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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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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,

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

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

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

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

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

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

9.2.2. TEFs for PCBs

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

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

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

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

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

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

9.2.3. The Most Recent Evaluation of TEFs for PCDDs, PCDFs, and PCBs

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

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

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

- Assigned as reported in the publication/manuscript (verified from available data).
- Calculated from the dose-response curves using linear interpolation of log doses comparing the same effect levels with correction for different control levels.
- Calculated from ratios of low or no observed effect levels (LOELs, NOELs) and effect concentration/dose 10%, 25% or 50% values ($ED/EC_{10,25,50}$).
- Calculated from ratios of tumor promotion indexes or maximal enzyme induction levels.
- Calculated from ratios of Ah receptor binding affinities (K_d).

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

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

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

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

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

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

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

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

This first section has outlined the process of assessing the relative potency of chemicals and the assignment of a consensus TEF value. There are still many questions on the use of the TEF method and the validity of some of the underlying assumptions. A detailed discussion and review of the data supporting the development and use of the TEF method, as well as the data 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

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

9.2.5. Variability in the REPs Across Endpoint, Species, Dosing Regimen and Laboratories.

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

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

9.2.6. Critical Considerations in the Application of the TEF Methodology.

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

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

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

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

9.3. SPECIFIC ISSUES

9.3.1. Ah Receptor and Toxicity Factors

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

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

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

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

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

9.3.3. Species Comparison of the AhR

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

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

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

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

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

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

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

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

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

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

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

9.3.4. Mode of Action and Implications for the TEF Methodology

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

9.3.5. Ah Receptor Ligands

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

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

9.3.5.1. *Industrial/Synthetic AhR Ligands*

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

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

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

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

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

9.3.5.2. *Naturally Occurring AhR Ligands*

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

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

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

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

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

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

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

9.3.5.3. *Industrial vs. Natural AhR Ligands*

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

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

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

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

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

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

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

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

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

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

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

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

9.4. TOTAL TEQ AND THE ADDITIVITY CONCEPT

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

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

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

9.4.1. Examination of Laboratory Mixtures of PCDDs and PCDFs

Bock and colleagues evaluated the TEF methodology in several systems using both individual congeners as well as laboratory-derived mixtures (Lipp et al., 1992; Schrenk et al., 1991, 1994). REPs or toxic equivalents or "TEs" (as designated by the authors) were determined for 2,3,7,8-substituted PCDDs based on enzyme induction in human HepG2 cells, rat H4IIE cells, and primary rat hepatocytes. Three laboratory-defined mixtures (M1, M2, and M3) were prepared and then examined in these same cell culture systems. TCDD contributed between 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 contributes approximately 60% of the TEQ, and 1,2,3,7,8-PCDD and 1,2,3,4,7,8-HxCDD contribute 10% each. In M2, 1,2,3,4,6,7,8-HpCDD contributes 45%, 1,2,3,7,8-PCDD and 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-HxCDD contribute less than 10% to the total TEQ. The TEQs in M3 are derived predominately 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%). These mixtures also contain up to 49 chlorinated dibenzo-*p*-dioxins. The TCDD equivalents of the mixtures were determined on the basis of the assumption of additivity using the TEF methodology and the laboratory derived REPs or TEs as well as experimentally by comparing the EC_{50}s of the mixtures with that of TCDD. According to the authors, in all three systems the data demonstrated that the components of the mixture act in an additive manner (Lipp, 1991; Schrenk et al., 1991). For example, in the human HepG2 cells the EC_{50} of a mixture of 49 different PCDDs was determined experimentally at 0.034 pg TEQ/plate, compared to the calculated or predicted EC_{50} of 0.028 pg TEQ/plate. Interestingly, the TEF methodology accurately predicted

