic alone, when added to the  
10 mixture further enhanced the synergistic response of hepatic porphyrin accumulation. Non-AhR-  
11 mediated induction of ALAS activity by both the dioxin-like mono ortho-substituted PCBs in the  
12 mixture and by PCB 153 was hypothesized to partially explain the synergism.

13 Decreases in thyroid hormone levels have been observed in both experimental animals and  
14 humans following exposure to both dioxin-like and non-dioxin-like compounds (Nagayama et  
15 al., 1998; Koopman-Esseboom et al., 1997). It is currently thought that multiple mechanisms,  
16 including induction of specific isozymes of hepatic UDP-glucuronyl transferase (UDPGT) and  
17 binding to thyroid hormone transport proteins (thyroid binding globulin, transthyretin) could be  
18 involved. Exposure of female rats to a food-related mixture of PCDDs, PCDFs, and dioxin-like  
19 PCBs for 90 days resulted in an approximately 85% decrease in decrease in plasma levels of  
20 thyroxine. In contrast, the TCDD equivalent dose produced no effect on serum thyroxine (van  
21 Birgelen et al., 1997). Increased induction of several isoforms of UDPGT by the HAH mixture  
22 as compared to TCDD was thought to only partially explain the observed response with  
23 thyroxine levels.

24 Several studies examining the interactions of dioxins and non-dioxins for rat liver tumor  
25 promotion and additive and nonadditive interactions have been reported. Synergistic interactions  
26 for tumor promotion have been observed for combinations of PCB 77 and PCB 52 (2,2',5,5'-  
27 tetrachlorobiphenyl) in rat liver (Sargent et al., 1992). Bager et al. (1995) reported greater than  
28 additive interactions of PCBs 126 and 153 in a rat liver tumor promotion model.

29 The assumption of additivity was examined in a laboratory-derived mixture of PCDDs,  
30 PCDFs, and PCBs in a rat liver tumor promotion model (van der Plas et al., 1999). The mixture  
31 contained TCDD, 1,2,3,7,8-PeCDD, 2,3,4,7,8-PeCDF, and PCBs 126, 118, and 156. The  
32 composition of the mixture was based on concentrations of these chemicals in Baltic herring.  
33 PCB 126 and 1,2,3,7,8-PeCDD accounted for 65% of the TEQ in the mixture and TCDD  
34 accounted for approximately 6.6%. Both TCDD and the TEQ mixture increased mean foci  
35 volume and the volume fraction of foci in the liver. However, the response was statistically  
36 significantly greater in the TCDD treated animals by approximately 2-fold. While the TEQ

1 mixture did not produce the exact same response level as TCDD, it is difficult to quantify the  
2 overestimation of the TEF methodology in this study since only a single dose level was  
3 examined. The authors also did a dose-response study with the mixture. However, they added  
4 PCB 153 to the mixture used for the dose response study. The concentration of PCB 153 was  
5 20,000 times the concentration of TCDD in these mixtures. Dose levels of 0.5, 1, and 2 ug  
6 TEQ/kg/week were administered to the animals. The presence of PCB 153 did not alter the  
7 effects of the 1 ug TEQ/kg/week dose since there was no statistical difference between the  
8 response of animals to the TEQ mixture with or without PCB 153. The highest dose examined, 2  
9 ug TEQ/kg/week produced an effect that was statistically equivalent to the animals treated with  
10 TCDD alone. Van der Plas et al (1999) also determined the concentration of chemicals in the  
11 liver at the termination of the study. Their data suggest that the lower response level of the  
12 mixture is due to pharmacokinetic interactions. Animals administered 1 ug TEQ/kg/week had  
13 approximately one third of the liver TEQ concentrations as the animals treated with TCDD.  
14 Animals treated with 2 ug/kg/week had equivalent TEQ concentrations in the liver and also had  
15 similar responses as animal treated with 1 ug TCDD/kg/week. Van der Plas and colleagues  
16 concluded that the TEF methodology predicted the tumor-promoting potency of the mixture quite  
17 well, within a factor of two, but pharmacokinetic interactions between dioxins may alter the  
18 accuracy of the methodology (van der Plas et al., 1999).

