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Dioxin-Like and Non-Dioxin-Like Toxic Effects of Polychlorinated Biphenyls (PCBs): Implications For Risk Assessment

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CONTENTS

I. INTRODUCTION 512

II. RISK ASSESSMENT BASED ON "TECHNICAL PCB MIXTURE" OR TOTAL PCBs 517
- A. Hazard Quotients 517
- B. Limitations of Total PCB Approach 518

III. TOXIC EQUIVALENCY FACTOR (TEF) APPROACH IN RISK ASSESSMENT 521
- A. Development of TEFs 521
  - 1. Mammalian TEFs 521
  - 2. Teleost TEFs 522
  - 3. Avian TEFs 524
- B. Applications of the TEF Approach 524
- C. Limitations of TEF Approach 525
  - 1. Interactive Effects 525
  - 2. Species- and Endpoint-Specific Variation 527
  - 3. Non-Ah-Receptor-Mediated Effects 527
  - 4. Toxicokinetics 528
  - 5. Dose-Response Relationships 528
  - 6. Extrapolation of Greater to Lesser Dose Ranges and Routes of Exposure 528

IV. RISK ASSESSMENT BASED ON *IN VITRO* BIOASSAYS 529
- A. Applications of Bioassays 529
- B. Limitations of *In Vitro* Bioassays 531

V. BIOLOGICAL EFFECTS OF NON-DIOXIN-LIKE PCBS 529 AND THEIR RISK ASSESSMENT 531
- A. Sources of Uncertainties in Deriving RfDs for Non-Dioxin-Like Effects 539

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VI. COMPARATIVE EVALUATION OF RISKS OF DIOXIN-LIKE AND NON-DIOXIN-LIKE PCBs: A CASE STUDY—MINK 544
A. Mink Reference Doses for Total PCBs, TEQs, and *ortho*-PCBs 544
B. Hazard Evaluation 545
VII. CONCLUSIONS 550

Abstract: Polychlorinated biphenyls (PCBs) are persistent, bioaccumulative, and toxic contaminants in the environment. Individual PCB congeners exhibit different physicochemical properties and biological activities that result in different environmental distributions and toxicity profiles. The variable composition of PCB residues in environmental matrices and their different mechanisms of toxicity complicate the development of scientifically based regulations for the risk assessment. In this article various approaches for the assessment of risks of PCBs have been critically examined. Recent developments in the toxic equivalency factor (TEF) approach for the assessment of toxic effects due to dioxin-like PCBs have been examined. PCB exposure studies that describe non-dioxin-like toxic effects, particularly neurobehavioral effects and their effective doses in animals were compiled. A comparative assessment of effective doses for dioxin-like and non-dioxin-like effects by PCBs has been made to evaluate the relative significance of non-*ortho*- and *ortho*-substituted PCBs in risk assessment. Using mink as an example, relative merits and implications of using TEF and total PCB approaches for assessing the potential for toxic effects in wildlife was examined. There are several advantages and limitations associated with each method used for PCB risk assessment. Toxic effects due to coplanar PCBs occur at relatively smaller concentrations than those due to non-dioxin-like PCBs and therefore the TEF approach derives the risk assessment of PCBs, in the environment. The need for the refinement of TEF approach for more accurate assessment of risks is discussed.

KEY WORDS: polychlorinated biphenyls, PCB congeners, risk assessment, neurotoxicity.

I. INTRODUCTION

Polychlorinated biphenyls (PCBs) are members of the group of halogenated aromatic hydrocarbons (HAHs) and consist of 209 isomers and congeners with different numbers and positions of chlorine atoms substituted on the biphenyl moiety. PCBs were produced commercially by the chlorination of biphenyl, which results in technical mixtures containing a given chlorine content, depending on the duration of the chlorination process.[1-3] Although all 209 of the PCB congeners can be synthesized in the laboratory,[4] the reaction conditions in commercial processes tend to favor specific substitution reactions leading to particular compositions in the technical mixtures, which are identified according to their chlorine content, expressed as percentage by weight. For example, Aroclors 1221, 1242, 1248, 1254, 1260, and 1268 are commercial PCB preparations that were formerly produced by the Monsanto Chemical Company in the U.S. (St. Louis, MO) that contain 21, 42, 48, 54, 60, and 68% chlorine by weight, respectively, as indicated by the last two digits in the numerical designation. Aroclor 1016, introduced in 1970 as an Aroclor 1242 substitute, contained 42% chlorine by weight. Use of the Aroclor tradename was not restricted to PCBs but designated to other polyhalogenated aromatic mixtures as well. For instance, Aroclor 5460 is a polychlorinated terphenyl mixture. In this report, however, Arolcor is used to denote PCB mixtures only. Technical PCB mixtures have also been produced by overseas manufacturers and these include Clophens (Bayer, Germany), Phenoclors and Pyralenes (Prodelec, France), Fenclors (Caffaro, Italy), Fenochlors (Cross, S.A., Spain), Kanechlors (Kanegafuchi, Japan), Sovol (Sovol, former USSR), Delor (Chemko, Czechoslovakia), and Chlorofen (Ząbkowice Śląskie, Poland). The production of PCBs in OECD (Organization for Economic Cooperation and Development) member countries is estimated to have been about 1.2 million metric tons, which is believed to contribute to the total world production substantially[5,6] (Figure 1). In addition, the former USSR produced 100,000 metric tons of Sovol, a technical PCB mixture resembling Arolcors 1242 and 1254, beginning in the 1940s to the early 1990s.[7] Production of PCBs in less industrialized countries in Asia, Africa, and South America is not known.

Although 209 congeners of PCBs are theoretically possible, only about 130 individual congeners have been identified in commercial PCB mixtures at concentrations ≥0.05%. PCBs with the same number of chlorines with different substitution patterns are called isomers, whereas PCBs with different numbers of chlorines are termed congeners. Groups of congeners with the same number of chlorines are referred to as homologs. Identification numbers have been assigned to individual chlorobiphenyls from 1 to 209 and termed CB (chlorobiphenyl) numbers.[8] These numbers were originally assigned by Ballschmiter and Zell[8] and subsequently adopted by the International Union of Pure and Applied Chemistry (IUPAC). However, it should be noted that 11 congeners (Nos. 33, 34, 76, 98, 122, 123, 124, 125, 177, 196, and 201) have different Ballschmiter and IUPAC numbers.[9] We have used IUPAC numbers in all discussions presented here.

PCBs were manufactured and used widely in industry as heat transfer fluids, hydraulic lubricants, dielectric fluids for transformers and capacitors, organic diluents, plasticizers, pesticide extenders, adhesives, dust-reducing agents, cutting oils, flame retardants, sealants, and in carbonless copy paper.[1] PCBs have been detected in environmental samples since 1966.[10] The chemical properties primarily responsible for many of the industrial applications of PCBs, such as inflammability, chemical and thermal stability, dielectric properties, and miscibility with organic compounds, are also the properties that have contributed to their ability to cause environmental problems. PCBs are persistent in the environment and are readily transported from localized or regional sites of contamination to remote areas,[11-13] which has led to their presence in almost every compartment in the environment.[6] Moreover, due to their lipophilicity, these compounds bioaccumulate and biomagnify in the food chain.

Individual PCB congeners exhibit different physicochemical properties that result in different profiles for environmental distribution and toxicity. PCBs have low water solubility, which decreases with increasing degree of chlorination. For example, the water solubilities of monochlorobiphenyl congeners are in the range of 1 to 5 g/l, but that of decachlorobiphenyl is only 0.015 mg/l.[14] Vapor pressure and degradability also decrease with increasing chlorine content.[15] Susceptibility to degradation and bioaccumulation depends on the structural arrangement of chlorine atoms among isomers. Due to the differences in the congener composition of PCBs among various commercial preparations and variations in physicochemical and biochemical properties (e.g., metabolism and biodegradation) of individual PCB congeners, their composition in environmental extracts are different among locations and from the original technical mixtures.[2,4,8,16-19] Several reviews have appeared on the production, properties, use, distribution, and fate of PCBs in the environment.[1,2,9,16,20-22]

While PCBs were first synthesized in Germany at the end of the nineteenth century, commercial production began in 1929, but large-scale production did not begin until 1945. Worldwide production increased an-

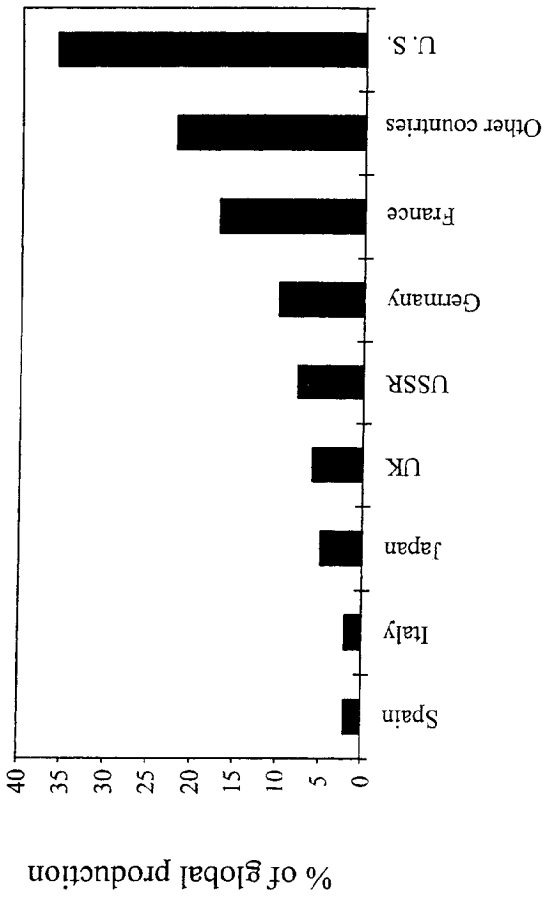

FIGURE 1. Production of PCBs in various countries (percent to global production of 1.2 million metric tons).

nually until the production was banned in Western Europe and North America in the 1970s, while the production in Eastern Europe and Russia continued until the early 1990s. Currently, there is no known production in the industrialized world. Due to their widespread distribution, persistence, bioaccumulation, and toxicity, potential impact of PCBs on humans and wildlife has been a concern for the last 3 decades.[23-27]

The differences in the composition of PCB residues in environmental matrices has implications for quantification and hazard evaluation, particularly when considering the differences in the biological activity, both qualitatively and quantitatively, among isomers as well as congeners. Several studies have demonstrated the differences in both mechanisms and toxic potentials of individual PCB congeners.[25,28-30] Thus, the impacts of PCBs on the environment and biota are due to the individual components of these mixtures and their additive and/or nonadditive (synergistic and antagonistic) interactions among themselves and other chemical classes of pollutants. Therefore, the development of scientifically based regulations for the risk assessment of PCBs requires analytical and toxicological data on the individual PCB congeners present in any technical mixture and information regarding interactive effects. Although developments in high-resolution isomer-specific PCB analysis have enabled identification and quantitation of individual PCB congeners present in commercial mixtures and environmental samples feasible, there are significant challenges associated with risk assessment of PCBs due to their different mechanisms of biological activity and toxicity. Most *in vivo* animal exposure and *in vitro* bioassay studies have exposed animals to commercially available technical PCB mixtures or individual congeners. Due to the differences in metabolism and/or biodegradation rates of individual congeners, the compositions of the original commercial technical mixtures are different from the compositions of the mixtures to which humans or wildlife are exposed. A further complication to the risk assessment is that many PCB congeners are metabolized to hydroxy and methyl sulfone metabolites. The available data on possible biological and toxicological effects of these metabolites are limited, and we have decided to preclude consideration of these metabolites in the present discussion. Because these metabolites would be expected to be in a dynamic equilibrium with their precursors, their effects may be included in assessments based on total PCBs. Only a few studies have investigated the effects of environmentally altered mixtures of PCBs. These studies have included field and controlled laboratory feeding experiments. However, in both cases, the co-occurrence of other toxicants such as DDT, Toxaphene, and dieldrin have complicated the interpretation of toxicity.

Health risks due to PCB exposure in humans or wildlife has been assessed based on either total PCB concentrations or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents (TEQs) using toxic equivalency factors (TEF). The U.S. Environmental Protection Agency (EPA) has adopted the TEF approach as an interim procedure for the calculation of risks of planar PCBs.[31,32] The concept of TEF was developed in the early 1980s for assessing the risks of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in waste incinerators.[33,34] The TEF model for PCBs presupposes a common mechanism of toxic action and additivity for the toxic effects of the individual congeners in the mixture and that PCBs act through the same mechanism of action as PCDDs/PCDFs. Further, the approach assumes that the dioxin-like effects of PCBs are the critical effects on animals. The critical effects are those that occur at the least concentration and would result in the least allowable total concentration of PCB mixtures.

The TEF approach has been validated for estimating the risks of non-*ortho*-substituted planar PCB congeners that exhibit

"dioxin-like" activities, based on their ability to interact with and activate the Ah receptor (AhR), sometimes referred to as the dioxin receptor because no endogenous ligand is currently known. Typical dioxin-like effects in rodents include chlor-acne (in hairless mice) and dermal lesions, body weight loss, thymic atrophy, immunosuppression, hepatotoxicity, reproductive and developmental toxicity, endocrine disruption (e.g., anti-estrogenicity), and induction of cytochrome P450 enzymes (e.g., CYP1A1).[25,35] The TEF approach does not consider potential adverse effects of *ortho*-substituted nonplanar PCB congeners that do not interact with the AhR, but elicit "non-dioxin-like" effects such as neurotoxicity, carcinogenicity and endocrine disruption.[30] Thus, the use of TEQs for assessing the potential toxicity of PCBs may not address all of the issues of potential adverse effects by PCBs. As an example, application of the TEF concept to examine the tumor promotion potential of Aroclor 1260 underpredicted the observed carcinogenic potential.[25,36] This is because *ortho*-substituted PCB congeners, such as 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB 153), which are major constituents of Aroclor 1260, are potent tumor promoters. Thus, theoretically if an estimate of the risk to humans or wildlife following exposure to complex mixtures of PCBs is based solely on the TEF approach, that risk may be biased or underestimated.[25,30,37] Further, studies have shown that exposure to *ortho*-substituted nonplanar PCB congeners can induce biochemical and morphological changes in *in vitro* cell cultures and in laboratory animals, which may result in neurological or behavioral dysfunctions in humans or wildlife and suggested the need to consider such effects of PCBs in risk assessment processes.[30,38,39,283]

Commercial PCB mixtures elicit a broad spectrum of toxic responses that are dependent on several factors, including chlorine content, purity, dose, species and strain, age and sex of animal, and route and duration of exposure. Immunotoxicity, carcinogenicity, and developmental toxicity as well as biochemical effects of commercial PCB mixtures have been investigated extensively in various laboratory animals, fish and wildlife species. The mechanisms of PCB toxicity and their dioxin-like effects and carcinogenicity have been reviewed previously.[29,35,40,41] However, information on the toxicological risk assessment of PCBs in wildlife is limited.[22] Particularly with the recent developments in the understanding of non-dioxin-like effects of PCBs, it is pertinent to examine critically the effective doses at which *ortho*-substituted PCBs could elicit non-dioxin-like effects in animals.