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

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

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

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

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

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

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

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

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

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

9.4.2. Examination of Commercial or Laboratory-Derived Mixtures of PCDDs, PCDFs, and PCBs

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

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

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

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

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

In in vitro systems, using H4IIe cells and rat hepatocytes, Schmitz et al. (1995, 1996) examined the assumption of additivity for individual congeners as well as commercial mixtures. After deriving REP values for enzyme induction, the authors concluded that a laboratory mixture of PCBs 77, 105, 118, 126, 156, and 169 demonstrated perfect additive behavior in these cell line systems (Schmitz et al., 1995). However, when the mixture was combined with a tenfold surplus of a mixture containing non-dioxin-like PCBs (PCB 28, 52, 101, 138, 153 and 180), the mixture demonstrated an approximate threefold higher TEQ than predicted. The authors concluded that a moderate synergistic interaction is responsible for the increased enzyme-inducing potency of the mixture containing dioxins and non-dioxin-like PCBs. Further studies by Schmitz et al. (1996) also demonstrated a slight synergistic deviation (less than threefold) from strict additivity when the calculated TEQ based on chemical analysis of Aroclor 1254 and Clophen A50 was compared to the CYP1A-induction TEQ derived in an established rat hepatoma cell line (H4IIE) (Schmitz et al., 1996).

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

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

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

9.4.3. Examination of Environmental Samples Containing PCDDs, PCDFs, and/or PCBs

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

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

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

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

9.4.4. Nonadditive Interactions With Non-Dioxin-Like Compounds

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

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

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

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

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

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

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

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

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

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

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

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

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

9.4.5. Examination of the TEF Methodology in Wildlife

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

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

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

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

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

9.4.6. Toxic Equivalency Functions

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

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

9.4.7. Species and Endpoint Specific TEFs

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

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

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

9.5. APPLICATION OF UNCERTAINTY ANALYSIS TO THE TEF METHODOLOGY

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

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

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

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

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

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

9.6. IMPLICATIONS FOR RISK ASSESSMENT

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

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

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

9.7. SUMMARY

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

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

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

Thus, in summary, the WHO_{98} TEF values, which include dioxins, furans and dioxin-like PCBs, are the recommended TEF values. These are the TEF values recommended for use in human health risk analysis.

Table 9-1. Estimated relative toxicity of PCDD and PCDF isomers to 2,3,7,8-T_4CDD[a]

Isomer groups	Toxicity factor relative to 2,3,7,8-T_4 CDD
DD	nontoxic
M_1CDD	0.0001
D_2CDD	0.001
T_3CDD	0.01
T_4CDD[b]	0.01
P_5CDD	0.1
H_6CDD	0.1
H_7CDD	0.01
O_8CDD	0.0001
DF	nontoxic
M_1CDF	0.0001
D_2CDF	0.0001
T_3CDF	0.01
T_4CDF	0.5
P_5CDF	0.5
H_6CDF	0.1
H_7CDF	0.01
O_8CDF	0.0001

[a] OME, 1984.
[b] Excluding 2,3,7,8-T_4CDD.

Table 9-2. Toxic equivalency factors (TEFs)

Congener		EPA/87 [a]	NATO/89 [b]	WHO/94 [c]	WHO/98[d]
PCDDs					
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
PCDFs					
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
PCBs					
IUPAC #	**Structure**				
77	3,3',4,4'-TCB			0.0005	0.0001
81	3,4,4',5-TCB			-	0.0001
105	2,3,3',4,4'-PeCB			0.0001	0.0001
114	2,3,4,4',5-PeCB			0.0005	0.0005
118	2,3',4,4',5-PeCB			0.0001	0.0001
123	2',3,4,4',5-PeCB			0.0001	0.0001
126	3,3',4,4',5-PeCB			0.1	0.1
156	2,3,3',4,4',5-HxCB			0.0005	0.0005
157	2,3,3',4,4',5'-HxCB			0.0005	0.0005
167	2,3',4,4',5,5'-HxCB			0.00001	0.00001
169	3,3',4,4',5,5'-HxCB			0.01	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	0.0001

[a] U.S. EPA, 1987.
[b] NATO/CCMS, 1989.
[c] Alhlborg et al., 1994.
[d] Van den Berg, 1998.

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

Chemical	Number of in vivo endpoints	Range of REPs (mean±std)	Number of end points from subchronic studies	Range of REPs (mean±std)	TEF
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