19 In another study, van der Plas and colleagues (2000) examined the interactions of co-  
20 planar and non-coplanar components of Aroclor 1260 in a tumor promotion study. In these  
21 studies, Aroclor 1260 was separated into planar (0-1 ortho chlorines) and non-planar (2-4 ortho  
22 chlorines) components. Rats were then exposed to either 1 ug TCDD/kg/week, 1 mg 0-  
23 1ortho/kg/week, 9 mg 2-4 ortho/kg/week, 10 mg 0-4 ortho/kg/week or 10 mg aroclor  
24 1260/kg/week. Mean foci volume and the volume fraction of the liver occupied by foci increased  
25 in animals treated with either TCDD, the 2-4 ortho mixture, the 0-4 ortho mixture and aroclor  
26 1260. The 0-1 ortho mixture did not alter foci development compared to the control animals.  
27 Van der Plas et al (2000) concluded that the results from their study indicate that 80% of the  
28 carcinogenicity of Aroclor 1260 is due to the non-dioxin congeners in the mixture.

29 In the study described above, Van der Plas et al (2000) used the CALUX assay to  
30 determine the TEQ of the different mixtures. The lot of Aroclor 1260 used in this study had very  
31 low TEQs based on the CALUX assay. For example, 10 mg Aroclor 1260/kg/week was  
32 equivalent to 0.0012 ug TEQ/kg/week or approximately 0.12 ppm TEQ. In addition, the 1 mg 0-  
33 1 ortho/kg/week dose is equivalent to 0.09 ng TEQ/kg/d. In contrast, the lot of Aroclor 1260  
34 used by Mayes et al (1998) had 7.2 ppm TEQ concentrations using the WHO TEF values and  
35 dose levels examined ranged from 10-42 ng TEQ/kg/d. The lot of Aroclor 1260 used by Mayes  
36 et al (1998) has approximately 60 times more TEQs than the lot used by van der Plas et al

1 (2000). In the Mayes et al (1998) studies the TEF methodology accurately predicts the  
2 carcinogenic response of the mixture. The differences in the van der Plas et al (2000) and the  
3 Mayes et al (1998) studies may be due to the different lots of Aroclor 1260 used by these two  
4 groups.

5 The interactions of dioxins with non-dioxin-like compounds results in additive and  
6 nonadditive responses. The antagonistic interactions, while endpoint specific, appear to occur at  
7 dose levels that greatly exceed most human exposures and should not affect the overall use of the  
8 TEF methodology. One of the difficulties in addressing the nonadditive interactions is  
9 understanding the mechanism behind these interactions. For the greater than additive  
10 interactions for induction of porphyria and decreases in serum thyroxine, there are hypotheses  
11 that may explain these effects. The mechanism of the antagonistic interactions of non-dioxin-  
12 like PCBs and TCDD on immunotoxicity and teratogenicity in mice is uncertain. For other  
13 responses, such as developmental reproductive toxicity, the interactions of PCDDs, PCDFs, and  
14 PCBs have not been examined. In addition, it has also been suggested that antagonism of Ah  
15 receptor-mediated events may be species specific. For example, addition of PCB 52, a congener  
16 commonly found in biotic samples, inhibited the TCDD-induced expression of a reporter gene  
17 under the regulatory control of the Ah receptor in mouse and rat cells, but not in guinea pig or  
18 human hepatoma cells (Aarts et al., 1995). Our limited understanding of the interactions  
19 between dioxins and non-dioxins for a variety of responses requires further research before their  
20 impact on the TEF methodology can be fully understood.

#### 21 22 **9.4.5. Examination of the TEF Methodology in Wildlife**

23 Many wildlife species also exhibit toxic effects associated with exposure to halogenated  
24 aromatic hydrocarbons. Early life stage (ELS) or sac fry mortality in fish, characterized by  
25 edema, structural malformations, and growth reduction prior to fry mortality can be induced in  
26 trout species following exposure to dioxin-like PCDDs, PCDFs, and PCBs (Walker and  
27 Peterson, 1991). Binary combinations of a variety of PCDDs, PCDFs, and both dioxin and non-  
28 dioxin-like PCB congeners injected into fertilized trout eggs were also capable of inducing ELS  
29 mortality, with the majority of interactions between the congeners described as strictly additive  
30 (Zabel et al., 1995). When a synthetic complex mixture of PCDDs, PCDFs, and PCBs, in  
31 congener ratios that approximated Great Lakes fish residues, was tested in the ELS mortality  
32 assay, the lethal potency observed for the mixture, compared to TCDD, deviated less than  
33 twofold from an additivity approach (Walker et al., 1996). Recently, the TCDD TEQ of an  
34 environmental complex mixture of PCDDs, PCDFs, and PCBs extracted from lake trout and  
35 applied to the ELS bioassay could also be predicted by an additivity approach (Tillitt and Wright,  
36 1997). These results suggest that additional halogenated aromatic compounds, including non-

1 dioxin-like PCBs, present in fish do not significantly detract from an additivity response for this  
2 AhR-mediated event.

