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Workshop Report on Toxicity Equivalency Factors for Polychlorinated Biphenyl Congeners

RISK ASSESSMENT FORUM

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Workshop Report on Toxicity Equivalency Factors for Polychlorinated Biphenyl Congeners

Assembled by:

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for the
Risk Assessment Forum

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This workshop was organized by Eastern Research Group, Inc., Arlington, Massachusetts, for the EPA Risk Assessment Forum. ERG also prepared and produced this workshop report. As requested by EPA, this summary report captures the main points of each scheduled presentation and highlights from the general discussion; the report is not a complete record of all details discussed. Relevant portions were reviewed by each workshop chairperson and speaker. Their time and contributions are gratefully acknowledged. The views presented are those of each contributor, not the U.S. Environmental Protection Agency.

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WORKSHOP REPORT ON TOXICITY EQUIVALENCY FACTORS FOR POLYCHLORINATED BIPHENYL CONGENERS

December 11-12, 1990
Washington, D.C.

INTRODUCTION

The purpose of the workshop was to examine the existing toxicity and exposure database on polychlorinated biphenyls (PCBs) to ascertain the feasibility of developing toxicity equivalency factors (TEFs) for PCB congeners. Given the widespread acceptance and acknowledged utility of the TEF method for assessing risks associated with exposures to complex mixtures of chlorinated dibenzo-p-dioxins and dibenzofurans, some experts have urged development of comparable TEF schemes for other structurally related chemicals, such as PCBs. Information from the workshop will contribute to Risk Assessment Forum recommendations on whether to pursue development of a TEF scheme for PCBs.

EPA's Risk Assessment Forum assembled approximately 30 experts in the fields of PCB toxicity and mechanisms of action, environmental exposure, and analytical methods for measuring PCBs in human and environmental samples. The agenda for the meeting can be found in Appendix A. Dr. Donald Barnes chaired the workshop. After presentations by Dr. Barnes and Dr. Stephen Safe, the participants divided into two work groups: the Work Group on Exposure/ Analytical Issues, chaired by Ms. Ann Alford-Stevens; and the Work Group on Toxicity/Mechanism of Action Issues, chaired by Dr. Linda Birnbaum. These groups discussed the following questions (outlined in more detail in Appendix B).

- Is the existing database on toxicity and mechanisms of action sufficient to support a TEF scheme for the PCBs?
- What is known about environmental exposures to specific PCB congeners?
- What analytical methods are available to identify and quantify individual congeners in environmental matrices?

- What are the important data gaps and what research is needed to fill them?

On the second day of the workshop, all participants reconvened and the work group chairs led the discussion of each group's findings and recommendations. Dr. Barnes closed the meeting with a summary of the workshop's conclusions and recommendations.

The remainder of this report contains summaries of the presentations given by Dr. Barnes, Dr. Safe, Ms. Alford-Stevens, and Dr. Birnbaum. A summary of the final discussion is also included. In addition, the following information can be found in the Appendices:

Appendix A	Agenda
Appendix B	Discussion Initiation Issues
Appendix C	List of Participating Scientists
Appendix D	List of Observers
Appendix E	Work Group Members

SUMMARY OF PRESENTATIONS

Opening Remarks
Donald Barnes, Workshop Chair

This workshop is one in a series of meetings to provide information and exchange ideas on toxicity equivalency factors (TEFs) for polychlorinated biphenyls (PCBs). While 1970s regulations banning PCBs were expected to end PCB exposure, the half-life of PCBs is so long that PCBs are still found throughout our environment. PCB mixtures found in the environment today, however, are not the same PCB mixtures that were released into the environment; they have changed over time. The PCB congeners in the environment, which are different from the original commercial mixtures, are the PCBs of interest to risk assessors and managers. (Risk assessment, not risk management, will be emphasized in the workshop.)

The TEF approach is a numerical procedure based upon scientific data and scientific judgment; it cannot be proven to be absolute and exact in a fundamental, scientific way. Nevertheless, by using the best scientific judgment based upon all available scientific data, TEFs can provide an interim procedure to reasonably assess the risk of mixtures of structurally related compounds. In addition, the use of TEFs allows the results of analysis of complex mixtures to be expressed in a common unit.

The use of TEFs was first considered in the late 1970s and early 1980s when data indicated consistent relative toxicity of different congeners of chlorinated dibenzo-p-dioxins and chlorinated dibenzofurans (CDDs/CDFs) when compared to an index chemical (i.e., 2,3,7,8-tetrachlorodibenzo-p-dioxin [TCDD]). This consistency across toxic end points indicated that a TEF could be used to evaluate complex mixtures and express them as a common measure. Subsequently, the TEF approach has been used successfully to estimate risks associated with CDDs/CDFs in a wide variety of environmental samples. The Science Advisory Board (SAB), with less than great enthusiasm, concurred in the use of TEFs for CDDs/CDFs, but indicated that it should be an interim procedure for no more than 2 to 5 years. In 1987, EPA adopted the use of TEFs on an interim basis. Since then, EPA and a number of regulatory groups, both in the U.S. and in countries around the world, have used the TEF concept on CDDs/CDFs because it provides a mechanism whereby one can summarize the toxicity of complex mixtures, and make risk management judgments. Nevertheless,

the SAB's 5-year time limit is fast approaching and the more definitive methods of assessing the toxicity of these complex mixtures remain beyond our grasp.