The thrust of this article is to describe, compare, and contrast several approaches to assessing the potential risks of PCBs to which wildlife or humans might be exposed. Specifically, the concept of a critical toxicant will be developed based on the determination of mechanisms of actions that are likely to cause biological effects at the lowest concentrations of PCBs. This was done by comparing reference doses (RfDs) for various toxic endpoints. A second level of assessment undertaken was to determine the effects of various environmental fate processes on outcomes of risk assessment based on total PCBs. Recent developments in the TEF approach for the assessment of dioxin-like effects of PCBs are also examined. Laboratory PCB-exposure studies for neurobehavioral effects and their effective doses in animals were compiled. A comparative assessment of effective doses for dioxin-like and non-dioxin-like PCBs has been made to evaluate their significance in the risk assessment process. Using mink as an example, the relative merits and implications of using the TEQ and total PCB approaches for assessing the potential for toxic effects in wildlife were examined.

II. RISK ASSESSMENT BASED ON "TECHNICAL PCB MIXTURE" OR TOTAL PCBs

Traditionally, ecological or human risk assessment of PCBs have involved comparison of exposure concentration in target species to a reference dose (RfD; Eq. 1). The RfD is an estimate of daily exposure, which during an entire lifetime is likely to be without an appreciable adverse effects. The RfD can be expressed as a mass of chemical per unit body mass per unit time (e.g., mg/kg bw/d). Alternatively, doses can be given as maximum acceptable toxicant concentrations (MATCs) or burdens in target tissue (mg) or as dietary exposures expressed as concentrations in the food (mg/kg in the diet).

$$\text{RfD} = \text{NOAEC (or LOAEC)/uncertainty or correction factor} \quad (1)$$

The RfD is estimated by dividing the no observable effect concentration (NOAEC) or the lowest observable effect concentration (LOAEC), which are usually derived from dietary exposure to animals with technical PCB mixtures such as Aroclors by correction (safety) factors. Correction factors, sometimes referred to as uncertainty factors, can be applied for (1) intraspecies variability, (2) interspecies variability, (3) situations where a LOAEC can be estimated but not NOAEC, and (4) extrapolations between different exposure durations (subchronic to chronic). RfDs are derived for various toxic responses or endpoints for the exposure period, which may be either the greatest NOAEC or the least LOAEC. In addition, uncertainty factors can be used to compensate for deficiencies in experimental designs and significance and sensitivity of endpoints.[42]

The dietary LOAEC and/or NOAEC values for commercial PCB preparations have been reported for various endpoints in a variety of animals.[2,21,25,43] The RfDs published by EPA, used to quantify cancer and noncancer risks to humans, are derived solely from animal studies.[44] Similarly, several national and international health agencies have proposed guidelines by setting tolerance and acceptable intake limits for PCBs in various foodstuffs.[45] Several reports have discussed the methods for assessing carcinogenic risks in humans based on total PCBs.[36,46–48]

The calculation of quotients has been conducted by utilizing the susceptibility of the most sensitive organism or group of organisms and comparing this to median, mean, or greatest exposure concentration. This may be made more conservative by the use of a safety factor, which is done to allow for unquantified uncertainty in the effect and exposure estimations. In the absence of an adequate range of toxicity tests, the risk assessment may be under- or overprotective, depending on the magnitude of safety factor used. However, where an acceptable range of toxicity data are available, the inherent variation in the response of organisms is better defined and the use of safety factors may be overly conservative. Thus, the quotient approach is acceptable for early tiers or preliminary risk assessments but fails to consider the range of variation that may exist in terms of real-world exposures and susceptibility, which need to be assessed probabilistically.[49]

A. Hazard Quotients

A toxic units approach was used to quantify the hazards due to PCB exposure in wild populations based on the NOAEC estimates from laboratory dietary exposure studies.[50–52] The Hazard Quotient (HQ) is defined as the ratio of the concentration in the tissue or diet divided by the RfD (Eq. 2). The units for the HQ are toxic units (TU).

$$\text{HQ} = [\text{concentration in tissue diet}]/\text{RfD} \quad (2)$$

An HQ of greater than one (1 TU) indicates that the concentration in the diet was expected to be sufficiently great to equal the threshold concentrations to elicit a statistically significant response. Population-level impacts at an HQ of 1 may not be observable, but, depending on the slope of the dose-response relationship, values of 10 to 20 TU are frequently required before population-level effects are observed.[51]

For estimation of HQs, laboratory studies reporting reference doses (RfD) and estimated tissue concentrations in the exposed animal or in their diet should be available. However, such informations are scarce. In addition, laboratory exposure studies may not reflect field exposures. Limitations of the total PCB approach are discussed below.

B. Limitations of Total PCB-Approach

Assessment of risks to humans and wildlife based on the RfDs derived for technical PCB mixtures under laboratory conditions is not explicit to predict effects in the real-world populations because the concentrations and composition of individual components change as a function of space and time. Thus, the mixture to which organisms are exposed at one time or at one location may be very different from that to which they are exposed at other times or locations. The pattern of relative proportions of PCBs in environmental mixtures is variable and does not resemble the composition of the original technical PCB mixtures that were released into the environment.[18,19,53-56] Furthermore, the relative concentrations of various PCB congeners differ according to trophic level and species. These differences are caused by several factors, including differential rates of environmental degradation, differences in physicochemical and biological properties, and changes in the composition of PCB residues in the food chain. Furthermore, toxicities of Aroclor preparation vary because of the differences in their composition. As an example, LC_{50} values for various Aroclors in the northern bobwhite quail (*Colinus virginianus*) exhibited about 10-fold differences in potencies (Figure 2).[57]

Due to changes in the relative proportions of individual congeners in PCB mixture, RfDs derived from laboratory studies for technical Aroclor mixtures may not be appropriate for the PCB mixture found in environmental samples. For instance, in certain aquatic animals, selective enrichment of AhR-active congeners resulted in their greater relative proportion in tissues than in technical mixtures.[55,56,58-64] In this case, estimation of hazard based on RfDs from laboratory exposure to technical PCB mixtures would underestimate the risk.

Uncertainty in estimates of risk is also contributed by the need to extrapolate from one species to another, including from wildlife to humans. The total PCB-mixture approach of risk assessment offers minimal insight into toxicokinetics, because animals exhibit interspecies differences in their abilities to metabolize specific PCB congeners. Toxicities of PCBs to different species may be modulated by species-specific differences in lipid metabolism, quantitative differences in binding of PCBs to receptors in target organs, enzyme induction, or other differences in toxicokinetics.

Another uncertainty associated with estimates of toxicity based on exposure to commercial PCB mixtures is related to the relative amounts of polychlorinated dibenzofurans (PCDFs) and polychlorinated naphthalenes (PCNs) identified as contaminants in technical PCB preparations or as covariates in complex environmental mixtures. Concentrations of total PCDFs and PCNs in Aroclor preparations were in the ranges of 0.6 to 7.5 and 2.6 to 170 μg/g, respectively.[9] Certain PCN congeners are bioaccumulative and exhibit toxic effects similar to those reported for PCBs.[65,66] In most studies, the PCDF and PCN contents were not quantified and their contribution to technical PCBs-induced toxicity is unknown.

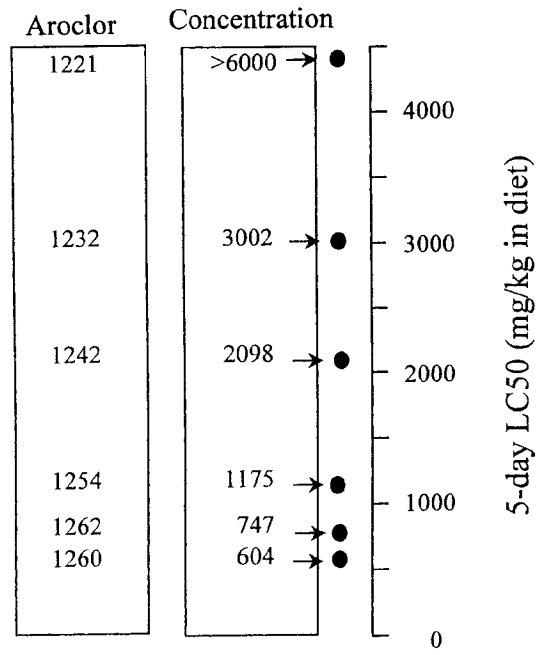

FIGURE 2. Toxic potencies (LC_{50}) of various Aroclors in bobwhite. (After Reference 57.)

Most animal exposure studies have not used appropriate statistical protocols or replications to reach dose-response relationships for the estimation of RfDs. Due to the lack of dose-response data from animal bioassays, it is presently not feasible to derive valid RfDs to perform scientifically based quantitative risk evaluations. As mentioned earlier, RfDs are based on different dose metrices. For example, the NOAEC applied for the risk assessment of TCDD, which was derived based on long-term exposures of rats was 1 ng/kg bw/d.[67] This value is about 200-fold greater than the background exposure in the U.S. population.[68] A recent approach uses a measure of body burden, as total TEQs/kg bw, for the risk assessment of TCDD-like compounds. Based on this approach, the general background population body burden was estimated to be about 13 ng TEQ/kg,[69] which was fivefold less than the estimated body burden of 60 ng TCDD/kg in rat exposure studies.[67] This indicates that validation of appropriate dose metrics is essential for the derivation of accurate RfDs. By using body burden as the dose metric, a strong relationship of some toxic and biochemical endpoints have been demonstrated in humans and laboratory animals.[69] Reference doses expressed as daily exposure doses may

poorly represent dose metrics because it does not account for bioavailability, metabolism, pharmacokinetics or pharmacodynamics, of the test compounds. A recent study suggested that the relationship between the external dose of TCDD and resulting TCDD concentrations in liver and adipose tissue of human and various species of rats and mice varied by as much as 725-fold, which illustrates that humans and experimental animals differ considerably in their ability to convert external doses of dioxins to tissue concentrations.[70]

The uptake or intestinal absorption efficiencies of PCB congeners vary depending on the species, health condition, amount, mode, and duration of exposure. The dietary uptake efficiencies of certain PCB congeners in fish varied between 45 and 80%,[71] whereas those in rat it varied from 66 to 96%.[72] Greatly chlorinated PCBs are less absorbed than less-chlorinated congeners.[72] The portion of PCBs not absorbed by the intestine and excreted unmetabolized may not account for toxic effects in animals. Thus, the portion retained in tissues may provide more realistic estimates for risk assessment. While PCBs partitioned into lipids or bound to proteins may not be directly available to cause toxicity, they are in dynamic equilibrium with an available pool of PCB congeners. However, the current database from laboratory animal studies rarely includes tissue concentrations at exposure doses. This complicates the risk assessment process by not considering toxicokinetics or requiring the prediction of tissue concentrations.

While some studies have examined the effects of commercial Aroclor mixtures in laboratory animals, few studies have used contaminated diet containing environmentally modified and physiologically accumulated mixtures of PCBs. Mink and chicken have been exposed to organochlorine-contaminated (mainly PCBs) carp from Saginaw Bay, Lake Huron, at different proportions in their diet, and threshold concentrations were established for various toxic endpoints.[73–77] Although this method provides environmentally realistic exposure to PCBs in test animals, effects due to the presence of several other contaminants in the diet are difficult to assess. In addition, the proportion of fish in the test diet may not be representative of field populations. For instance, wild mink are opportunistic feeders and the proportion of fish in their diet can vary from 0 to 100%, with an average of approximately 35% fish in the diet of wild mink.

In addition to uncertainties about the hazard portion of the risk assessment, there are uncertainties in exposure due to differences in the methods of quantifying PCBs in complex mixtures derived from different environmental matrices. Most routine analytical surveys report "total PCB" concentrations using peak matching techniques with commercial Aroclors as standards. Another method is to determine the concentrations of specific congeners for quantitation and report the results as total PCBs. This method is referred to as the peak summing method. Another method is to use a single Aroclor or a mixture of Aroclors with known chlorobiphenyl composition and content for quantitation. If the gas chromatograms of the environmental PCB residues cannot be matched with an appropriate mixture of known amounts of Aroclor standards, quantitation will be less accurate.[78] As an example, concentrations of PCBs determined based on the COMSTAR algorithm, a statistical procedure to determine total PCBs based on marker congeners and peak ratios[79] overestimated the concentrations determined by summing the concentrations of individual congeners. This artifact occurs because COMSTAR estimates total concentrations of unweathered PCB congeners that would have been present in the original technical Aroclor mixtures.[80] The discrepancies in the congener composition between the commercial mixture and real-world environmental exposures imply that the predictive value of studies based on commercial mixtures may be limited with respect to estimating risks from environmental exposure. Determination of all PCB isomers and congeners present in environmental matrices using a mixture of technical PCB preparations (such as an equivalent mixture of Aroclors 1016, 1242, 1254, and 1260) as standards may provide a better estimate of PCB concentrations for use in risk assessment.

In recent years, the importance of including 'critical body residues' in risk assessment has been emphasized.[69,81] This approach suggests the need to estimate residue-effect relationships in laboratory exposure studies. However, earlier studies of laboratory animal exposure to PCBs did not estimate final body or tissue concentrations or burdens to derive RfDs based on such residue concentrations. This approach would eliminate several uncertainties due to bioavailability, accumulation kinetics, and metabolism. Body residue-based RfDs have been developed for mink[82] and a few species of birds[83] but not for other organisms.

The advantages of total PCB-based risk assessment include its simplicity by being used as a conventional method. This approach also incorporates risks due to metabolites and interactions among congeners. The ability of animals to metabolize PCBs does not necessarily imply that the metabolites can get excreted and therefore the risk can be minimal. Hydroxylated and methylsulfonyl metabolites of PCBs have been shown to accumulate in humans and wildlife[84–86] and are reported to be toxic.[87]

The potential adverse effects of PCBs on wildlife is dependent on several factors, including the overall levels of PCB exposure, the toxicities of the individual congeners present in the mixture, and their interactive effects. Due to several limitations of total PCB-based approach in risk assessment, application of congener specific risk assessment methods has been suggested.

III. TOXIC EQUIVALENCY FACTOR (TEF) APPROACH IN RISK ASSESSMENT

A. Development of TEFs

One approach to congener-specific hazard assessment for complex mixtures of PCBs has been to develop relative potency factors for individual congeners. This is dependent on the mechanism of action of these compounds. If each congener causes different toxic responses and acts via independent mechanisms, then the relative toxicities of every congener must be determined separately. Due to the highly complex nature of PCB mixtures found in environmental and biological samples, this would be a daunting, if not impossible, task. Earlier studies have recognized that 2,3,7,8-tetrachloro dibenzo-*p*-dioxin (TCDD) and structurally related halogenated aromatics invoke a number of common toxic responses, which are mediated through a high-affinity cytosolic receptor protein, the AhR.[35,88–93] Structure-receptor-binding relationships for different classes of halogenated aromatics, including certain PCB congeners, have been developed using [^{3}H]-2,3,7,8-TCDD as the radioligand and rat hepatic cytosol as a source of the AhR.[35,91] It has been recognized that TCDD is the most competitive ligand for the AhR and also the most toxic member of the group of HAHs. Other structural congeners of PCDDs, PCDFs and PCBs, that are similar to TCDD also cause similar effects but with varying potencies. Based on studies that indicated the pivotal role of the AhR in mediating most, if not all, of the toxic and biochemical effects induced by PCBs, PCDDs, and PCDFs, and the structure-receptor binding and structure-activity relationships, a TCDD equivalency factor (TEF) approach was developed. This approach allows the expression of toxic potential of a complex mixture of individual congeners as one integrated parameter, the toxic equiva-

lency (TEQ) value, in which the toxic potency of the mixture corresponds to the potency of the most toxic congener, TCDD. In this way, definitive studies of TCDD for several species and endpoints can be used to derive a MATC. If relative potencies can be derived for PCB congeners for a few endpoints and species that are found to be intercorrelated and if congeners can be established to have the same rank-order among endpoints and species, the relative potencies can be used to develop TEF for each congener. As an example of the technique, if the ED_{50} values for immunosupressive activity of TCDD and 1,2,3,7,8-penta-CDD were 1 and 2 µg/kg, respectively, then the TEF for the latter compound would be the ratio ED_{50} (TCDD): ED_{50}(1,2,3,7,8-pentaCDD) or 0.5. TEF values have been determined for several different AhR-mediated responses. However, for every PCB congener tested, the TEF values are response and species dependent.[94] As an example, TEFs for 2,3,7,8-TCDF obtained from *in vivo* and *in vitro* studies varied from 0.17 to 0.016 and 0.43 to 0.006, respectively.[25] Regulatory agencies have chosen consensus TEF values for individual congeners. Selection criteria have been based on the importance of data obtained for specific responses (e.g., carcinogenicity, reproductive, and developmental toxicity).