3       There are also numerous studies that have examined the effects of environmental mixtures  
4 in marine mammals and avian species (Ross, 2000; Giesy and Kannan, 1998; Ross et al., 1996;  
5 Shipp et al., 1998a,b; Restum et al., 1998; Summer et al., 1996a,b). Ross and colleagues  
6 examined captive harbor seals fed herring from either the Atlantic Ocean (low levels of  
7 PCDDs/PCDFs/PCBs) or the Baltic Sea (high levels of PCDDs/PCDFs/PCBs). The seals fed  
8 herring from the Baltic Sea displayed immunotoxic responses including impaired natural killer  
9 cell activity and antibody responses to specific antigens. These effects were correlated with the  
10 TEQ concentrations in the herring. Using mink as a model, Aulerich, Bursian, and colleagues  
11 have also examined the TEF methodology. Minks were fed diets containing carp from Saginaw  
12 Bay to provide exposures of 0.25, 0.5, or 1 ppm PCB in the diet. In a series of reports, the  
13 authors demonstrated that the diet induced dioxin-like effects ranging from enzyme induction to  
14 reproductive and developmental effects, and that these effects were correlated with the dietary  
15 intake of TEQs (Giesy and Kannan, 1998). Similar studies in White Leghorn hens also  
16 demonstrated that the TEQ approach provided accurate estimates of the potency of the mixtures  
17 (Summer et al., 1996).

18       In summary, current experimental evidence suggests that for PCDDs, PCDFs, coplanar  
19 dioxin-like PCBs, and strictly AhR-mediated events, the concept of TEF additivity adequately  
20 estimates the dioxin-like toxicity of either synthetic mixtures or environmental extracts, despite  
21 the variations in relative contributions of each congener. Addition of the more prevalent mono-  
22 and di-ortho-substituted PCBs to a mixture, at least in the case of environmental extracts and  
23 wildlife, does not seem to significantly detract from this assumption of additivity. Interactions  
24 other than additivity (antagonism, synergism) have been observed with a variety of effects  
25 (teratogenicity, immunotoxicity, hepatic porphyrin accumulation, thyroid hormone metabolism)  
26 in both binary combinations and complex synthetic mixtures of dioxin and partial or non-Ah  
27 receptor agonists (commercial PCBs, PCB 153). However, it appears that at these high-dose  
28 exposures, multiple mechanisms of action not under the direct control of the Ah receptor are  
29 responsible for these nonadditive effects.

30       Additional research efforts should focus on complex mixtures common to both  
31 environmental and human samples and the interactions observed with biological and  
32 toxicological events known to be under Ah receptor control. In the interim, the additive  
33 approach with TEFs derived by scientific consensus of all available data appears to offer a good  
34 estimation of the dioxin-like toxicity potential of complex mixtures, keeping in mind that other  
35 effects may be elicited by non-dioxin-like components of the mixture.

36

1       **9.4.6. Toxic Equivalency Functions**

2           The TEF methodology has been described as an “interim” methodology. Since this  
3 interim method has been applied, there have been few proposed alternatives. One recent  
4 proposal suggests that the TEF value be replaced by a toxic equivalency function (Putzrath,  
5 1997). It has been proposed that the REPs for PCDDs/PCDFs are better described by a function  
6 as compared to a factor or single-point estimate (Putzrath, 1996). The use of a factor to describe  
7 the relative potency of a chemical implies that its relative potency is independent of dose.  
8 Putzrath (1997) suggests that data exists which indicates that the REPs are dose dependent and  
9 that the REPs must be described as a function of dose. Recent studies have examined this  
10 possibility for a series of PCDDs/PCDFs and PCBs (DeVito et al., 1997; DeVito et al., 2000).  
11 For the PCDDs/PCDFs, the data indicate that the REPs estimated from enzyme induction data in  
12 mice are best described by a factor and not a function. For some of the PCBs examined, a  
13 function fit better, but the change in the REP was within a factor of two to five for most of the  
14 four enzymatic responses examined (DeVito et al., 2000). In addition, the dose dependency was  
15 observed only at the high-dose and not in the low-dose region (DeVito et al., 2000).

16           Even though these studies suggest that a TE function may be useful, there are numerous  
17 difficulties in applying this method. If the REPs are really functions and not factors, there must  
18 be a mechanistic basis for these differences, and these mechanisms would most likely be  
19 response specific and perhaps species specific. This would then require that for all critical  
20 responses, every chemical considered in the TEF methodology would have to be examined.  
21 Once again, it is highly unlikely that 2-year bioassays and multigenerational studies will be  
22 performed on all the TEF congeners in the foreseeable future. The use of a TEF function  
23 requires extensive data sets that are not available and are unlikely to be collected.