One aspect of an interim procedure is that it is modified as new data become available. The TEFs adopted by EPA in 1987 for CDDs/CDFs (so called "EPA-TEFs/87") were modified in 1989 through an international consensus (so called international TEFs, or I-TEFs/89). I hope that no further modifications will need to be made before the whole procedure is replaced by a more definitive approach.

Because people have been discussing the application of TEFs to polycyclic aromatic hydrocarbons (PAHs), metals (as a group), and many other combinations of chemicals, an Agency group proposed seven guiding criteria for the successful application of TEFs to any given complex mixture.

1. A demonstrated need. An interim TEF procedure should not be used unless there is a clear need to do so. For example, 15 or 20 years ago 2,3,7,8-TCDD was the only known "dioxin," but in the late 70s, it became increasingly clear that CDDs/CDFs were a complex mixture of over 200 chemicals that needed to be addressed in toto. There was a clear regulatory need, a clear public policy need, as well as a toxicological need to use TEFs. The issue is whether or not there is a demonstrated need for PCBs.

2. A well-defined group of chemicals. There are 210 CDDs/CDFs. There are 209 PCBs, so they, too, constitute a well-defined group of chemicals.

3. A broad base of toxicological data. Large quantities of data first attracted attention to the possibility of TEFs for CDDs/CDFs. Tens of millions of dollars have been spent on the analysis and toxicity of CDDs/CDFs; comparable amounts of money have been spent on PCBs. Whether or not the array of toxicological data is comparable in the two cases is something that the Toxicology Work Group will discuss.

4. Consistency in the relative toxicity of congeners across toxicological end points, both in vivo and in vitro. This consistency in relative toxicity is critical in determining the applicability of TEFs. The Toxicology Work Group will examine whether or not such consistency across toxic end points exists for the PCB congeners.

5. Demonstrated additivity between the toxicity of individual congeners. The use of TEFs implicitly presumes additivity, so there should be some evidence that additivity is a reasonable assumption for the group of chemicals in question, in this case PCBs.

6. Some mechanistic rationale as to why TEFs would be applicable to a particular group of chemicals. In the area of CDDs/CDFs, for example, the common mechanism relates to a receptor mediating a panoply of different toxicities, which are sometimes known as "dioxin-like" toxic end points. Can such a common mechanism be found for PCBs?

7. Some method of gaining a consensus as to what the TEFs ought to be. One problem is that sometimes different groups use different TEFs, generating different risk numbers. As a result, scientists have a difficult time comparing assessments made by different groups. In the case of TEFs for the CDDs/CDFs, a mechanism was developed for achieving international consensus. The need for such an international consensus for TEFs for PCBs lies in the future. However by maintaining open communications through meetings like this, we hope to be able to move along together to reach that consensus, when appropriate.

In the plenary session, we should ask ourselves whether or not these criteria have been met in the case of PCBs. Even if we are able to develop TEFs for PCBs, we have to recognize that they must be applied with caution. As scientists and risk assessors, we have a responsibility in terms of risk communication to our clients, the risk managers. It's up to us as risk assessors to state this as clearly as possible and as persistently as necessary to get the correct message across. We have found in the case of TEFs for CDDs/CDFs that, even with all the instructions provided in the Risk Assessment Forum report entitled, Interim Proceedings for Estimating Risks (U.S. EPA, 1989) and even with the NATO document (Pilot Study on International Information Exchange on Dioxins and Related Compounds: International Toxicity Equivalency Factor (I-TEF)

Method of Risk Assessment for Complex Mixtures of Dioxins and Related Compounds. Report No. 176, August 1988. North Atlantic Treaty Organization (NATO) Committee on the Challenges of Modern Society.), the results can be misused by some risk managers.

As we move forward, we need to make clear in our own minds what it is that we need to tell risk managers and in what context and fashion we should express our concerns. For example, for toxicity equivalents (TEs) for CDDs/CDFs, we state clearly that we ought to express not only the total equivalents, but also what percentage of those equivalents are due to 2,3,7,8-TCDD in the sample. The clear implication is that a risk manager might be well advised to react differently to a TE that is made up of a large percentage or even 100 percent of 2,3,7,8-TCDD, compared to a TE for a sample containing very little or no 2,3,7,8-TCDD. In the former case, we are dealing with a material with demonstrated carcinogenicity. In the latter case, we are dealing with a material which we have reason to believe is carcinogenic.