The TEF approach was first utilized to assess the risks associated with air emissions of PCDDs and PCDFs formed during high-temperature incineration of industrial and municipal waste.[95,96] Subsequently, the USEPA proposed interim guidelines for estimating risks associated with mixtures of PCDDs and PCDFs for other media as well. Several international agencies have also adopted the TEF approach for the risk assessment of PCDDs and PCDFs.[33,34,97,98] The mechanistic considerations for the development of TEFs for the risk assessment of PCBs have been described elsewhere.[25,94] A brief description of the development of TEFs using mammalian models[25] and the recent progress in studies relating to fish- and bird-specific TEFs for PCBs are reviewed in this article. The TEFs proposed by the WHO for mammals, birds, and fish are given (Table 1).[99] These values are tentative and will be updated when more data are available. Some of the TEFs for fish and birds were derived mainly from *in vitro* studies, suggesting the need for more *in vivo* studies to validate avian and teleost TEFs.

1. Mammalian TEFs

Initially, all PCB congeners were regarded as toxic. Early text books suggested that the toxicity of PCB congeners was proportional to the degree of chlorination. However, *in vivo* studies conducted with rodents in the 1970s and the 1980s found that the toxicity of PCB congeners varied greatly, while a small group of congeners had great toxic potential.[41,100] It was found that the location of chlorine atoms was more important than the number of chlorine atoms. Studies using mammalian models found a correlation between the structure-AhR binding for certain PCB congeners that exhibit "dioxin-like" activities such as induction of AHH (aryl hydrocarbon hydroxylase) and EROD (ethoxyresorufin-*O*-deethylase), body weight loss, hypothyroidism, decreased hepatic or plasma vitamin A levels, porphyria, thymic atrophy, immunotoxicity, and teratogenicity.[29,89,101–106] The non-*ortho*-substituted coplanar PCBs, 3,4,4′,5-tetraCB (PCB 81), 3,3′,4,4′-tetraCB (PCB 77), 3,3′,4,4′,5-pentaCB (PCB 126), and 3,3′,4,4′,5,5′-hexaCB (PCB 169), which are substituted in both *para*, at least 2 *meta*, and no *ortho* positions are the most toxic PCB congeners. It is hypothesized that the lack of chlorine substitution at opposing *ortho* positions allows the two phenyl rings to rotate into the same plane, and so these congeners are commonly referred to as coplanar PCBs. The toxic potencies derived from *in vivo* and *in vitro* assays for coplanar PCBs are variable and dependent on both the species (rats, mouse, monkeys) and endpoint.[25] As an example, the potency ratios of PCB 126: TCDD for different responses were: 66 (body weight loss, rat); 8.1 (thymic atrophy, rat); 10 (mouse fetal thymic lymphoid development); 125 (AHH induction, rat), and 3.3 (AHH induction, rat hepatoma H4IIE cells). Based on these toxicity data, TEFs in the range of 0.008 to 0.3 could be derived, depending on the species and endpoint selected. A consensus TEF for mammalian of 0.1 was assigned to this congener.[99]

Similar to non-*ortho* coplanar PCBs, chlorobiphenyl congeners with chlorine substitution at only one *ortho* position (mono-*ortho* PCBs) may achieve partial coplanarity and also exhibit AhR agonist activity. Based on the potency of PCB congeners relative to TCDD for several AhR-mediated responses in *in vivo* and *in vitro* mammalian models, TEFs have been proposed for non-*ortho* and mono-*ortho* PCBs.[25] As mentioned earlier, the potency ranges of these congeners varied by 2 to 3 orders of magnitude depending on the species and the end point used to derive values. Data that were considered in determining TEF values were prioritized based on *in vivo* studies being given greater weight than *in vitro* information, and effects that are clearly adverse being given more strength than biochemical changes. In mammalian models, long-term *in vivo* exposures were given more weight than acute exposure studies. Currently, TEF values assigned to PCB congeners are tentative and subject to modification as new data become available. Recognizing the need for a more consistent approach for setting internationally accepted

TABLE 1
International Toxic Equivalency Factors (TEFs) for Non- and Mono-*Ortho* PCBs for Mammals, Fish, and Birds

PCB congener	IUPAC No.	Mammals	Fish	Birds
Non-*ortho* PCBs				
3,4,4′,5-	81	0.0001[a,b,c,e]	0.0005	0.1[e]
3,3′,4,4′-	77	0.0001	0.0001	0.05
3,3′,4,4′,5-	126	0.1	0.005	0.1
3,3′,4,4′,5,5′-	169	0.01	0.00005	0.001
Mono-*ortho* PCBs				
2,3,3′,4,4′-	105	0.0001	<0.000005	0.0001
2,3,4,4′,5-	114	0.0005[a,b,c,d]	<0.000005[b]	0.0001[f]
2,3′,4,4′,5-	118	0.0001	<0.000005	0.00001
2′,3,4,4′,5-	123	0.0001[a,c,d]	<0.000005[b]	0.00001[f]
2,3,3′,4,4′,5-	156	0.0005[b,c]	<0.000005	0.0001
2,3,3′,4,4′,5′-	157	0.0005[b,c,d]	<0.000005[b,c]	0.0001
2,3′,4,4′,5,5′-	167	0.00001[a,d]	<0.000005[b]	0.00001[f]
2,3,3′,4,4′,5,5′-	189	0.0001[a,c]	<0.000005	0.00001[f]

[a] Limited data set.
[b] Structural similarity.
[c] QSAR modeling prediction from CYP1A induction (monkey, pig, chicken, or fish).
[d] No new data from 1993 review.
[e] *In vitro* CYP1A induction.
[f] QSAR modeling prediction from class specific TEFs.

After Reference 99.

TEFs, the World Health Organization-European Centre for Environment and Health (WHO-ECEH) and the International Program of Chemical Safety (IPCS) initiated a project in the early 1990s to create a database containing information relevant to the setting of TEFs, and, based on the available information, to assess the relative potencies and to derive consensus TEFs for halogenated aromatics.[107] The first international TEFs for dioxin-like PCBs were proposed in 1994, which have been revised and updated.[99]

2. Teleost TEFs

Most of the data describing the structure-activity relationships for the toxicity of coplanar PCBs have been determined with mammalian *in vivo* and *in vitro* models. As the toxicity of coplanar PCBs vary among vertebrate taxa, recent studies have focused on determining TEFs for coplanar PCBs in fish and bird models.[108–113] The AhR has also been shown to be present in several fish species and fish cell lines,[114–116] and therefore the mechanistic basis for TEFs in aquatic species should be similar to that observed in mammalian systems. Nevertheless, in contrast to mammalian studies, at present few reports are available for the development of TEFs for PCB congeners in fish. Toxic endpoints used to estimate fish-specific TEFs include *in vivo* induction of AHH and EROD in salmonid species[110,117,118] and embryo mortalities in salmonids[111,119–120] and Japanese medaka.[121] Generally, coplanar PCB TEFs derived from early life stage mortality studies with rainbow trout are less than mammalian TEFs.[111] Little information is available on the toxic potencies of mono-*ortho*-substituted congeners in fish to speculate on appropriate teleost TEFs.[122] Mono-*ortho*-substituted congeners IUPAC Nos. 105,118, and 156 did not induce EROD activity in rainbow trout at the dose ranges of 0.45 to 4.4 mg/kg.[110] Studies have shown that *ortho*-substituted PCBs lack biological activity in trout as estimated by CYP1A induction and mortality.[110,111,119,123] However, based on P4501A-mRNA induction, congener 156 has been found to be active in a rainbow trout gonadal cell line (RTG-2), but the potency was weak relative to non-*ortho*-substituted congeners.[120] Therefore, use of TEFs for di-*ortho* PCBs derived from mammalian exposure studies would overestimate the potency of these mixtures relative to their impact on fish. Fish-specific TEFs have been developed for non-*ortho* and mono-*ortho* PCBs (Table 1)[119,124] and have been tentatively adopted for use by the EPA.[125] It should also be noted that TEF values for fish are posed primarily on acute exposure doses of test compounds. Long-term toxicity studies with rainbow trout exposed to less concentrations of TCDD indicated that many toxic responses developed only after a few weeks.[126] Therefore, additional research is needed to develop consensus teleost TEFs.

3. Avian TEFs

End points used to estimate TEFs for birds include *in vitro* and *in ovo* EROD induction[112,113,127,128] and embryo mortalities.[129] In fact, most of the avian TEF data were derived based on EROD induction potencies of PCB congeners. In chicken embryo hepatocytes, PCB 169 was shown to be less potent than PCB 77[112,113,130] which is different from that found in several rodent bioassays.[25] The lesser potency of PCB 169 relative to that of PCB 77 in birds was further supported by the embryo lethality data.[129] Similarly, TCDF was more potent than TCDD in avian models based on EROD induction.[131] Mono-*ortho* PCBs were less potent inducers of EROD than non-*ortho* congeners in bird models, but TEFs for mono-*ortho* congeners were relatively great in birds compared with teleost and rodent TEFs (Table 1). For this reason, the PCB congener that contributes the greater proportion of TEQs based on avian TEFs in most environmental mixtures of PCBs is congener 77 (3,3′,4,4′-tetra-CB). Therefore, more information on the TEF and environmental fate of this congener, particularly on its pharmacokinetics in birds, is necessary for an accurate risk assessment.

Avian TEFs are difficult to estimate because there is considerable interspecific variation in the toxicity of PCB congeners among birds.[108] Some TEFs for PCB congeners in birds are based on EROD induction with eggs and cell cultures from chicken. The preferred endpoint is embryo lethality based on *in ovo* exposure. Domestic chickens and their embryos are considerably more sensitive to AhR-mediated responses than other avian species.[112,113,132] For example, based on *in vitro* EROD induction potency of coplanar PCB congeners in several bird species, the order of sensitivity was shown to be: domestic chicken > ring-necked pheasant > turkey ≈ double-crested cormorant ≈ great blue heron ≈ ring-billed gull ≈ duck ≈ herring gull ≈ common tern > Forster's tern.[133] In general, fish-eating bird species examined so far are at least an order of magnitude less sensitive than the domestic chicken. Thus, toxicological information for the chicken is less appropriate than other species for risk assessment of avian wildlife species, and the use of RfD values based on chicken would be overprotective of most species. Because the chicken is much more responsive than other birds, if chicken TEFs and RfDs are used as a surrogate for wild birds no uncertainty factors should be applied.

B. Applications of the TEF Approach

TEFs have been used to assess the toxic risk associated with mixtures of PCB congeners measured in biota and or environmental matrices by multiplying the concentration of each non- or mono-*ortho* congener detected in the biota by the corresponding TEF to yield a TCDD equivalent concentration or TEQ (Table 2).[134] A total TEQ for all toxic congeners in the sample can be calculated by summing all of the individual TEQs. TEFs were first used to determine non-*ortho* coplanar PCB-derived TEQs[134,135–137] in environmental samples to compare with those obtained for PCDDs/PCDFs, utilizing TEFs derived from the relative potencies of PCB congener-induced AHH and EROD activities in rat hepatoma H4IIE cells.[138] These results showed that the TEQs for PCBs in most extracts from environmental samples or human tissues exceeded the TEQs calculated for the PCDDs/PCDFs in these same extracts.[134,137] The data indicated that the TEQs contributed by the PCBs are greater than those contributed by PCDDs/PCDFs. Comparable results have been obtained in several other studies.[18,139–144]

The utility of the TEF approach to environmental risk assessment is shown by the correlation between total TEQs and adverse effects in populations of birds.[62,145,146] A negative correlation was reported between the incidence of deformities in cormorant populations from the Great Lakes and the total TEQ in cormorant eggs.[53] Poor hatching success in a population of Forster's tern is directly correlated with TEQ in the eggs.[139] In a laboratory and field study on populations of common terns from the Netherlands, egg volume was negatively correlated with the total TEQ in the egg yolk.[108] A weak negative correlation was observed between total TEQs and survival of early life stages in populations of lake trout from the Great Lakes.[147]

C. Limitations of TEF Approach

1. Interactive Effects

Despite the ability of the TEF approach to predict the potency of some mixtures of planar HAHs, there are limitations to its

TABLE 2
An Example for Deriving 2,3,7,8-TCDD Equivalents (TEQs) by the TEF Approach

Congener	TEF[a]	Concentration (pg/g, wet wt)[b]	TEQ (pg/g, wet wt)
Dioxins			
2,3,7,8-tetraCDD	1	3.7	3.7
1,2,3,7,8-pentaCDD	1	6.4	6.4
1,2,3,4,7,8-hexaCDD	0.1	3.9	0.39
1,2,3,6,7,8-hexaCDD	0.1	34	3.4
1,2,3,7,8,9-hexaCDD	0.1	5.7	0.57
1,2,3,4,6,7,8-heptaCDD	0.01	33	0.33
OCDD	0.0001	510	0.051
Furans			
2,3,7,8-tetraCDF	0.1	3.1	0.31
1,2,3,7,8-pentaCDF	0.05	0.5	0.025
2,3,4,7,8-pentaCDF	0.5	11	5.5
1,2,3,4,7,8-hexaCDF	0.1	5.6	0.56
2,3,4,6,7,8-hexaCDF	0.1	1.4	0.14
1,2,3,6,7,8-hexaCDF	0.1	5.3	0.53
1,2,3,7,8,9-hexaCDF	0.1	ND	—
1,2,3,4,6,7,8-heptaCDF	0.01	2.9	0.029
1,2,3,4,7,8,9-heptaCDF	0.01	ND	—
OCDF	0.0001	ND	—
Non-*ortho* PCBs			
3,3′,4,4′-tetraCB	0.0001	350	0.035
3,3′,4,4′,5-pentaCB	0.1	330	33
3,3′,4,4′,5,5′-hexaCB	0.01	90	0.9
Total TEQs			**55.87**

[a] From Reference 99.
[b] From Reference 134 for human adipose tissue.

application. The assumption that toxic responses to planar HAHs are additive and that other classes of contaminants do not modify or add to the toxicity may or may not be valid.[62] There are data from rodent studies that indicate that toxic responses to mixtures of planar HAHs are additive.[148,149] However, there are other rodent data showing either less than additive (antagonistic) responses[150,151] or greater than additive (synergistic) responses.[152,153] Based on the review of experimental evidence, both additive and nonadditive interactions among planar HAHs have been observed.[25] Recent studies have also reported both additive[124] and other interactive effects[154-159] of planar congeners in experimental animals or in cell lines derived from various animals, including mammals and fish. While TEQs estimated based on instrumental analysis do not account for these interactions, bioassay-derived TCDD-equivalents (TCDD-EQs) integrate potential additive and nonadditive interactions among AhR agonists and between AhR agonists and other compounds by measuring a final receptor-mediated response.[145,160] Comparison of bioassay-derived TCDD-EQs with those of instrumental TEQs estimated for the same samples also suggested the existence of both nonadditive and additive interactions in biota.[58,145,146] Details regarding bioassays and their applications in risk assessment are discussed in a later section. The exclusion of nonadditive mixture interactions in the present TEF approach has been justified by (1) the antagonistic or synergistic effects are observed at only very high dose levels and the magnitude of these interactions are smaller than the uncertainties already present in the TEF values, (2) the observed nonadditive effects are highly species-, response-, and dose-dependent and their relevance might be of minimal importance, and (3) the mechanism responsible for these nonadditive effects are unknown.[161] In general, complex mixtures of PCBs are slightly infraadditive (less than additive), so the TEQ of an additive model is conservative (protective).