24  
25       **9.4.7. Species and Endpoint Specific TEFs**

26           It is often suggested that species and endpoint TEFs may be required for the TEF concept  
27 to provide accurate estimates of risk. In fact, the WHO does have class specific TEFs based on  
28 fish, birds and mammals (van den Berg et al., 1998). The most notable differences are the lack  
29 of effect of some mono-ortho PCBs in fish (van den Berg et al., 1998). Hahn and colleagues  
30 recently examined the influence of affinity and intrinsic activity on the relative potency of PCBs  
31 in PLHC-1 cells (Hestermann et al., 2000). Using this cell line derived from fish, Hahn and  
32 colleagues demonstrated clear differences in the response of these cells to mono-ortho PCBs.  
33 The insensitivity of these fish cells to the mono-ortho PCBs is due to both reduced affinity and  
34 reduced intrinsic efficacy. Using information on affinity and intrinsic efficacy allowed for better  
35 predictions of mixtures of these chemicals than did the application of the TEF methodology  
36 (Hestermann et al., 2000). Future studies examining species differences applying the approach of

1 Herstermann et al., (2000) may provide insight into species specific TEFs as well as alternative  
2 approaches to the TEF methodology.

3 There are numerous examples of endpoint specific relative potencies for receptor mediated  
4 pharmacological agents, such as the antiestrogen, tamoxifen. It is reasonable to assume that the  
5 Ah receptor and its ligands would be no different from these other receptor systems.  
6 Examination of the WHO data base suggests that even for the chemicals with the largest data sets  
7 this question cannot be adequately addressed (See section 9.2.5). Endpoint specific TEFs would  
8 require a much more complete data set than is available at this time. In addition, these studies  
9 would have to be designed to test the hypothesis that the REPs are equivalent across endpoints.  
10 This requires controlling the species and dosing regimen employed as well as other factors. One  
11 of the reasons the TEF methodology was developed was because limited toxicity data was  
12 available for the other dioxin-like chemicals and it was unlikely that all relevant chemicals would  
13 be tested for all responses in all species, including humans. For example, it is extremely unlikely  
14 that 2-year bioassays for carcinogenesis or multi-generational studies will be performed on all  
15 chemicals included in the TEF methodology. Even though there are significant data  
16 demonstrating that a number of chemicals produce dioxin-like toxic effects, clearly the data set is  
17 not complete. For this reason, WHO recommends revisiting the TEF values every 5 years.

#### 19 **9.5. APPLICATION OF UNCERTAINTY ANALYSIS TO THE TEF METHODOLOGY**

20 TEFs are presented as point estimates, in spite of the fact that variability in the REP values  
21 estimated from the supporting experimental data can range several orders of magnitude for a  
22 particular congener. It has been proposed that some of this variability in the REP values can be  
23 attributed to differences in exposure regimens, test species, or purity of the test compound. In  
24 addition, others have argued that the variability of the REPs may be due to differences in the REP  
25 across endpoints. The reasons for much of this variability have not been adequately examined  
26 experimentally and remain unknown. For example, in the WHO database, PCB 126 has the  
27 largest data set of REP values. However, while there are numerous studies estimating the REPs  
28 for this chemical, these individual studies were not designed to address the variability in the REP  
29 values. Close examination of these studies indicates that it is difficult to attribute the variability  
30 of the REP to either species, endpoint, dosing regimen or laboratory differences. For example,  
31 there are four studies that examined the REP of 126 for immune effects in mice in the WHO data  
32 base (Harper et al., 1994; 1995; Mayura et al., 1993; Steinburg et al., 1993). The range of the  
33 REPs from these studies is 0.05 - 0.99 with a mean of  $0.23 \pm 0.22$ . It is not clear why the range  
34 is so large. In fact, three of the studies and the two extreme REPs (0.05 and 0.99) come from the  
35 same laboratory (Harper et al., 1994; 1995; Mayura et al., 1993). Similarly, there are four studies  
36 examining the REP of PCB 126 for hepatic EROD induction in mice following an acute

1 exposure and the REPs are 0.0005, 0.012, 0.38 and 0.55. Once again, there is no clear reason for  
2 the three order of magnitude range in the REPs for this endpoint. Because the experiments used  
3 to estimate the REPs were not designed to address the variability, further studies will be required  
4 to determine what is causing the variability.