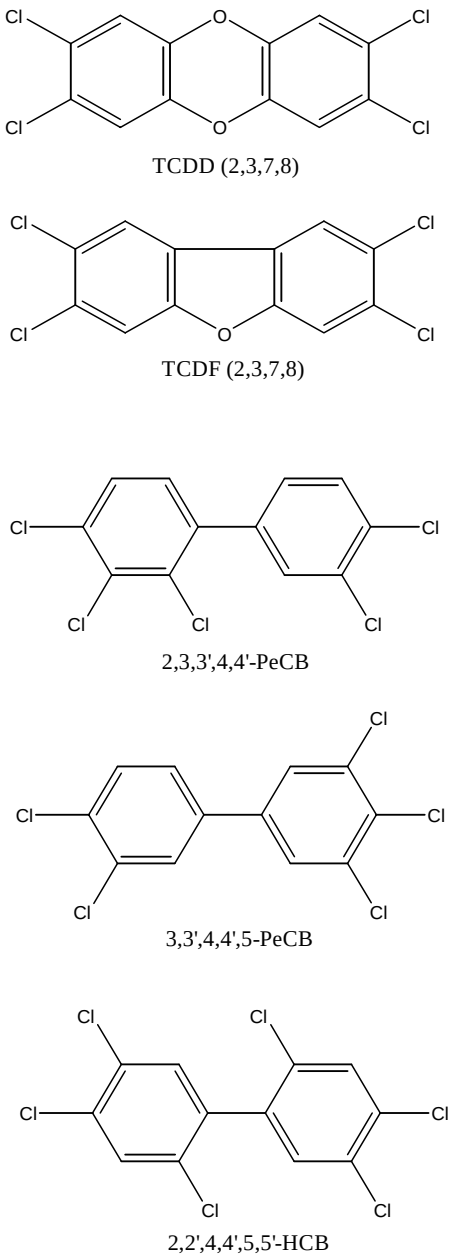

Figure 9-1. Structures of polychlorinated dibenzo-*p*-dioxins, dibenzofurans and biphenyls. The prototype chemical 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD[2,3,7,8]), and example of a dioxin-like dibenzofuran 2,3,7,8-tetrachlorodibenzfuran (TCDF[2,3,7,8]), a mono-ortho dioxin-like PCB, 2,3,3',4,4'-pentachlorobiphenyl (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).

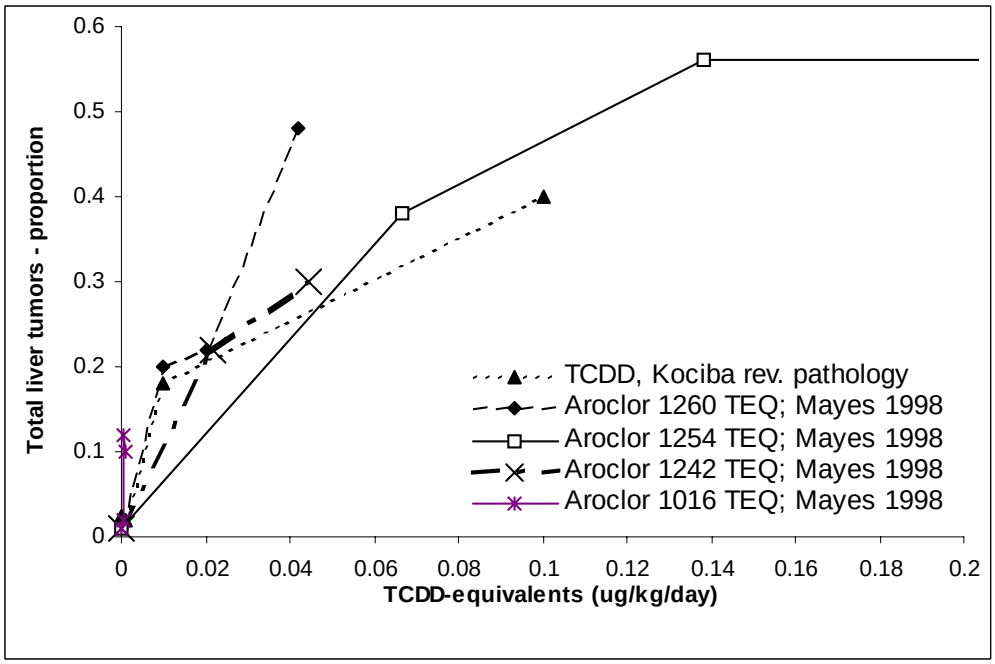

Figure 9-2: TEQ-based bioassay results. (Kociba et al.,1978 and Mayes et al.,1998) Presentation of the comparison of the dose-response relationship for hepatic tumors for TCDD (Kociba et al., 1978) with Aroclor 1016, 1242, 1254, and 1260 (Mayes et al., 1998) when dose is expressed as TCDD equivalents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

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