2. Species- and Endpoint-Specific Variations

The TEF approach assumes that the rank order of relative potencies of congeners are the same among species. However, there are quantitative differences in the relative potencies of PCB congeners among species and endpoints. There are considerable variations in the potency of mono- and non-*ortho* PCBs among as well as within mammalian, teleost, and avian models. The application of mammalian-derived TEFs from rodent bioassays for the assessment of risks in aquatic mammals (e.g., dolphins, whales) may not be appropriate due to differences in the responsiveness of these animals to PCB congeners. Similarly, the differences in the potencies of PCB congeners for various endpoints lead to a range of relative potency values from which a congener-specific TEF is derived. Therefore, the predictive ability of the TEF approach is species- and endpoint-dependent.[25,122,162] Uncertainties of a few orders of magnitude between species and for specific end points are a major drawback in using TEF approach in the risk assessment.

Age- and sex-specific differences in sensitivities could also influence the toxic effects of PCB exposure. EROD induction potencies of planar HAHs in primary chicken hepatocyte cultures were age-dependent.[131] EROD activities were less in hepatocyte cultures prepared from 14-d-old embryos than those from 19-d-old embryos or 1-d-old hatchlings. In the white leghorn chicken, TCDF was 1.2- to 3.4-fold more potent than TCDD, which was different from that observed in mammalian and teleost cell lines.[131]

It is also imperative to note that the induction of P450 enzymes may not necessarily indicate a toxic effect, but may be an adaptive mechanism. Moreover, the induction of P450 enzymes is sometimes nonspecific. Therefore, the use of EROD (an example of P450 induction) induction to measure TEF, and eventually in risk assessment, requires careful interpretation.

3. Non-Ah-Receptor Mediated Effects

The TEF approach does not address potential non-TCDD-like effects of PCBs. Because only a small portion of the total mass of PCB mixtures are coplanar non-*ortho* congeners that elicit dioxin-like activities,[37,94,163,164] the TEF approach based solely on AhR-mediated responses cannot be applied for the risk assessment of non-AhR-mediated toxic effects. Thus, ignoring the non-dioxin-like effects of PCBs could result in an underestimate of the potential adverse effects of environmental mixtures. Therefore, the potential for non-TCDD-like effects need to be evaluated. If the dioxin-like PCBs are the critical contaminant, then variation among mixtures can be reduced by the TEQ approach. However, if the critical mechanism of action, that occurring at lesser concentrations relative to environmental

exposures, is caused by non-TCDD-like compounds, the use of the TEQ approach would not be accurate.

Nonplanar *ortho*-substituted PCBs have been shown to elicit a diverse spectrum of 'non-Ah-receptor-mediated' toxic responses in experimental animals, including neurobehavioral,[165–167] neurotoxic,[30,168–170] carcinogenic,[41,171] and endocrinal changes.[172,173] While AhR-mediated toxicity is peliotropic, effects due to non-dioxin-like PCBs may involve multiple unrelated mechanisms of action. In addition, certain of the metabolites of PCBs have antiestrogenic properties[174] and cause hypothyroidism and decreased plasma vitamin A levels.[158,172,175] These alterations in vitamin A and thyroid hormone concentrations may significantly modulate tumor promotion and developmental and adult neurobehavioral changes.[161] Recent studies have addressed the need for developing an alternate or parallel TEF approach for non-dioxin-like PCB congeners. Neurotoxic effects of PCB congeners and their implications for the risk assessment are reviewed later.

4. Toxicokinetics

The TEF values for dioxin-like PCBs have been derived mainly from short-term tests and *in vitro* assays.[25] Such studies may not reflect delivery of a toxicant to a target organ due to pharmacokinetics, metabolism, and excretion.[69,70] Also, for extrapolations among species, the toxicokinetics must be identical or differences have to be taken into account. Some of the factors that would affect the interspecies differences have been reviewed recently.[40] In addition, species- and tissue-specific differences in the binding properties, specificity and physicochemical properties of the AhR, and the contribution of other P-450 genes to HAH-induced activities challenge the generalities of assumptions of the TEF approach.

5. Dose-Response Relationships

The TEF approach assumes that the relative potencies of individual congeners can be derived. To develop relative potency values, several assumptions must be made. Regardless of the methods applied, the maximum achievable response for the end point of interest is identical for the chemicals evaluated and TCDD, that is, the congener of interest must have the same efficacy as TCDD. A second assumption of parallel lines and slope-ratio methods is that the dose-response relationships are parallel or that they have the same origin. Based on both theoretical analyses and empirical examples from certain studies that developed TEFs, it has been demonstrated that these assumptions for dose-response relationships put forth in the TEF approach are seldom met.[176] Furthermore, the slopes of the dose-response curves for many endpoints were different.[177] It has been suggested that the relative potency among chemicals would be more accurately represented by a function rather than a point estimate such as the EC_{50} or LD_{50}, which are generally used to estimate relative potency.[163,176] This can be accomplished by the use of probability functions.

6. Extrapolation of Dose Ranges and Routes of Exposure

Most of the information used for establishing TEF has come from *in vitro* studies of the induction of monooxygenases, and more recently from subchronic toxicity studies. Most of the *in vivo* studies have produced information on acute effects induced at the greater dose ranges such as lethality. Since, in real-world scenarios, biological effects at chronic, low-level exposures are more relevant, TEFs derived from great exposure doses may be questionable. The dose regimen used in exposure studies are different, which may influence the derived TEF value and eventually the risk assessment process.

Although the TEF concept has several constraints in its application, at this stage this has been a feasible approach for the risk assessment of planar HAHs. Some of the uncertainties due to interactive effects among planar HAHs can be calculated and specified by *in vitro* bioassay techniques.

IV. RISK ASSESSMENT BASED ON *IN VITRO* BIOASSAYS

Although the TEF approach does not account for interactive effects such as synergism, additivity, or antagonism between and among active and inactive compounds, *in vitro* cell systems have been developed to integrate concentrations and potencies of all planar HAHs and their interactions in complex mixtures. In addition, development of bioassays has several implications in risk assessment. The current techniques for detection and quantitation of dioxin-like compounds involve costly and time-consuming instrumental analysis methods such as gas chromatography separation and electron capture or mass spectrometry identification. For a number of biologically active compounds, neither routine methods nor authentic standards are available. Bioassays are sensitive, cost-effective, rapid screening tools and integrate the biological effects of complex mixtures of PCBs and other HAHs.[145,175,178] Numerous bioassay systems have been developed, which are mainly based on the AhR-dependent mechanism for the detection of dioxin-like planar HAHs.[109,112,113,179–186] The majority of the AhR-dependent bioassays are based on measurement of the induction of gene expression, and in these assays the magnitude of induction by the mixture is expressed relative to TCDD, and the calculated values are expressed as TCDD-EQs (Figure 3). The induction of cytochrome P4501A1-dependent EROD activity, in rat hepatoma H4IIE cells, is one such response that has been utilized extensively for this purpose.[25,145,187] The minimal detection limit of TCDD in the EROD assay has been reported to be 3.2 pg.[94] Later, a fluorescence assay microplate was developed for measurement of dioxin-like HAHs-dependent EROD induction and porphyrin accumulation in chicken embryo hepatocytes, which was reported to be 100-fold more sensitive (detection limit = 0.16 pg TCDD) than the conventional EROD assay.[160]

With the recent advancements in understanding of the molecular mechanism of AhR action[188,189] combined with exogenous reporter genes,[190] several recombinant PCB/PCDD/PCDF-inducible expression vectors, which contain an easily measurable reporter gene have been developed.[160,182,184] In addition, these recombinant bioassay systems can be easily manipulated to increase the sensitivity by increasing the number of dioxin-responsive elements (DREs) regulating reporter gene expression, as well as increasing the number of copies of the expression plasmid in the cells by amplification. Furthermore, the concentrations of AhR in these cell lines can be significantly increased by introduction of constitutively active expression vectors that respond proportionally to the toxicity of a mixture by measuring the ability of chemicals in a sample extract to either bind to the AhR[180,183] or to bind to the AhR and stimulate its DNA binding, and subsequent changes in a reporter gene that can be quantified.[189]

A. Applications of Bioassays

In vitro bioassays are faster and less expensive than instrumental analyses. Bioassays integrate various interactions among and between planar HAHs in sample extracts, and therefore measurement of a biochemical response is more biologically relevant. Bioassays are rapid screening tools

FIGURE 3. Schematic illustration for the estimation of TCDD-EQs by biossays. (RLU = Relative Luminescence Units).

for detection of PCB/PCDD/PCDF containing sample extracts, thus, are complimentary tools for instrumental analysis. Types of bioassay systems that are used to measure the potency of dioxin-like compounds and to detect their presence in environmental matrices are shown.[175] Advantages and limitations of each type of bioassay are listed elsewhere.[175]

B. Limitations of *In Vitro* Bioassays

Some of the limitations of the TEF approach are also limitations of *in vitro* bioassays. Much of the information used to derive TEF values has been derived from *in vitro* bioassays. AhR-dependent bioassays are not appropriate for the detection or estimation of the biopotency of compounds that do not act by this mechanism. These bioassays provide overall TCDD-EQs for a complex mixture, but do not provide specific information on the class of compounds that contribute to the toxicity because several classes of planar HAHs can act via the AhR-mediated mechanism.[62] Interferences due to AhR antagonists presumably by interfering with the ability of TCDD-like chemicals to bind to the AhR may produce false indications. *In vitro* bioassays do not account for toxicokinetics and species-specific variability in sensitivities.

V. BIOLOGICAL EFFECTS OF NON-DIOXIN-LIKE PCBs AND THEIR RISK ASSESSMENT

The TEF approach fails to recognize the potential health effects of *ortho*-substituted congeners that do not interact at the AhR.[30] Recent studies have provided data on the potency and possible mechanisms by which *ortho*-substituted congeners exhibit toxic effects.[30,170] PCBs with two or more *ortho* chlorines do not interact with the AhR and elicit a different pattern of toxicity. Developmental and cognitive dysfunctions observed in children born to mothers who consumed PCB-contaminated rice oil in Japan (Yusho) and Taiwan (Yu-Cheng) have been associated with exposure to complex mixtures of HAHs.[191,192] The rice oil in both the Yusho and Yu-Cheng incidents was contaminated with a mixture of HAHs, including PCBs, PCDFs, and PCQs (polychlorinated quarterphenyls),[193] and possibly PCNs (polychlorinated naphthalenes), and thus it has been difficult to establish which contaminants in the rice oil are responsible for the persistent alterations in behavior and cognitive development in the exposed children. The neurotoxic effects of *ortho*-substituted PCBs observed in laboratory studies were compared with accidental PCB poisoning incidences such as Yusho and Yu-Cheng and epidemiological reports, which indicated that the developing nervous system is sensitive to exposure of PCBs.[30,195–200] A poor or non-existent correlation between the presence of AhR mediated physical signs of exposure such as chloracne, hyperpigmentation, and the observed cognitive dysfunctions suggested the possibility that the alterations in neurological function in the Yusho and Yu-Cheng children may be due to exposure to nonplanar *ortho*-substituted PCB congeners present in many commercial mixtures of PCBs rather than the coplanar contaminants that interact at the AhR.[194,195] Details regarding the mechanisms of neurotoxic effects of *ortho*-substituted PCBs and their linkage to epidemiological studies in which behavioral alterations in children prenatally exposed to PCBs are given elsewhere.[170,201–211]

Aroclor 1254 reduced cellular dopamine (DA) activity of pheochromocytoma (PC12) cells.[212–214] This is a continuous cell line derived from a rat adrenal gland tumor that synthesizes, stores, releases, and metabolizes biogenic amine neurotransmitters, including DA, in a manner similar to that of the mammalian central nervous system. Subsequent studies using a neuroblastoma cell line (NIE-N115), a continuous cell line derived from a

mouse neuroblastoma, deficient in the enzyme L-aromatic amino acid decarboxylase, which converts the intermediate product L-DOPA (L-dihydroxy phenylalanine) to DA, demonstrated that exposure to 2,2′-diCB (PCB 4) resulted in a significant decrease in media concentrations of L-DOPA, which suggested that the reductions in the synthetic capability of the rate-limiting enzyme for DA, tyrosine hydroxylase.[215] Additional studies examined the relationship between the structure of individual PCB congeners and their ability to alter PC12 cellular DA content. About 50 individual PCB congeners were tested for their ability to reduce cellular DA content in PC12 cells and found that di-*ortho*- through tetra-*ortho*-substituted congeners were the most potent, whereas coplanar PCB congeners were ineffective.[216] In addition, chlorination in a *meta*-position decreased the potency of *ortho*-substituted congeners, but *meta*-substitution had little effect on congeners with both *ortho*- and *para*-substitutions. Further experiments with PCB 4, a di *ortho*-substituted congener that was potent in decreasing DA concentrations *in vitro*, indicated that the active agent was not a metabolite.[216] These results suggested that PCB congeners, predicted to have little dioxin-like activity based on the structural configuration, decreased DA levels in the nervous system and that neurotoxicity might be due to a mechanism independent of AhR activation. Investigations on the effects of various PCB congeners on Ca^{2+} homeostasis and protein kinase C (PKC) translocation in cerebellar granule cells found a similar structure-activity relationship that showed that the *ortho*-substituted PCBs have potential to alter Ca^{2+} homeostasis in the brain, while the AhR-active congeners were reported to be inactive.[217] Based on these studies, two different PCB binding sites in the brain were suggested. Further, the effect of *ortho*-substituted PCBs in reducing DA levels in brain was shown to be additive.[169] As an example, a mixture of PCB congeners 2,4,4′-(PCB 28), 2,2′,4,4′-(PCB 47), and 2,2′,5,5′-(PCB 52) was more potent in reducing brain DA content than the equal amounts of each congener in *in vitro* systems.[169]

Neurotoxicological effects of various technical PCB mixtures in laboratory animals and in *in vitro* studies and their effects and effective doses (NOAEC, LOAEC, EC_{50}) have been compiled (Table 3). The data suggest that *ortho*-substituted PCB congeners are potential neurotoxicants when exposed prenatally. It is also evident that neurotoxicological effects may be of considerable significance following neonatal exposure and acute accidental exposures. In general, these studies have found that *in utero* exposure to PCB mixtures or congeners could alter motor activity,[218] neurological development and cognitive function[219] in offspring. Following acute exposure to PCBs in mice, changes in neurotransmitters such as DA content have been associated with neurobehavioral changes.[215,220–225] Studies conducted in adult non-human primates also suggested that *ortho*-substituted congeners are capable of reducing brain DA concentrations. Adult pigtailed macaques (*Macaca nemestrina*) were exposed to Aroclor 1016 or Aroclor 1260 at doses of 0.8, 1.6, or 3.2 mg/kg bw/d for 20 weeks. Significant reductions in DA concentrations in certain regions in the brain, where DA synthesis occurs, were observed. Several *ortho*-substituted PCB congeners, including 2,4,4′-(PCB 28), 2,2′,4,4′-(PCB 47), and 2,2′,5,5′-(PCB 52) have been found to accumulate in the brain.[224] The DA-reducing effects of PCBs were found to be persistent even after the exposure was terminated, which suggested that the neurobehavioral alterations following exposure to *ortho*-substituted PCBs may be a long-term and irreversible effect.[225] While non-human primates exposed to a dose of 3.2 mg/kg bw/d of Aroclor 1016 or Arolcor 1260 showed reductions in DA, offspring of rats exposed up to 25 mg/kg bw/d did not exhibit changes in brain DA content,[207,208] which suggests that