5 One of the difficulties in quantitatively describing the uncertainties in the TEF  
6 methodology is due to the method by which the TEF values are assigned. First and foremost is  
7 the fact that TEFs are assigned and not derived. While there is a clear description of the  
8 qualitative weighting scheme used in assigning the TEFs, quantitatively describing how the  
9 actual committee actually assessed this weighting scheme is impossible. Consequently, the TEF  
10 approach, as currently practiced, does not provide for a quantitative description of the uncertainty  
11 for individual TEF values.

12 There has been several proposals for incorporating quantitative uncertainty descriptors into  
13 TEFs. Suggestions have been made to use meta-analytic approaches or Monte Carlo techniques,  
14 however (Finley et al., 1999), these approaches are only as good as the data available. For some  
15 chemicals, such as PCB 126, PeCDD and 4-PeCDF, there are sufficient data to apply these  
16 methods. In contrast, chemicals such as OCDD and OCDF have only a few studies and  
17 application of these statistical methods would be inappropriate. Another shortcoming to the  
18 application of meta-analytic approaches or Monte Carlo techniques is that they would also have  
19 to incorporate the weighting scheme described by the WHO workgroup (van den Berg et al.,  
20 1998). The weighting scheme gives qualitatively greater weight to studies that examine toxic  
21 endpoints following repeated exposures. Because our concern is generally for potential toxic  
22 effects following repeated exposures, this weighting scheme is appropriate. Incorporating a  
23 quantitative description of the weighting scheme into a meta-analytic approaches or a Monte  
24 Carlo approach to describe the uncertainty is not a trivial task (Finley et al., 2000). Future efforts  
25 by WHO or USEPA which develop guidelines and approaches to incorporating these weighting  
26 schemes into quantitative uncertainty analysis are an important step in understanding the  
27 uncertainties of the TEF methodology.

28 Qualitative statements of confidence are embodied in the discussions associated with the  
29 establishment and revision of TEFs. These qualitative judgments, when examined in the context  
30 of a specific risk assessment, can provide valuable insight into the overall uncertainty of some  
31 TEQ estimates. For example, using WHO TEFs (van den Berg et al., 1998) to look at  
32 background exposure from a typical U.S. diet, it is clear that only a limited number of congeners  
33 significantly contributed to the total TEQ. Approximately 80% of the TEQ-WHO<sub>98</sub> associated  
34 with background dietary exposure (1 pg/kg/d) comes from only five congeners: 2,3,7,8-TCDD,  
35 1,2,3,7,8-PCDD, 2,3,4,7,8-PeCDF, and PCB 126 (see Part I, Volume 3). The variability of the  
36 REP values found in the literature for these congeners is much lower than for congeners that are

1 minor contributors to background TEQ. Furthermore, the assigned TEF values for the chemicals  
2 contributing 80% to the TEQ intake are similar to the mean of their in vivo REP values. The  
3 confidence in the TEF methodology is also increased by empirical examination. A number of  
4 studies have examined complex mixtures of dioxin and non-dioxin-like compounds and the TEF  
5 methodology consistently results in TEQ estimates within a factor 2-3 for these mixtures. Based  
6 on these mixture studies it is unlikely that the estimated TEQ over or under estimates the “true”  
7 TEQ by more than a factor of five. Finally, the uncertainty in TEQ estimates is only one  
8 component of the overall uncertainty in a dioxin risk assessment. The TEQ uncertainty only  
9 addresses the confidences associated in ascribing 2,3,7,8-TCDD equivalents to a mixture. It does  
10 not address the uncertainty associated with quantitatively linking health effects to 2,3,7,8-TCDD  
11 exposure, or the uncertainties associated with exposure estimates themselves.