TABLE 3
Summary of Effects of Peri- and Postnatal Exposures to PCBs on Neurotoxic Effects in Animals

PCB congener/ mixture	Species, sex, age	Dose and exposure	Effects and effective doses	Ref.
***In vivo* studies**				
3,3′,4,4′-(PCB77)	CD-1 mice, pregnant female	32 mg/kg bw, oral, prenatal exposure, 10 to 16 days of gestation	Hyperactivity in offspring, neuromuscular dysfunction, learning and performance deficits, 'spinning' syndrome	271
3,3′,4,4′-(PCB77)	CD-1 mice, pregnant female	32 mg/kg bw, oral, prenatal exposure, 10 to 16 days of gestation	Hyperactivity in offspring, reduction in brain dopamine, behavioral alterations	243
3,3′,4,4′-(PCB77)	NMRI mice, male, 10 days	0.41–41 mg/kg bw, oral, single postnatal exposure	Cholinergic system affected at 0.41 mg/kg bw, disturbed behavior	245
2,4,4′-(PCB 28)	NMRI mice, male, 10 days	0.18, 0.36, 3.6 mg/kg bw, oral, single postnatal exposure	After 4 months aberrations in spontaneous behavior, lack of effect on memory and learning and on nicotinic receptors, no effect on dopamine or serotonin, ≥0.36 mg/kg bw reduced total activity	205
2,2′,5,5′-(PCB 52)	NMRI mice, male, 10 days	0.2, 0.41, 4.1 mg/kg bw, oral, single postnatal exposure	After 4 months aberrations in spontaneous behavior, deficits in memory and learning function, cholinergic nicotinic receptors affected, no effect on dopamine or serotonin, ≥4.1 mg/kg bw reduced total activity	205
2,3′,4,4′,5-(PCB 118)	NMRI mice, male, 10 days	0.23, 0.46, 4.6 mg/kg bw, oral, single postnatal exposure	No significant changes in spontaneous and swim-maze behavior up to the dose of 4.6 mg/kg bw	205
2,3,3′,4,4′,5-(PCB 156)	NMRI mice, male, 10 days	0.25, 0.51, 5.1 mg/kg bw, oral, single postnatal exposure	No significant change in spontaneous and swim-maze behavior up to the dose of 4.6 mg/kg bw	205

TABLE 3 (continued)
Summary of Effects of Peri- and Postnatal Exposures to PCBs on Neurotoxic Effects in Animals

PCB congener/ mixture	Species, sex, age	Dose and exposure	Effects and effective doses	Ref.
***In vivo* studies**				
2,2′,5,5′- (PCB 52)	NMRI mice, male, 10 days	4.1 mg/kg bw, oral, single postnatal exposure	At 4 months decrease in rearing, locomotion, and total activity	204
3,3′,4,4′,5- (PCB 126)	Sprague-Dawley rats, both sexes, 5–7 weeks (weanling)	0.1–100 ng/g in diet for 13 weeks, oral, postnatal	Growth suppression, thymic atrophy, increased liver weight, anemia, no significant alterations in biogenic amines, NOAEL = 0.1 ng/g in diet or 0.01 μg/kg bw/d	272
3,3′,4,4′- (PCB 77)	Sprague-Dawley rats, both sexes, 5–7 weeks (weanling)	10–10,000 ng/g in diet for 13 weeks, oral, postnatal	Increased EROD activity, decreased vitamin A, altered dopamine and homovanillic acid in brain, histopathological changes in thyroid and liver, NOAEL = 100 ng/g in diet or 8.7 μg/kg bw/d	202
2,3′,4,4′,5- (PCB 118)	Sprague-Dawley rats, both sexes, 5–7 weeks (weanling)	10–10,000 ng/g in diet for 13 weeks for males, 2–2000 ng/g for females, oral, postnatal	Increased EROD activity, reduced dopamine, and homovanillic acid in brain, histopathological changes in thyroid and liver, brain residues at the highest dose 0.36 – 1 μg/g, NOAEL = 200 ng/g in diet or 17 μg/kg bw/d	202
2,2′,4,4′,5,5′- (PCB 153)	Sprague-Dawley rats, both sexes, 5–7 weeks (weanling)	50–50000 ng/g in diet for 13 weeks, oral, postnatal	Increased EROD activity, reduction in hepatic vitamin A, decreased dopamine and its metabolites, females more sensitive, histological changes in thyroid and liver, highest dose brain residues 16–29 μg/g, NOAEL = 500 ng/g in diet or 34 μg/kg bw/d	203
2,2′,3,3′,4,4′- (PCB 128)	Sprague-Dawley rats, both sexes, 5–7 weeks (weanling)	50–50000 ng/g in diet for 13 weeks, oral, postnatal	Increased EROD activity, reduction in hepatic vitamin A, decreased dopamine and its metabolites, females more sensitive, histological changes in thyroid and liver, highest dose brain residues 5–10 μg/g, NOAEL = 500 ng/g in diet or 42 μg/kg bw/d	273
3,3′,4,4′,5- (PCB 126)	Lewis rats, adult female	10 and 20 μg/kg bw on days 9,11,13,15, 17, and 19 days of gestation, oral, prenatal	Fetotoxicity, delayed physical maturation, reduced body weight in offspring, increased liver weight and EROD activity, no effect on learning or neurobehavioral performance, no residues in brain, exhibited sex differences in neurotoxicity	274
3,3′,4,4′,5- (PCB 126)	Lewis rats, adult female	2 μg/kg bw on days 10,12,14,16,18, and 20 days of gestation, oral, prenatal	Neurotoxic effects in offspring, no fetotoxicity, behavioral alterations, hyperactivity, impaired discrimination learning, no brain residues	219
2,3′,4,4′,5- (PCB 118)	Lewis rats, adult female	1 and 5 mg/kg bw on days 10,12,14,16,18, and 20 days of gestation, oral, prenatal	Neurotoxic effects in offspring, no fetotoxicity, behavioral alterations, hyperactivity, impaired discrimination learning, brain residues 6–982 ng/g	219
3,3′,4,4′- (PCB 77)	Wistar rats, adult female	1 mg/kg bw, days 7 to 18 of gestation, subcutaneous injection, prenatal	Behavioral effects in offspring, PCB concentrations in brain 0.15 μg/g	275
2,2′,4,4′- (PCB 28)	Wistar rats, adult female	1 mg/kg bw, days 7 to 18 of gestation, subcutaneous injection, prenatal	Behavioral effects in offspring, PCB concentrations in brain 0.61 μg/g	275
Fenclor 42	Fischer rats, adult female	5–10 mg/kg bw/d intake or 25–50 mg/kg, i.p., five injections daily, 2 weeks prior to mating, prenatal	Neurotoxicity and behavioral alterations, 40 mg/kg resulted in significant postweaning behavioral effects, LOAEL = 10 mg/kg bw/d	276

TABLE 3 (continued)
Summary of Effects of Peri- and Postnatal Exposures to PCBs on Neurotoxic Effects in Animals

PCB congener/ mixture	Species, sex, age	Dose and exposure	Effects and effective doses	Ref.
***In vivo* studies**				
Aroclor 1254	Wistar rats, adult female	0.2–26 μg/g in diet, preweaning, perinatal exposure	Impaired neurological development, LOAEL = 2.5 μg/g	277
Aroclors 1254 and 1260	Wistar rats, adult male	500–1000 mg/kg bw, single oral exposure, postnatal	Decrease in dopamine, norepinephrine, and serotonin concentrations in specific regions in brain up to 14 days after exposure	222
Aroclor 1254	Wistar rats, adult male	500–1000 mg/kg bw, oral exposure for 30 days, postnatal	Dopamine and its metabolites decreased, PCB concentrations in brain after 30 days were 75–82 μg/g, 6 di-ortho and 3 mono-ortho congeners dominated	223
Aroclor 1254	Wistar rats, adult female	5 and 25 mg/kg bw from day 10 to 16 of gestation, prenatal, oral	Alterations in seratonin metabolism in the brains of offspring after 21 and 90 days of birth, other biogenic amines (e.g., dopamine norepinephrine) in brain were unaffected, effect was significant at dose 25 mg/kg bw	207
Aroclor 1254 and 3,3′,4,4′-(PCB 77)	Wistar rats, adult female	5 and 25 mg/kg bw from day 10 to 16 of gestation, prenatal, oral	Reduced plasma thyroid hormone, plasma concentrations of hydroxylated metabolite of PCB 153 was greater than the 153 in fetus, neonates, and weanling rats, fetus brain thyroid residues affected, effect of OH-PCBs on brain is discussed	208
Clophen A30	Wistar rats, adult female	5 and 30 mg/kg bw in diet or intake of 0.4 and 2.4 mg/kg/d, from 60 d prior to mating until 21 days after birth, oral	Behavioral effects, PCDF contamination in Clophen — 2.5 mg/kg, brain concentration = 60 ng/g after 420 d of exposure, PCBs 28, 52, and 101 were the prevalent ones	278
Aroclor 1016	Pig-tailed macaque (*Macaca nemestrina*), male, 3–5 years	0.8–3.2 mg/kg bw/d, for 20 weeks, oral, postnatal	Persistent reduction in brain dopamine, brain PCB concentrations 1–5 μg/g, only PCBs 28, 47, and 52 accumulated in brain, lightly chlorinated PCB mixtures are more effective than heavily chlorinated ones	169
Aroclor 1260	Pig-tailed macaque (*Macaca nemestrina*), male, 3–5 years	0.8–3.2 mg/kg bw/d, for 20 weeks, oral, postnatal	Persistent reduction in brain dopamine, brain PCB concentrations 18–28 μg/g, di-*ortho*-substituted hexa- and heptaCBs accumulated in brain, less effective to reduce dopamine when compared with Arolcor 1016 exposure	224
Aroclor 1248	Rhesus monkeys, adult female	0.5–2.5 mg/kg in diet, exposed before and during gestation, oral, perinatal, cumulative PCB intake was 293 mg	Hyperactivity in offspring, behavioral deficits, PCB concentrations in body fat was 20 μg/g	166
***In vitro* or *ex vivo* studies**				
Aroclors 1254: 1260 (1:1)	Wistar rats, male, 65 days	10–100 μg/g in media, *ex vivo* brain tissue, 6 h exposure	Decrease in dopamine and its metabolites at 20 μg/g or above, brain total PCB concentration at the effective dose was >15 μg/g	206
Aroclor 1254	PC-12 cells	1–100 μg/g, *in vitro*, 6 h exposure	Increase followed by a decrease in cellular catecholamine	214
2,2′-(PCB 4)	Long-Evans hooded rats, adult male	50–200 μ*M*, *in vitro*, cerebellar granule cells exposed	Altered Ca^{2+} homeostasis in cerebellar granule cells, IC_{50} = 6.17 μ*M*, more effective than PCB 126	226, 227
3,3′,4,4′,5-(PCB 126)	Long-Evans hooded rats, adult male	50–200 μ*M*, *in vitro*, cerebellar granule cells exposed	Altered Ca^{2+} homeostasis in cerebellar granule cells, IC50 = 7.61 μ*M*	226, 227
2,2′-(PCB 4)	Long-Evans hooded, male, adult rats, 40–90 days	10–100 μ*M*, *in vitro*, mitochondrial and synaptosomal preparations from brain exposed	Mg^{2+}-ATPase activity inhibited, but not Na^{+}/K^{+}-ATPase activity, ED_{50} is roughly 5 μ*M*	201
3,3′,4,4′,5-(PCB 126)	Long-Evans hooded, male, adult rats, 40–90 days	10–100 μ*M*, *in vitro*, mitochondrial and synaptosomal preparations from brain exposed	Mg^{2+}-ATPase activity was not inhibited up to the dose of 100 μ*M*	201

TABLE 3 (continued)
Summary of Effects of Peri- and Postnatal Exposures to PCBs on Neurotoxic Effects in Animals

PCB congener/ mixture	Species, sex, age	Dose and exposure	Effects and effective doses	Ref.
In vitro or *ex vivo* studies				
2,2′,3,5′,6- (PCB 95)	Sprague-Dawley rats, male	1–200 μM, *in vitro*, microsomes of rat brain hippocampus	Alterations in neuronal Ca^{2+} signal and neuroplasticity, EC_{50} = 12 μ*M*	210
2,3′,4,4′- (PCB 66)	Sprague-Dawley rats, male	1–200 μ*M*, *in vitro*, microsomes of rat brain hippocampus	No effect was found on [^{3}H] ryanodine receptors, suggesting no alterations in neuronal Ca^{2+} signal up to 200 μ*M*	210

rats may require greater exposure doses of PCBs to elicit the same response. These results also implied that the potency of *ortho*-substituted PCBs in reducing brain DA may be species-specific. Similarly, effects of PCBs on learning behavior has been shown to be sex-specific, with females being more sensitive than males.[218] Neurotoxic effects were prominent following prenatal exposure, while adults were relatively less susceptible to neurotoxic effects following exposure to PCBs.[30]

In addition to reducing DA content of the brain, *ortho*-substituted PCBs have been shown to alter the translocation/activation of PKC and intraneuronal sequestration of Ca^{2+} in brain cerebellar granule cells.[228–229] The di-*ortho* substituted congener 2,2′,3,5′,6- (PCB 95) altered microsomal Ca^{2+} transport by interfering with the ryanodine receptor in rat brain.[210] Similarly, another di-*ortho* substituted congener 2,2′-diCB, interfered with oxidative phosphorylation by inhibiting mitochondrial Mg^{2+}-ATPase activity in mitochondrial and synaptosomal preparations of rat brain.[201] Alterations in hormone levels, including thyroid hormones, which play an important role in regulating neuronal growth and development, have also been suggested to be responsible for PCB-induced neurotoxicity.[30] Particularly, alterations in hormone levels during early development of animals or humans may have long-term consequences on the behavior and neurochemistry of adult animals.[30] Because of multiple mechanisms, a pure quantitative structure-activity relationship for neurotoxic effects is not possible with the limited data available. Thus, there are currently no relative potency factors for PCB congeners to cause these effects.

A. Sources of Uncertainties in Deriving RfDs for Non-Dioxin-Like Effects

Laboratory studies describing neurotoxicological effects of PCBs were based on dietary exposure of rats, mice, or non-human primates with technical mixtures of PCBs, which may not represent the PCB mixtures found in environmental matrices. Doses of PCBs to laboratory animals in these studies were greater than those observed in real-world situations. Similarly, the *in vitro* assays with rat cerebellar granules or PC12 cells have used greater doses and the EC_{50} values for various end points were generally great (>50μ*M*). The EC_{50} values based on *in vitro* studies of neurotoxicological effects of PCBs are presented in Table 4. In *in vitro* systems, less-chlorinated *ortho*-substituted congeners were more potent in reducing DA than more chlorinated congeners. Aroclor 1016 (42% chlorine by weight) was more potent than the more chlorinated Aroclor 1260 (60% chlorine) in producing neurobehavioral effects and reducing brain dopamine in pig-tailed macaques.[224] This was attributed to the greater abundance of lesser-chlorinated *ortho*-substituted PCBs in Aroclor 1016 than in Aroclor 1260 (Table 5). Accumulation of lesser-chlorinated *ortho*-substituted PCB congeners, IUPAC Nos. 28, 47 and 52, in brains of pig-tailed macaques following exposure at 3.2 mg/kg bw/d for 20 weeks has also been observed.[169]

Few studies have examined the presence of PCB congeners in brains of humans and wildlife. PCBs were not detected in brain tissues obtained from two men with Parkinson's disease.[230] Concentrations of total PCBs in the brain of a Yu-Cheng victim was 80 ng/g, whereas those in fat tissues ranged up to 11 μg/g.[231] PCB 153 (2,2′,4,4′,5,5′-HxCB 1.6 ng/g, wet wt) and PCB 138 (2,2′,3,4,4′,5′-HxCB 0.96 ng/g, wet wt) were the only two congeners detected in the brain of grey seals, at a concentration of 1% of that measured in the blubber.[232] Similarly, the PCB profile in brain tissue resembled those in other body tissues with PCB 153 > PCB 138 > PCB 187, in harbor porpoises,[233] which suggested that there was no preferential enrichment of lesser-chlorinated *ortho*-substituted PCBs in wildlife.

TABLE 4
EC_{50} Values for PCB-Congener-Mediated Decreases in Dopamine Content in PC-12 Cells *In Vitro* and [^{3}H]phorbol Ester Binding in Rat Cerebellar Granule Cells and IC_{50} Values for Microsomal $^{45}Ca^{2+}$ Uptake in Rat Cerebellar Granule Cells[a]

Congener/PCB mixture	IUPAC No.	EC_{50} (dopamine content) μM	EC_{50} ([^{3}H]phorbol binding) μM	IC_{50} ($^{45}Ca^{2+}$ uptake) μM
2,2′-	4	64	43	8.0 (6.2)[b]
2,2′,4,6-	50	71	41	7.3
2,3′,4,6-	69	78	na	na
2,2′,4,6,6′-	104	93	38	5.5
2,2′,5-	18	82	na	na
2,2′,5,5′-	52	86	28	4.9
2,2′,4,5′-	49	97	na	na
2,2′,4,4′-	47	115	89	5.8
2,6-	10	106	na	na
2,4,4′,6-	75	118	na	na
2,2′,3,3′,4,4′,6-	171	134	na	na
2,4,6-	30	150	na	na
2,2′,3,5′-	44	114	na	na
2-	1	182	na	na
2,4,4′-	28	196	>100	6.9
2,4-	7	200	na	na
2,4′-	8	200	na	na
2,2′,4,4′,6,6′-	155	156	na	na
2,2′,4,4′,6-	100	158	na	na
2,2′,4,5′,6-	103	157	na	na
2,3-	5	173	na	na
2,4′,5-	31	176	na	na
2,3′-	6	173	na	na
2,3′,5-	26	161	na	na
2,3,6-	24	160	na	na
3,4-	12	169	na	na
3,3′-	11	195	60	13
2′,3,4-	33	185	na	na
4-	3	335	na	na
2,3′,4,4′-	66	>200	na	na
2,3′,4-	25	>200	na	na
2,2′,3,3′-	40	>200	na	na
2,3,4-	21	>200	na	na
2,2′,3,4,4′,5,6-	181	370	na	na
2,5-	9	>200	na	na
3,5-	14	>200	74	17
3-	2	300	na	na
3,4′-	13	410	na	na
3,4′,5-	39	310	na	na
4,4′-	15	>1000	>100 (NEO)	>100 (NEO)
2,2′,6,6′-	54	>1000	>100 (NEO)	>100 (NEO)
3,3′,4,4′-	77	>1000	>100 (NEO)	>100 (NEO)
3,3′,4,4′,5-	126	>1000	>100 (NEO)	>100 (7.6)[b]
2,2′,6-	19		58	7
2,2′,4,6′-	51		50	2.4
3,3′,5,5′-	80		72	>100
2,3,3′,4,4′-	105		95	5.3
2,3′,4,4′,5-	118		>100	6.6
2,2′,3,3′,4,4′-	128		>100	4.9

TABLE 4 (continued)
EC_{50} Values for PCB-Congener-Mediated Decreases in Dopamine Content in PC-12 Cells *In Vitro* and [^{3}H]phorbol Ester Binding in Rat Cerebellar Granule Cells and IC_{50} Values for Microsomal $^{45}Ca^{2+}$ Uptake in Rat Cerebellar Granule Cells[a]

Congener/PCB mixture	IUPAC No.	EC_{50} (dopamine content) μM	EC_{50} ([^{3}H]phorbol binding) μM	IC_{50} ($^{45}Ca^{2+}$ uptake) μM
2,2′,3,3′,5,5′-	133		>100	5.1
2,2′,3,3′,6,6′-	136		58	6.3
2,2′,4,4′,5,5′-	153		>100	6.6
2,3,3′,4,4′,5-	156		>100	5.4
3,3′,4,4′,5,5′-	169		>100 (NEO)	>100 (NEO)
2,2′,3,4,4′,5,5′-	180		>100 (NEO)	4.8
Aroclor 1016			71	6.8
Aroclor 1254			56	6.3
Aroclor 1260			>100	7.6

Notes: NEO: No effect observed up to 100 μM.

[a] From References 216, 217, and 229 (only mean values are presented).
[b] Values in parentheses were from Reference 227.

na: Data not available.

PCB concentrations in brain were 1.5% of that found in the blubber.[233] Similarly, concentrations of total PCBs in the brain of marine mammals from Greek waters were 1 to 2% of those found in the blubber.[234] Less-chlorinated *ortho*-substituted PCB congeners are metabolized in humans,[235] birds,[236] and dolphins.[54,235,237–239] These results suggest that the accumulation of the lesser-chlorinated PCBs is small following chronic exposure. Laboratory studies have shown the presence of greater than 1 μg/g, wet wt of PCBs in brains of exposed rats and mouse, which could be due to the exposure at greater concentrations (Table 3). Therefore, neurotoxicological effects found in laboratory animals may be expected to occur only at relatively great exposure scenarios, as observed for Yusho and Yu-Cheng incidences as well as from occupational exposures. The results reviewed here indicate that it is unlikely that the effects of *ortho*-substituted PCBs will be the critical toxic effects of PCBs. This is due to the following factors: First, relatively great concentrations of lesser-chlorinated di-*ortho* -substituted congeners need to accumulate in the brain to cause the observed effects. Second, the congeners that are active do not tend to be accumulated in brains of animals exposed to complex mixtures of PCBs in the environment (field studies). Finally, the most neurotoxic congeners are the less-chlorinated PCBs, which are more easily degraded in the environment and less bioconcentrated and more readily metabolized and excreted. The relative potential for adverse effects through AhR-mediated and non-AhR-mediated effects of environmentally weathered mixtures is assessed in subsequent sections.

Exposure of experimental animals to weathered PCBs may provide more realistic estimates for the risk assessment of *ortho*-substituted PCB congeners. A few studies have examined behavioral alterations in rats following exposure to contaminated fish from the Great Lakes.[240,241] Rats fed different rations of Great Lakes fish (8, 15, and 30% of

TABLE 5
Abundance of *Ortho*-Substituted PCB Congeners in Various Aroclor Mixtures

Chlorobiphenyl (CB) congener	*o,o'*-Cl	Composition (weight %) Aroclor 1016	Aroclor 1242	Aroclor 1254	Aroclor 1260	Aroclor 1268
Di CB	1	14.3	10.2	—	—	—
	2	4.26	3.21	—	—	—
Tri CB	1	30.6	21.9	0.61	0.1	—
	2	20.7	14.1	0.6	—	—
	3	0.96	0.53	—	—	—
Tetra CB	1	4.28	11.3	5.84	0.09	—
	2	20.3	18.4	10.7	0.9	—
	3	3.27	2.52	0.09	—	—
Penta CB	1	—	3.18	12.3	0.94	—
	2	0.15	6.85	29.7	9.28	—
	3	0.84	3.76	8.9	3.29	—
	4	—	—	0.08	—	—
Hexa CB	1	—	0.09	1.83	1.28	—
	2	0.19	1.22	13.3	24	—
	3	—	1.01	7.63	18.5	—
	4	—	0.07	1.12	2.23	4
Hepta CB	1	—	—	—	0.11	—
	2	—	0.17	0.82	13.5	—
	3	—	—	3.03	17.5	8
	4	—	—	0.53	2.74	—
Octa CB	2	—	—	—	1.45	3.5
	3	—	—	—	3.76	31
	4	—	—	0.68	2.06	11
Nona CB	3	—	—	—	0.45	21
	4	—	—	—	0.22	14
Deca CB	4	—	—	—	0.05	4.8

[a] Congeners that contributed to <0.05% of the total composition were not included.

After Reference 17 for Aroclors 1016, 1242, 1254, and 1260 and after 19 for Aroclor 1268.

the diet) for 20 days exhibited behavioral alterations. The effects included reduced exploratory activity and decreased rearing and nose-poke behavior in comparison with controls. PCB concentrations in fish were in the range of 4 to 19 μg/g, wet wt, and total PCB concentrations in rat brain after the exposure period was 50-78 ng/g, wet wt. On the contrary, no significant effect in behavioral measures following a 90-day subchronic exposure to PCB-contaminated Great Lakes fish was observed, although the accumulation of *ortho*-substituted congeners such as 2,2′,4,4′-(PCB 47), 2,2′,5,5′-(PCB 48), 2,2′,4,4′,5,5′-(PCB 153), 2,2′,5,5′-(PCB 52), 2,4,4′,5-(PCB 74), and 2,2′,4,5′-(PCB 49) was in the range of 2.5 to 18 ng/g, wet wt, in the brain of rats to which fish were fed.[242] Confounding factors in these studies could be the presence of several other contaminants such as methyl mercury in the diet. Synthetic pyrethroids, organophosphorus pesticides, organometallics such as tributyltin and methyl mercury, aluminium, and monosodium glutamate have been known to alter Ca^{2+} homeostatis and to alter neurobehavioral

responses in exposed laboratory animals.[39,226,227] Thus, the results of these studies are considered equivocal.

In addition to *ortho*-substituted PCBs, the non-*ortho* coplanar congener, 3,3′,4,4′-(PCB 77) also alters DA concentrations, depending on the species, the developmental status of the animal at the time of exposure and the dose.[243,244] Early postnatal exposure of mice to non-*ortho* coplanar congeners has been observed to alter cholinergic function.[205,245,246] A decrease in cellular DA concentrations in PC12 cells following exposure to 3,3′,4,4′,5-(PCB 126) has been reported.[247] Although the decrease in DA by PCB 126 in this study was attributed to the cytotoxicity, this implies that the non-*ortho* PCBs could be lethal to cells at concentrations that are neurotoxic for certain *ortho*-substituted PCBs. Coplanar HAHs inhibit estradiol-induced cell clumping in the MCF-7 breast cancer cell line.[248] This suggests that they are antiestrogenic (based on *in vitro* bioassays). This decrease was due to the metabolism of estradiol to 2- and 4-hydroxy estradiol, which alters the synthesis of biogenic amines.[249,250] Similarly, the hydroxy metabolites of PCBs are antiestrogenic,[174] which could alter steroid hormone homeostasis, and consequently affect neurochemical behavior. Hypothalamic brain tissues convert endogenous estrogens to catechol estrogens,[250] which have been shown to inhibit tyrosine hydroxylase, a rate-limiting enzyme for DA synthesis. Therefore, alterations in endogenous estrogen concentrations and metabolism in animals could modulate biogenic amines in brain, which subsequently would lead to neurotoxic effects.

TCDD- and TEQ-related induction of morphometric brain abnormalities has been reported in cormorants and great blue herons following exposure to PCBs, PCDDs, and PCDFs in the environment.[251,252] These results suggested neurotoxic effects due to dioxins and dioxin-like compounds and brain asymmetries as a biomarker for the effects of TCDD-related compounds on neuromorphological development. However, this result could not be duplicated in a later study.[83] In fact, it was concluded that avian brains are inherently asymmetrical and that the effect was not due to exposure to xenobiotics.[33] In any case, the existence of several mechanisms by different groups of compounds for the alterations in neurobehavioral responses imposes challenges to risk assessment for neurotoxicological effects of *ortho*-substituted congeners.

Few studies have examined the effects of *ortho*-substituted PCBs on behavioral alterations or neurotoxic effects in wildlife. Dietary exposure of mink to 2,2′,4,4′,5,5′-HxCB (PCB 153) and 2,2′,3,3′,6,6′-HxCB (PCB 136) at 5 μg/g for over 3 months did not produce significant changes in concentrations of dopamine, norepinephrine, or seratonin in the brain.[253] Di-*ortho* congeners did not elicit reproductive effects in fish. Exposure of di-*ortho* congeners, 2,2′,5,5′-(PCB 52), 2,2′,4,5,5′-(PCB 101), 2,2′,3,3′,4,4′-(PCB 128), 2,2′,3,4,4′,5′-(PCB 138), 2,2′,4,4′,5,5′-(PCB 153), 2,2′,3,4,4′,5,5′-(PCB 180) did not affect survival, growth, or reproduction in the fathead minnow, *Pimephales promelas*,[254] despite their great accumulation in tissues with concentrations, as great as 13 to 183 μg/g, wet wt. However, behavioral effects were not examined in that study. Studies have shown the presence of *ortho*-substituted tetra- through hexa-CB congeners in the brains of fish. While congener 2,2′,4,4′,5,5′-(PCB 153) was the predominant di-*ortho* congener, lesser-chlorinated *ortho*-substituted congeners such as 2,4,4′-(PCB 28) and 2,2′,5,5′-(PCB 52) did not accumulate.[255] Metabolism of slightly chlorinated PCBs has been shown in fish.[256]

Most studies describing neurototoxic effects of PCBs using *in vitro* models have not accounted for toxicokinetics or dose-response relationships. The presence of impurities such as PCDFs and PCNs in technical mixtures of PCBs has also not been

addressed. Moreover, most laboratory studies describing neurotoxic effects of *ortho*-substituted PCBs have used mammalian models. Few studies have examined the effects of *ortho*-substituted PCBs in fish[168] and birds.[257] Dietary exposure of Japanese quail (*Coturnix coturnix japonica*) to Aroclor 1254 at 200 μg/g for 8 days showed suppressed avoidance response.[257] Further studies on the behavioral effects of *ortho*-substituted PCB congeners in fish and other wildlife are needed for risk assessment.

VI. COMPARATIVE EVALUATION OF RISKS OF DIOXIN-LIKE AND NON-DIOXIN-LIKE PCBs: A CASE STUDY — MINK

Mink (*Mustela vison*) are piscivorous mammals that inhabit the margins of aquatic ecosystems and are particularly sensitive to the effects of PCBs.[50,253] Numerous laboratory feeding studies have demonstrated that mink reproduction is disrupted by small amounts of dietary PCBs either as commercial mixtures or as weathered mixtures, physiologically accumulated in fish (Table 6). Relatively more information is available on the RfDs for dietary total PCBs or as TEQs for mink than any other wildlife species.[82,258–264] Similarly, effects of dietary exposure to *ortho*-substituted PCB congeners, 2,2′,4,4′,5,5′-(PCB 153) and 2,2′,3,3′,6,6′-(PCB 136) on biogenic amines such as norepinephrine, dopamine, and seratonin in different parts of the brain of mink has been reported.[253] This provides an opportunity to evaluate and compare the risks of dioxin-like and non-dioxin-like PCBs in mink. In this exercise, we have estimated and compared the hazard associated with PCB exposure in mink based on total PCBs — both as Aroclor technical mixtures and as weathered mixtures, dioxin-like PCBs and non-dioxin-like, *ortho*-substituted PCBs (*o*-PCBs). The issue is not whether these effects occur, but that they can cause effects at lesser concentrations than the mass of total PCBs than do the dioxin-like responses.