## 12 13 **9.6. IMPLICATIONS FOR RISK ASSESSMENT**

14 The TEF methodology provides a mechanism to estimate potential health or ecological  
15 effects of exposure to a complex mixture of dioxin-like compounds. However, the TEF method  
16 must be used with an understanding of its limitations. This methodology estimates the dioxin-  
17 like effects of a mixture by assuming dose-additivity and describes the mixture in terms of an  
18 equivalent mass of 2,3,7,8-TCDD. Although the mixture may have the toxicological potential of  
19 2,3,7,8-TCDD it should not be assumed for exposure purposes to have the same environmental  
20 fate as 2,3,7,8-TCDD. The environmental fate of the mixture is still the product of the  
21 environmental fate of each of its constituent congeners. Different congeners have different  
22 physical properties such as vapor pressure, practical vapor partition, water octanol coefficient,  
23 photolysis rate, binding affinity to organic mater, water solubility, etc. Consequently, both the  
24 absolute concentration of a mixture in an environmental medium and the relative concentration  
25 of congeners making up an emission will change as the release moves through the environment.  
26 For some situations, treating emission as equivalent to exposure, which assumes that modeling  
27 fate and exposure can be reasonably accomplished by treating a mixture as if it were all  
28 2,3,7,8,-TCDD, is a useful but uncertain assumption. However, for many risk assessments the  
29 differences in fate and transport of different congeners must be taken into consideration and TEQ  
30 must be calculated at the point of exposure if more accurate assessments are to be achieved.  
31 Similarly, many dioxin releases are associated with the release of non-dioxin-like compounds  
32 such as pesticides, metals, and non-dioxin-like PHAHs, and their risk potential may also need to  
33 be assessed in addition to dioxin-related risk.

34 There are instances where exposures to PCBs are the major problem. The TEF  
35 methodology provides risk assessors with a useful tool to estimate potential dioxin-related health  
36 risks associated with these exposures. Typically, the congener makeup of environmental

1 exposures to PCBs does not resemble the congener profile of any of the commercial mixtures  
2 produced. Because the environmental mixtures do not resemble the commercial mixtures, it is  
3 not clear that using total PCB concentrations and comparing them to any of the commercial  
4 mixtures provides an accurate assessment of the potential risks. However, the use of the TEF  
5 methodology allows for the estimation of the risk associated with the dioxin-like effects of the  
6 mixture and may provide a more accurate assessment of the risk in conjunction with the use of  
7 total PCBs. The Agency has recently published an application of this approach to the evaluation  
8 of PCB carcinogenicity (U.S. EPA, 1996; Cogliano, 1998)

### 9 10 **9.7. SUMMARY**

11 The AhR mediates the biochemical and toxicological actions of dioxin-like compounds  
12 and provides the scientific basis for the TEF/TEQ methodology. In its 20-year history, this  
13 approach has evolved, and decision criteria supporting the scientific judgment and expert opinion  
14 used in assigning TEFs have become more transparent. Numerous countries and several  
15 international organizations have evaluated and adopted this approach to evaluating complex  
16 mixtures of dioxin and related compounds. It has become the accepted, interim methodology,  
17 although the need for research to explore alternative approaches is widely endorsed. Although  
18 this method has been described as a “conservative, order of magnitude estimate” of the TCDD  
19 dose, experimental studies examining both environmental mixtures and laboratory-defined  
20 mixtures indicate that the method provides a greater degree of accuracy when all effects are  
21 considered and may not be as conservative as sometimes described. Clearly, basing risk on  
22 TCDD alone or assuming all chemicals are as potent as TCDD is inappropriate on the basis of  
23 available data. Although uncertainties in the TEF methodology have been identified, one must  
24 examine the utility of this method in the broader context of the need to evaluate the public health  
25 impact of complex mixtures of persistent bioaccumulative chemicals. The TEF methodology  
26 decreases the overall uncertainties in the risk assessment process (U.S. EPA, 1999); however,  
27 this decrease cannot be quantified. One of the limitations of the TEF methodology in risk  
28 assessment is that the risk from non-dioxin-like compounds is not evaluated. This applies to  
29 both industrial/synthetic as well as natural ligands which are not considered to be dioxin-like, in  
30 addition to non-AhR ligands which may be interacting with dioxin-like chemicals in modulating  
31 their impacts on biological systems. Future research should focus on the development of methods  
32 that will allow risks to be predicted when multiple mechanisms are present from a variety of  
33 contaminants.

34 Since TEFs were first proposed in the 1980's, there have been several expert panels  
35 charged with evaluating and assigning TEF values to dioxin-like congeners. The development of  
36 the TEF methodology can be seen as an iterative process in which as more data was collected and

1 our knowledge base on the mode of action and biological effects of these chemicals accumulated,  
2 the later panels provided more accurate assessments of the chemicals included in the TEF  
3 methodology. For example, the initial TEF proposals assigned values to all tetra-, penta-, hexa-,  
4 hepta- and octa-chlorinated dioxin and dibenzofuran congeners. Later evaluations assigned TEF  
5 values only to the 2,3,7,8-chlorine substituted congeners. The most recent expert panel to re-  
6 evaluate and assign TEF values to dioxin-like congeners was the WHO panel convened in 1997  
7 (Van den berg, 1998). This group of experts assigned TEF values to dioxin-like PCBs and  
8 revised TEF values for several of the chlorinated dioxins and dibenzofurans. The WHO<sub>98</sub> TEF  
9 values are based on the most recent data available and it is recommended that these values  
10 supercede previous TEF values.

11 Thus, in summary, the WHO<sub>98</sub> TEF values, which include dioxins, furans and dioxin-like  
12 PCBs, are the recommended TEF values. These are the TEF values recommended for use in  
13 human health risk analysis.

**Table 9-1. Estimated relative toxicity of PCDD and PCDF isomers to 2,3,7,8-T<sub>4</sub>CDD<sup>a</sup>**

Isomer groups	Toxicity factor relative to 2,3,7,8-T <sub>4</sub> CDD
DD	nontoxic
M <sub>1</sub> CDD	0.0001
D <sub>2</sub> CDD	0.001
T <sub>3</sub> CDD	0.01
T <sub>4</sub> CDD <sup>b</sup>	0.01
P <sub>5</sub> CDD	0.1
H <sub>6</sub> CDD	0.1
H <sub>7</sub> CDD	0.01
O <sub>8</sub> CDD	0.0001
DF	nontoxic
M <sub>1</sub> CDF	0.0001
D <sub>2</sub> CDF	0.0001
T <sub>3</sub> CDF	0.01
T <sub>4</sub> CDF	0.5
P <sub>5</sub> CDF	0.5
H <sub>6</sub> CDF	0.1
H <sub>7</sub> CDF	0.01
O <sub>8</sub> CDF	0.0001

<sup>a</sup> OME, 1984.

<sup>b</sup> Excluding 2,3,7,8-T<sub>4</sub>CDD.

**Table 9-2. Toxic equivalency factors (TEFs)**

Congener	EPA/87 <sup>a</sup>	NATO/89 <sup>b</sup>	WHO/94 <sup>c</sup>	WHO/98 <sup>d</sup>
<b>PCDDs</b>				
2,3,7,8-TCDD	1	1		1
1,2,3,7,8-PeCDD	0.5	0.5		1
1,2,3,4,7,8-HxCDD	0.04	0.1		0.1
1,2,3,7,8,9-HxCDD	0.04	0.1		0.1
1,2,3,6,7,8-HxCDD	0.04	0.1		0.1
1,2,3,4,6,7,8-HpCDD	0.001	0.1		0.01
1,2,3,4,6,7,8,9-OCDD	0	0.001		0.0001
<b>PCDFs</b>				
2,3,7,8-TCDF	0.1	0.1		0.1
1,2,3,7,8-PeCDF	0.1	0.05		0.05
2,3,4,7,8-PeCDF	0.1	0.5		0.5
1,2,3,4,7,8-HxCDF	0.01	0.1		0.1
1,2,3,7,8,9-HxCDF	0.01	0.1		0.1
1,2,3,6,7,8-HxCDF	0.01	0.1		0.1
2,3,4,6,7,8-HxCDF	0.01	0.1		0.1
1,2,3,4,6,7,8-HpCDF	0.001	0.01		0.01
1,2,3,4,7,8,9-HpCDF	0.001	0.01		0.01
1,2,3,4,6,7,8,9-OCDF	0	0.001		0.0001
<b>PCBs</b>				
<b>IUPAC #</b>	<b>Structure</b>			
77	3,3',4,4'-TCB			0.0005
81	3,4,4',5-TCB			-
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			0.0001
180	2,2',3,4,4',5,5'-HpCB			0.00001
189	2,3,3',4,4',5,5'-HpCB			0.0001

<sup>a</sup> U.S. EPA, 1987.

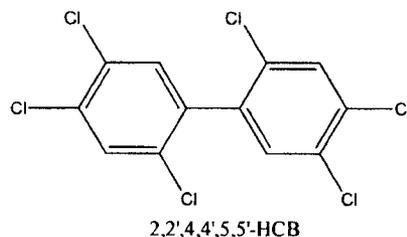
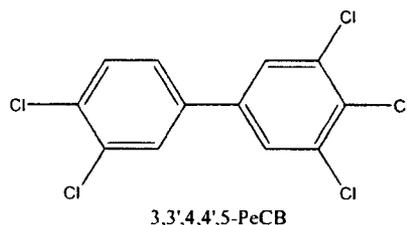
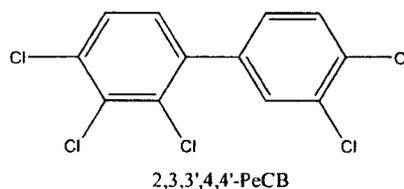
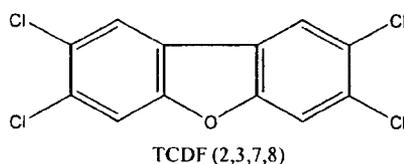
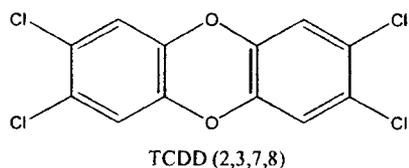
<sup>b</sup> NATO/CCMS, 1989.