A. Mink Reference Doses for Total PCBs, TEQs, and *Ortho*-PCBs

The LOAEC, NOAEC, or EC_{50} values derived for various toxic endpoints in mink following exposure to technical PCB mixtures, individual congeners, or contaminated diet as weathered PCBs have been compiled in order to derive RfDs for a comparative assessment of dioxin-like and non-dioxin-like effects (Table 6). The effective concentrations of a weathered mixture of PCBs in the diet was less than that of total PCBs based on Aroclor technical mixtures. The dietary 28-day LC_{50} of Aroclor 1254 in mink was 79 μg/g, wet wt, expressed as total PCBs in the diet.[259] Aroclor 1254 was also exposed secondarily by feeding tissues of Aroclor 1254-exposed rabbits to mink at similar daily dose ranges. The LC_{50} for Aroclor 1254 fed as a weathered mixture in rabbit tissues was 47 μg/g, wet wt. This suggests that PCBs of which the pattern of relative concentrations has been altered by metabolism are more toxic than the original mixtures when exposed as a total mass basis. Nevertheless, the differences in the LC_{50}s for minks due to Aroclor 1254 exposure and the rabbit diet contaminated by Aroclor 1254 could also be influenced by the method of PCB quantitation in the diet (such as the number of peaks selected). In any case, several other studies have found that weathered PCBs in the diet are more toxic than technical PCB mixtures.[50,77] Thus, the RfDs derived from exposure to technical mixtures may underestimate the actual risks. This observation is the primary reason that TEQs have been reported to be a more accurate predictor of adverse effects of PCB mixtures. Also, the types of effects observed are those that are known to be caused by the dioxin-like PCB congeners through an AhR-mediated process. It is thought that the weathering process selectively removes congeners with less toxic

TABLE 6
LOAEC, NOAEC or EC_{50} Values for Toxic Effects of Dietary Exposure of Commercial PCB Mixtures or Congeners in Mink

PCB mixture/congener	NOAEC, LOAEC or EC_{50}	Ref.
Commerial mixture in diet		
Aroclor 1016[a]	LOAEC = 2 μg/g	279
Aroclor 1254[b]	NOAEC = <1 μg/g	258
Aroclor 1254	LOAEC = 0.1 mg/kg/d or 1 μg/g in diet	280
Aroclor 1254	LC_{50} = 79 μg/g (28 d)	259
3,3′,4,4′,5,5′-(PCB 169)	LD_{50} = 0.05 μg/g; NOAEC = 0.01 μg/g	260
2,2′,3,3′,6,6′-(PCB 136)[c]	LOAEC = 5 μg/g	253
2,2′,4,4′,5,5′-(PCB 153)[c]	LOAEC = 5 μg/g	253
2,3,7,8-TCDD	LD_{50} = 4.2 ng/g, bw	263
Weathered PCBs/TEQs		
Aroclor 1254-fed rabbit diet	LC_{50} = 47 μg/g (28 d)	259
Contaminated fish diet total PCBs	NOAEC = 72 ng/g	50
Contaminated fish diet TEQs	NOAEC = 0.3 pg/g or 0.08 ng/kg bw/d	77
Contaminated fish diet TCDD-EQs (H4IIE-bioassay derived)	NOAEC = 2 pg/g or 0.54 ng/kg bw/d	50
Body residues based PCBs/TEQs		
All technical PCB mixtures[d]	Relative litter size EC_{50} =1.2 μg/g; Kit survival EC_{50} = 2.4 μg/g	82
TEQs[d]	Relative litter size EC_{50} = 0.16 ng/g; Kit survival EC_{50} = 0.20 ng/g	82

[a] Aroclors 1242 and 1254 caused significant reproductive failure at 2 μg/g in the diet.
[b] Assessment based on several studies reported by Aulerich and co-workers.
[c] No effect on survival and reproduction, but slightly altered brain dopamine concentrations.
[d] Estimated based on several technical mixture-exposure studies, and the values were derived based on a bioaccumulation model.

potency, which results in a greater toxic potency of the mixture when exposure is normalized to a weathered total PCB. Generally it has been found that the ratio of TEQs to total mass of PCBs increase with trophic level.[59,265–267]

B. Hazard Evaluation

The relative hazard of concentrations of total PCBs, TEQs, and *o*-PCBs to mink was determined by calculating hazard quotients (HQ) by dividing the concentrations in mink diet by an estimated NOAEC. Generally, a value of 1.0 would indicate that the populations were just at the threshold for adverse effects. Hazard quotient values greater than 1.0 would indicate a probability that adverse effects could be caused by exposure to such concentrations of PCBs. Population-level effects are generally not observed until the HQ is between 10 and 20, depending on the acute to chronic ratio (ACR) and slope of the

dose-response relationships. However, our primary intention is to compare HQs derived for total PCBs, TEQs and *o*-PCBs to evaluate the relative importance of these values in risk assessment strategies and to assess the relative importance of *ortho*- and non-*ortho*-substituted PCBs in eliciting toxic responses in wild populations. Because this exercise was intended to be used for comparison rather than protection, the absolute HQ values would not be the final values applied in a risk assessment.

Until now, most hazard assessment for mink has been based on total PCBs. Furthermore, few studies have measured the concentrations of *ortho*-substituted congeners in mink tissues. Due to the lack of data on the complete profile of PCB congener distribution in mink, we adopted a diet-specific biomagnification model in which the biomagnification factor (BMF) for individual PCB congeners multiplied by the concentration in prey species (diet) is used. BMF values for a few congeners have been reported,[77] and additional congener-specific BMFs have been developed for another mustelid, the otter (*Lutra lutra*) from a field study.[239] Because mink belong to the same family as otters (Mustelidae) and have similar food habits (piscivores), BMFs from these studies were used to predict concentrations in mink. The exercise presented here is purely heuristic and the use of predicted concentrations reduced uncertainty without compromising accuracy. Development of site-specific BMFs, using the PCB congener data measured in fish, would improve the accuracy of risk assessments.

For demonstrative purposes, congener-specific concentrations of PCBs reported for middle-aged (5 to 9 years) carp, *Cyprinus carpio*, from the Buffalo River, New York,[63] was used to estimate the relative risks. As mentioned earlier, this exercise has been intended to examine the relative risks of non-*ortho* and *ortho*-PCBs and therefore details on the consumption of carp by mink or the occurrence of mink in the Buffalo River area are not considered. Therefore, it is assumed that mink are present in the Buffalo River and feed on carp from the Buffalo River. Because the congener-specific data for PCBs in mink were not available, it was estimated based on BMFs reported elsewhere.[77,239] The mean concentration of the sum of major PCB congeners in the whole carp from the Buffalo River was 3600 ng/g, wet wt (Table 7).[63] Concentrations of individual congeners in mink were derived based on the assumption that carp were the sole source of PCB exposure to mink. A mean total PCB concentration of 59,300 ng/g, wet wt, was derived for mink. The BMFs for lesser-chlorinated PCB congeners are small due to metabolism and excretion in predatory species.[82,239] The concentrations of all di- through tetra-*ortho*-substituted congeners were summed to estimate the risk due to *o*-PCBs. Total concentrations of di- through tetra-*ortho* substituted congeners in fish and mink were 2970 and 55,900 ng/g, respectively, which accounted for 83 and 94% of the total PCB concentrations, respectively. Despite the lesser concentrations of di-*ortho* PCBs in fish, greater BMFs of congeners, such as PCB 153, PCB 138, PCB 170, and PCB 180, contributed to the greater proportion of di-*ortho* congeners in total PCBs in mink tissues. Non-*ortho* PCBs, 3,3′,4,4′-(PCB 77), 3,3′,4,4′,5-(PCB 126), and 3,3′,4,4′,5,5′-(PCB 169) were also detected in fish (Table 7). Based on the WHO-TEFs, TEQs for non- and mono-*ortho* congeners in fish and mink were estimated, which were 57 and 1730 pg/g, wet wt, respectively (Table 8).

Several studies have involved exposing mink to PCBs under laboratory conditions or inferred the effect concentrations from exposure to PCB that were weathered and occurred with other toxicants in fishes. Concentrations of 0.64 µg/g, wet wt, in the fish-containing diet or 0.66 µg/g, wet wt, of Aroclor 1254 caused complete reproductive failure in mink.[268] The LOAEC, based on

TABLE 7
Congener Specific Concentrations (ng/g, wet wt) of PCBs in Carp from the Buffalo River, New York, and the Values Calculated for Mink Based on Biomagnification Factors (BMFs)[a]

IUPAC No.	Structure	BMF[b]	Carp	Mink[c]
TriCB				
19	2,2′,6-	0.05	2.7	0.1
18	2,2′,5-	0.05	53	2.7
17	2,2′,4-	0.05	15	0.8
24	2,3,6-	0.05	8.9	0.4
16/32	2,2′,3-/2,4′,6-	0.05	43	2.2
34	2′,3,5-	0.05	12	0.6
28/31	2,4,4′-/2,4′,5-	0.05	140	7.0
20	2,3,3′-	0.05	15	0.8
33	2′,3,4-	0.05	21	1.1
TetraCB				
53	2,2′,5,6′-	0.02	27	0.54
51	2,2′,4,6′-	0.02	19	0.38
52	2,2′,5,5′-	0.02	125	2.5
49/69	2,2′,4,5′-/2,3′,4,6-	0.02	92	1.8
47	2,2′,4,4′-	0.02	62	1.2
44	2,2′,3,5′-	0.02	74	1.5
42	2,2′,3,4′-	0.02	84	1.7
41/64	2,2′,3,4-/2,3,4′,6-	0.02	76	1.5
40	2,2′,3,3′-	0.02	18	0.4
58/74	2,3,3′,5′-/2,4,4′,5-	0.02	41	0.8
70	2,3′,4′,5-	0.02	77	1.5
66	2,3′,4,4′-	0.02	83	1.7
60	2,3,4,4′-	0.02	41	0.8
PentaCB				
91/95	2,2′,3,4′,6-/2,2′,3,5′,6-	9	96	860
102	2,2′,4,5,6′-	9	21	190
84/92/90	2,2′,3,3′,6-/2,2′,3,5,5′-/2,2′,3,4′,5-	9	120	1080
101	2,2′,4,5,5′-	0.07	155	11
99	2,2′,4,4′,5-	9	92	830
83	2,2′,3,3′,5-	9	22	200
97/113	2,2′,3′,4,5-/2,3,3′,5′,6-	9	34	310
87/117	2,2′,3,4,5′-/2,3,4′,5,6-	9	53	477
85	2,2′,3,4,4′-	9	28	250
118	2,3′,4,4′,5-	15	135	2000
105	2,3,3′,4,4′-	12	60	720
HexaCB				
136	2,2′,3,3′,6,6′-	12	21	250
151	2,2′,3,5,5′,6-	12	57	680
135	2,2′,3,3′,5,6′-	12	30	360
144/149	2,2′,3,4,5′,6-/2,2′,3,4′,5′,6-	12	170	2000
132	2,2′,3,3′,4,6′-	12	46	550
128	2,2′,3,3′,4,4′-	12	50	600
153	2,2′,4,4′,5,5′-	15	310	4650
141	2,2′,3,4,5,5′-	12	40	480
137	2,2′,3,4,4′,5-	12	17	200
138	2,2′,3,4,4′,5′-	26	260	6760
156	2,3,3′,4,4′,5-	30	20	600

TABLE 7
Congener Specific Concentrations (ng/g, wet wt) of PCBs in Carp from the Buffalo River, New York, and the Values Calculated for Mink Based on Biomagnification Factors (BMFs)[a]

IUPAC No.	Structure	BMF[b]	Carp	Mink[c]
HeptaCB				
176	2,2′,3,3′,4,6,6′-	14	42	590
178	2,2′,3,3′,5,5′,6-	14	31	430
187	2,2′,3,4′,5,5′,6-	14	62	870
177	2,2′,3,3′,4′,5,6-	14	47	660
173	2,2′,3,3′,4,5,6-	14	29	410
180	2,2′,3,4,4′,5,5′-	123	230	28300
170	2,2′,3,3′,4,4′,5-	15	75	1130
OctaCB				
198	2,2′,3,3′,4,5,5′,6-	21	53	1110
201	2,2′,3,3′,4,5′,6,6′-	21	45	950
194	2,2′,3,3′,4,4′,5,5′-	21	36	760
Coplanar PCBs				
77	3,3′,4,4′-	1.4	2.3	3.2
126	3.3′,4,4′,5-	70	0.15	11
169	3,3′,4,4′,5,5′-	348	0.03	10
Total			**3600**	**59300**

[a] From Reference 63.
[b] From Reference 239. When BMF for individual congeners were not available, a mean BMF for the homolog group was calculated and used.
[c] Values have been rounded.

survival and growth of kits was found to be 0.72 μg/g, wet wt, when carp from Saginaw Bay, Michigan, was fed to mink.[75,76] The NOAEC was estimated from the LOAEC by using a 10× safety factor.[42,269] This NOAEC takes into account of weathering of the PCBs and thus is more directly comparable to total concentrations of PCBs in the diet than would be a NOAEC derived from exposures to technical mixtures of PCBs. We have selected a value of 72 ng/g, wet wt, expressed as total weathered and physiologically altered PCBs in the diet as the best estimate of the NOAEC. Similarly, a NOAEC for dietary TEQs were based on a study involving exposure of mink to weathered PCBs from the diet and a value of 0.3 pg/g, wet wt, was derived.[77]

Reproductive toxicity of PCBs in mink was reviewed recently.[82] Dose-response relationships were examined between litter size and/or kit survival and technical Aroclor mixtures in food. However, when all data are plotted, the slope of the curves were abrupt so that no dose-response relationship could be discerned. Because the congener composition of commercial Aroclor mixtures has been reported, the corresponding TEQs can be calculated using TEFs. Replotting of the relative litter size data on a TEQ basis provided a steep but discernible dose-response curve.[82] These results suggested that the toxicity of PCBs is dependent on the congener composition of the mixture and is not well described by the total PCB exposure. Due to the lack of appropriate dose-response relationships for total PCBs in the diet,[82] accurate RfDs for Aroclor mixtures were difficult to predict. However, from the plot of litter size and kit survival to PCB concentrations in the diet, a LOAEC of 2 μg/g was selected. This was done based on the observations that indicated kit survival and litter size at 2 μg/g in the diet. A NOAEC of 200 ng/g was derived for commercial PCB

TABLE 8
TEQs of Non- and Mono-*ortho* PCBs (pg/g, wet wt) in Carp and Mink[a]

IUPAC no.	Structure	TEF[b]	Carp	Mink
Non-*ortho*				
77	3,3′,4,4′-	0.0001	0.23	0.32
126	3,3′,4,4′,5-	0.1	15	1050
169	3,3′,4,4′,5,5′-	0.01	0.3	104
Mono-*ortho*				
60	2,3,4,4′-	0.0001	4.1	0.08
66	2,3′,4,4′-	0.0001	8.3	0.17
105	2,3,3′,4,4′-	0.0001	6.0	72
118	2,3′,4,4′,5-	0.0001	13.5	203
156	2,3,3′,4,4′,5-	0.0005	10	300
Total			**57**	**1730**

[a] Mink concentrations were calculated by multiplying fish concentration with BMFs from Reference 239.
[b] Reference 99; TEFs for IUPAC Nos. 60 and 66 were assigned as that of 105.

mixtures, from the LOAEC by using a 10× safety factor.[42]

RfDs based on critical body residues were also derived using BMF values to estimate whole-body concentrations in mink from concentrations in food. Critical body residues (EC_{50}) for mink litter size of 1.2 μg/g for total PCBs and 160 pg/g for TEQs have been proposed.[82]

RfDs for neurotoxic effects of *ortho*-substituted PCBs were derived from a study that exposed mink to 2,2′,4,4′,5,5′-HxCB (PCB 153) and 2,2′,3,3′,6,6′-(PCB 136) each at 2.5 and 5 μg/g in the diet for over 3 months and measured brain biogenic amines such as norepinephrine, dopamine, and seratonin. No significant effect was observed for either of the *o*-PCBs at a dietary concentration of 5 μg/g, although there was a slight alteration in biogenic amine concentrations.[253] Thus, a dietary LOAEC of 5 μg *o*-PCBs/g was derived for brain neurotransmitter effects. A correction factor of 10× (most conservative) was applied to derive a RfD of 500 ng/g in the diet. It may be argued that the lesser-chlorinated *o*-PCB congeners are more potent neurotoxicants based on the results from *in vitro* studies using PC12 cells, and that the RfDs derived for di-*ortho*-substituted hexachlorobiphenyls may not be appropriate (Table 4). However, lesser-chlorinated *o*-PCB congeners were not accumulated in mink due to their lesser BMFs relative more chlorinated congeners.

Hazard quotients were estimated for total PCBs as weathered and technical mixtures, TEQs, and *ortho*-PCBs in mink (Table 9). Hazard due to non-*ortho* PCBs estimated as TEQs was 31-fold greater than those due to *o*-PCB congeners in mink. As expected, the hazard due to total PCBs estimated as weathered mixtures was greater than that estimated for non-weathered commercial mixtures, which is due to the enrichment of AhR active congeners in the diet. This may suggest that dioxin-like PCB congeners are the critical contaminants driving risk assessment of PCBs in wildlife populations. The reference doses for non-dioxin-like PCBs were not available for effects in neonatal animals. Due to the great abundance of *o*-PCB congeners in technical PCB mixtures (Table 5), their exposure is much greater than non-*ortho* congeners. *o*-PCBs

TABLE 9
Hazard Quotients (HQ) for Total PCBs, TEQs and Di- through Tetra-*ortho* PCBs in Mink Based on Concentrations in the Diet

Compound	NOAEC	HQ
Total PCBs (weathered)	72 ng/g	50
Total PCBs (technical mixtures)	200 ng/g	18
TEQ	0.3 pg/g	190
Di- through tetra-*ortho* PCBs	500 ng/g	5.9

in technical mixtures are at least 2 orders of magnitude greater in abundance than non- and mono-*ortho* PCBs. On the other hand, the AhR-binding potencies of non-*ortho* PCBs are at least 2 orders of magnitude stronger than the binding potentcies estimated for *o*-PCBs using an *in vitro* model that used [^{3}H] phorbol binding in cerebellar granule cells as a measure of neurotoxicity (Table 10). *o*-PCBs are neurotoxic at acute exposures such as those observed in Yusho and Yu-Cheng. At acute exposure scenarios, *o*-PCBs may cross blood-brain and/or placental barriers, which could lead to neurotoxic effects. However, neurotoxic effects following sub-chronic, real-world exposures are not clearly understood. Due to the lack of determinable structure-activity relationships,[270] the risk assessment for *o*-PCBs that cause neurotoxic effects need further validation. Nevertheless, it appears that neurotoxic effects occur only at relatively great exposures and it is unlikely that the neurotoxic effects of PCBs would drive risk assessment. Reproductive effects of PCBs in several wildlife species appear to be largely AhR dependent and occur at the least total PCB conentrations, which could be best predicted on a TEQ basis. Although the TEF approach provides a convinient and scientifically defensible means to evaluate ecological risks of PCBs on a congener-specific basis, it must be used with considerable caution. It is considerd that the TEF approach is a useful normalization technique that corrects for environmental weathering and provides a relatively more accurate predictions of hazard in wildlife.

VII. CONCLUSIONS

The complex nature of PCB mixtures complicates the risk evaluation for humans, fish, and wildlife.[284] In order to evaluate risks due to PCBs, a fundamental understanding of the mechanism of action is a prerequisite. At present, sufficient evidence is available that there is a common mechanism for non- and mono-*ortho* PCB congeners, involving binding to the Ah-receptor as an initial step. When applying the TEF concept, the toxicity of these coplanar congeners relative to that of 2,3,7,8-TCDD is determined on the basis of available *in vivo* or *in vitro* data. However, it should also be understood that the TEF concept is based on a number of assumptions and has limitations.

Studies have also shown that apart from non- and mono-*ortho* PCBs, *ortho*-substituted nonplanar PCB congeners elicit neurotoxic effects in exposed animals and in cell cultures. Although a well-defined TEF has not been derived for nonplanar PCB congeners, it appears that at greater exposures these congeners may cause neurotoxic effects in humans or wildlife. Therefore, for a complete evaluation of risks due to PCBs, consideration of the effects of both *ortho*- and non-*ortho*-substituted congeners are needed.

Based on an example, using mink as a model, it was found that the hazard quotients

TABLE 10
EC_{50} Values for Ah Receptor Binding, AHH- and EROD-Induction Potencies of Certain Planar, Mono-, and Di-*ortho*-Substituted PCB Congeners in Rat Hepatoma Cells[a]

Congener/PCB mixture	IUPAC no.	Receptor binding affinity (μM)	AHH (μM)	EROD (μM)
Non-*ortho* congeners				
3,3′,4,4′-	77	0.43	0.035	0.089
3,3′,4,4′,5-	126	0.12	0.00024	0.00025
3,3′,4,4′,5,5′-	169	na	0.06	0.024
Mono-*ortho* congeners				
2,3,3′,4,4′-	105	4.3	0.088	0.12
2,3,4,4′,5-	114	4.1	0.97	0.57
2,3,3′,4,4′,5-	156	7.1	2.1	0.9
2′,3,4,4′,5-	123	1.4	3.9	1.1
2,3,3′,4,4′,5′-	157	5.0	0.71	1.3
2,3,4,4′-	60	28	na	na
2,3′,4,4′,5-	118	9.1	12	8.9
2,3′,4,4′,5,5′-	167	16	13	9
Di-*ortho* congeners				
2,2′,4,4′,5,5′-	153	79	na	na
2,2′,4,4′-	47	130	na	na
PCB mixtures				
Aroclor 1242		na	320 (84 mg/kg)	1320 (346 mg/kg)
Aroclor 1248		na	180 (51 mg/kg)	870 (251 mg/kg)
Aroclor 1254		6.0	280 (92 mg/kg)	420 (137 mg/kg)
Aroclor 1260		na	920 (343 mg/kg)	1190 (442 mg/kg)

Note: na: Data not available.

[a] From References 103, 281, 282.

(HQs) (Table 9) of dioxin-like PCBs were greater than those of non-dioxin-like PCBs, indicating that the coplanar PCBs are critical in the risk assessment of PCBs. Nevertheless, it should be noted that mink are sensitive to reproductive effects of PCBs,[285,286] and therefore the effects due to coplanar PCBs have been critical. Further, the RfDs derived for non-dioxin-like effects of *ortho*-PCBs in mink were based on adult exposure. Because developing organisms are more sensitive to neurotoxic effects of *ortho*-PCBs, RfDs from developmental exposures (pre- and/or perinatal) is necessary. However, RfDs for the neurotoxic effects of *ortho*-PCBs are not available for mink or other wildlife. Further studies are needed to derive RfDs for neurotoxic effects of *ortho*-PCBs in wildlife. In any case, laboratory exposure studies with rodents and other mammals and *in vitro* bioassays have indicated that the neurotoxic effects have occurred only at great exposures. Therefore, it is considered that TEQs for dioxin-like PCBs are critical in setting environmental quality criteria. In other words, establishment of threshold limits for PCBs based on dioxin-like effects would be able to protect the animals from non-dioxin-like effects. The following

points summarize the issues regarding the risk assessment of PCBs:

- Hazard assessment based on total PCBs as technical mixtures are generic and generally underestimate the actual risks of weathered PCB mixtures. Estimates of risks based on weathered mixture of PCBs is more realistic, but this approach involves several confounding parameters.
- *In vitro* bioassays with recombinant cell lines are integrative tools for the risk assessment of dioxin-like compounds and are inexpensive and rapid but cannot identify the risk due to specific compounds. *In vitro* bioassay-derived TCDD-EQs can be used in conjunction with instrumentally derived TEQs to identify various interactive effects of planar HAHs. The results of bioassays can be compared to instrumentally derived TEQs in a mass balance approach to determine if all of the TEQ had been accounted for. This approach would allow us to identify other unidentified dioxin-like contaminants that act through the AhR-mediated mechanisms.
- Reproductive effects of PCBs in birds and fish-eating mammals appear to be largely AhR-dependent and best predicted on a TEQ basis by the TEF approach. Internationally accepted TEFs are available for birds, fish, and mammals for use in risk assessment. Uncertainties in TEFs due to endpoint, life stage, species, sex, etc. can result in the use of uncertainty factors in deriving TEQs for use in risk assessments. This approach often leads to very conservative maximum allowable toxicant concentrations (MATC). Consensus TEFs are selected to be protective and thus include a degree of conservation. If species-specific TEFs are available they should be used.
- The relative sensitivity of the four exposures of PCBs (total PCBs-as technical mixtures, weathered total PCBs — as contaminated diet, TEQs, and *ortho*-PCBs) ranged by about a factor of 10, which is a relative uncertainty compared with other uncertainties in the risk assessment process. The relative hazard quotients (HQ) derived for these exposures to the sensitive species, mink, were 1:0.26:0.10:0.03 for TEQs: total PCBs-weathered: total PCBs-technical Aroclors: *ortho* PCBs (Table 9).
- The neurobehavioral effects of *ortho*-substituted PCBs are unlikely to occur at concentrations of weathered PCBs that do not cause AhR-mediated effects. Neurotoxic effects have occurred at great exposures, while dioxin-like effects due to coplanar PCBs have occurred at small concentrations.
- TEQs derived for dioxin-like effects are the critical parameters for the risk assessment of PCBs, that is, the least concentration of total weathered PCBs would be allowed based on the presence of TEQs.

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REFERENCES

1. **Hutzinger, O., Safe, S., and Zitko, V.**, *The Chemistry of PCBs*. CRC Press, Boca Raton, FL, 1974, 269.
2. **Safe, S. and Hutzinger, O.**, *Polychlorinated Biphenyls (PCBs): Mammalian and Environmental Toxicology*. Environmental Toxin Series, Vol. I., Springer-Verlag, Berlin. 1987, 152.
3. **de Voogt, P. and Brinkman, U. A. Th.**, Production, properties and usage of polychlorinated biphenyls. In: *Halogenated Biphenyls and Related Products*, 2nd ed., Kimbrough, R. D. and Jensen, A. A., Eds., Elsevier, Amsterdam, The Netherlands, 1989, pp. 3–45.
4. **Mullin, M. D., Pochini, C. M., McCrindle, S., Romkes, M., Safe, S. H., and Safe, L. M.**, High-resolution PCB analysis: synthesis and chromatographic properties of all 209 PCB congeners, *Environ. Sci. Technol.*, 18, 468, 1984.
5. **Bletchly, J. D.**, Polychlorinated biphenyls: production, current use and possible rate of future disposal in OECD member countries. In: *Proceedings of PCB Seminar*. Barros, M. C., Koemann, H., and Visser, R., Eds., Ministry of Housing, Physical Planning and Environment, The Netherlands, 1984, pp. 343–372.
6. **Tanabe, S.**, PCB problems in the future: foresight from current knowledge, *Environ. Pollut.*, 50, 5, 1988.
7. **Ivanov, V. and Sandell, E.**, Characterization of polychlorinated biphenyl isomers in Sovol and Trichlorodiphenyl formulations by high resolution gas chromatography with electron capture detection and high-resolution gas chromatography-mass spectrometry techniques, *Environ. Sci. Technol.*, 26, 2012, 1992.
8. **Ballschmiter, K. and Zell, M.**, Analysis of polychlorinated biphenyls (PCB) by glass capillary gas chromatography, *Fresenius Z. Anal. Chem.*, 302, 20, 1980.
9. **Erickson, M. D.**, *Analytical Chemistry of PCBs*. Lewis Publishers, Boca Raton, Florida, 1997, 667.
10. **Jensen, S.**, Report of a new chemical hazard, *New Scientist*, 32, 612, 1966.
11. **Risebrough, R. W., Rieche, P., Peakall, D. B., Herman, S. G., and Kirven, M. N.**, Polychlorinated biphenyls in the global ecosystem, *Nature*, 220, 1098, 1968.
12. **Atlas, E. and Giam, C. S.**, Global transport of organic pollutants: ambient concentrations in remote marine atmosphere, *Science*, 211, 163, 1981.
13. **Tanabe, S., Hidaka, H., and Tatsukawa, R.**, PCBs and chlorinated hydrocarbon pesticides in Antarctic atmosphere and hydrosphere, *Chemosphere*, 12, 277, 1983.
14. **Mackay, D., Shiu, W. Y., and Ma, K. C.**, Monoaromatic hydrocarbons, chlorobenzenes and PCBs. In *Illustrated Handbook of Physicochemical Properties and Environmental Fate for Organic Chemicals*, Vol. 1, Lewis Publishers, Chelsea, MI, 1992.
15. **Loganathan, B. G. and Kannan, K.**, Global organochlorine contamination trends: an overview, *Ambio*, 23, 187, 1994.
16. **Waid, J. S.**, *PCBs and the Environment*. Volumes I to III, CRC Press, Boca Raton, Florida, 1987.
17. **Schulz, D. E., Petrick, G., and Duinker, J. C.**, Complete characterization of polychlorinated biphenyl congeners in commercial Aroclor and Clophen mixtures by multidimensional gas chromatography-electron capture detection, *Environ. Sci. Technol.*, 23, 852, 1989.
18. **McFarland, V. A. and Clarke, J. U.**, Environmental occurrence, abundance, and potential toxicity of polychlorinated biphenyl congeners: considerations for a congener-specific analysis, *Environ. Health Perspect.*, 81, 225, 1989.
19. **Kannan, K., Maruya, K., and Tanabe, S.**, Distribution and characterization of polychlorinated biphenyl congeners in soil and sediments from a Superfund site contaminated with Aroclor 1268, *Environ. Sci. Technol.*, 31, 1483, 1997.
20. **Kimbrough, R. D. and Jensen, A. A.**, *Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins and Related Products*, 2nd edition, Elsevier, Amsterdam, The Netherlands, 1989.
21. **World Health Organization**, *Environmental Health Criteria 140. Polychlorinated Biphenyls and Terphenyls*, 2nd edition, WHO, Geneva, 1993, 682.
22. **Delzell, E., Doull, J., Giesy, J. P., Mackay, D., Munro, I., and Williams, G.**, Interpretive review of the potential adverse effects of chlorinated organic chemicals on human health and environment, *Reg. Toxicol. Pharmacol.*, 20, 1, 1994.