<sup>c</sup> Ahlborg et al., 1994.

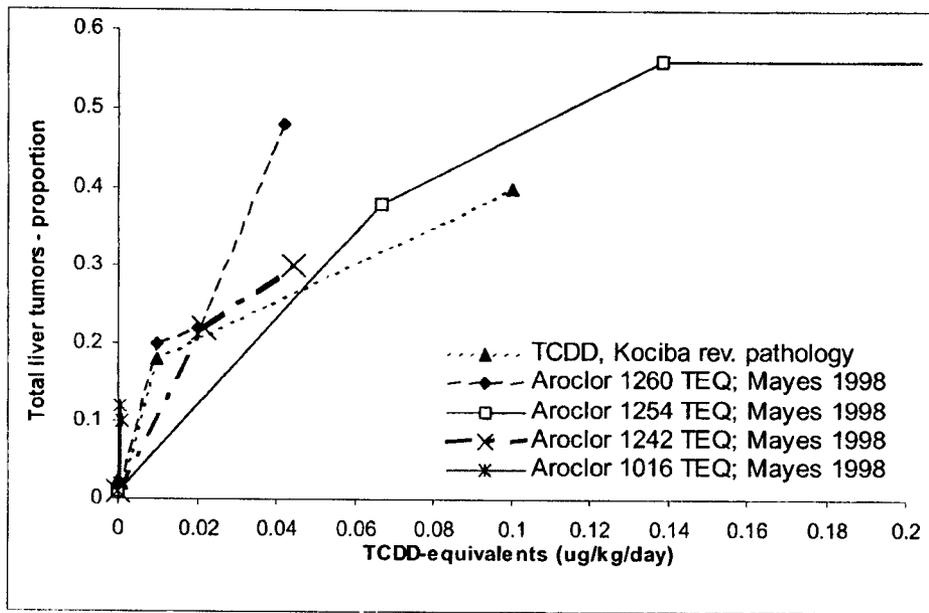
<sup>d</sup> Van den Berg, 1998.

**Table 9-3. The range of the in vivo REP values for the major TEQ contributors**

<b>Chemical</b>	<b>Number of in vivo endpoints</b>	<b>Range of REPs (mean±std)</b>	<b>Number of end points from subchronic studies</b>	<b>Range of REPs (mean±std)</b>	<b>TEF</b>
1,2,3,7,8-PCDD	22	0.16-0.9 (0.5±0.22)	16	0.19-0.9 (0.53±0.24)	1
2,3,4,7,8-PCDF	40	0.018-4.0 (0.4±0.7)	20	0.018-0.6 (0.20±0.13)	0.5
1,2,3,6,7,8-HxCDD	3	0.015-0.16	1	0.04	0.1
PCB 126	62	0.0024-0.98 (0.20±0.20)	31	0.004-0.18 (0.13±0.13)	0.1



**Figure 9-1. Structures of polychlorinated dibenzo-*p*-dioxins, dibenzofurans and biphenyls.** The prototype chemical 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD[2,3,7,8]), and example of a dioxin-like dibenzofuran 2,3,7,8-tetrachlorodibenzofuran (TCDF[2,3,7,8]), a mono-ortho dioxin-like PCB, 2,3,3',4,4'-pentachlorobiphenyl (2,3,3',4,4'-PeCB), a dioxin-like coplanar PCB, 3,3',4,4',5-pentachlorobiphenyl (3,3',4,4',5-PeCB) and an example of a non-dioxin-like di-ortho substituted PCB, 2,2',4,4',5,5'-hexachlorobiphenyl (2,2',4,4',5,5'-HCB).



**Figure 9-2: TEQ-based bioassay results.** (Kociba et al.,1978 and Mayes et al.,1998)  
 Presentation of the comparison of the dose-response relationship for hepatic tumors for TCDD (Kociba et al., 1978) with Aroclor 1016, 1242, 1254, and 1260 (Mayes et al., 1998) when dose is expressed as TCDD equivalents using the TEF methodology (Ahlborg et al., 1994).

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