Scientists also have a responsibility to recognize the interim nature of the TEF approach. Frankly, as I look back over the last 5 years, I submit that we have fallen short in this area. Even though we said with great protestations and continue to say that the TEF procedure for CDDs/CDFs is an interim procedure, 5 years have been lost in which we could have conducted concerted research to develop a replacement procedure. My sense is that a replacement procedure is within our grasp and that with directed research, we could develop an alternative procedure within a reasonably short period of time and, in the case of CDDs/CDFs, put all of this TEF business behind us.

After this workshop today, the Risk Assessment Forum work group will decide whether or not we should press on with the idea of developing TEFs for PCBs and if so, what the next steps ought to be. That work group, if they decide to move forward, will develop a position, present it to the Forum, in order to reach an Agency-wide consensus among EPA scientists. That scientific consensus will then go to the Risk Assessment Council along with an analysis of what the risk management implications might be. Then the Council will determine whether to send the scheme to EPA's SAB and/or whether or not to adopt it as Agency policy.

This workshop is designed to focus on four major questions:

1. Is the scientific database on the toxicity and mechanism of PCB actions sufficient to support a TEF scheme for PCBs?

2. What is known about environmental exposures to specific PCB congeners? (The exposure group will be looking at the issue of where the PCB congeners are in the environment and what the likelihood of exposure is to humans and nonhumans.)

3. What analytical methods are available to identify and quantify individual congeners in environmental matrices? This goes to the issue of whether congener-specific data of sufficient precision and accuracy are available to make TEFs a workable approach.

4. What are the important data gaps and what research is needed to fill those gaps?

Next, I would like to introduce Stephen Safe of Texas A & M University, who will present his perspective on TEFs for PCBs.

A Perspective on Toxicity Equivalency Factors for PCBs
Stephen Safe, Texas A&M University

In considering TEFs for PCBs, we should immediately recognize that the complex mixtures of PCB isomers and congeners that were formerly produced commercially in the United States (these mixtures are called Aroclors) differ substantially from mixtures in environmental residues. Although this was recognized several years ago, all PCB regulations have been based on commercial mixtures. Nevertheless, our exposures reflect PCBs in the environment, not those in commercial mixtures. Therefore, meaningful risk assessments of PCBs must be based on the actual environmental mixtures to which humans are exposed. Once these compounds enter the body, some are retained and biomagnified, whereas others are metabolized and excreted.

The common toxic responses to halogenated aromatic hydrocarbons, of which PCBs are one member, are numerous and include: body weight loss, thymic atrophy, immunotoxicity, porphyria, dermal toxicity, endocrine effects, reproductive toxicity, carcinogenicity, and tissue-specific hyperplastic and hypoplastic responses. There are also a number of known biochemical responses including induction of: Phase II drug-metabolizing enzymes, cytochrome P-450-dependent monooxygenases and associated cytochrome P-450 isozymes (P1-450 mouse; P-450c rat), cellular responses (EGF, estrogen, glucocorticoid), and other enzymes (e.g., ODC). The most familiar biochemical response is the induction of aryl hydrocarbon hydroxylase (AHH), a good marker of exposure to toxic halogenated aromatics. Some of the commercial PCB mixtures and individual congeners resemble 2,3,7,8-TCDD and related toxic halogenated aromatic hydrocarbons and elicit the same broad spectrum of dioxin-like responses.

This presentation summarizes the TEF process in general terms. A premise for the development of TEFs is that there is good evidence that some PCB congeners and mixtures are like TCDD in that they elicit their responses through a common, receptor-mediated mechanism. For the PCBs, as with dioxins, furans, and other halogenated aromatics, some of the mixtures and some of the congeners produce dioxin-like effects, as evidenced by extensive structure-receptor binding relationships; structure-induction relationships, usually the induction of aryl hydrocarbon hydroxylase (AHH), a well-known and well-characterized TCDD-mediated response; numerous structure-toxicity relationships; and genetic evidence with inbred strains of mice. This evidence is well-documented in the literature.

There is good evidence that PCBs, dioxins, and related compounds act through a common mechanism, a receptor-mediated response as illustrated in Figure 1. The inducer, in this case a PCB, binds to a receptor protein, undergoes some transformation, is transported into the nucleus, occupies a nuclear binding site (probably upstream from the P-4501A1 gene), and turns on gene expression similar to the way steroid hormone-receptor complexes turn on genes. While we understand little about the induced proteins or the induced messages involved in nondioxin-like responses, research is underway using TCDD as a prototype; some of the dioxin-like PCBs could also be used as prototypes.

For compounds or mixtures that elicit a dioxin-like response, the TEFs for individual congeners or commercial mixtures can be derived by comparing their ED_{50} or other dose comparable end point (e.g., ED_{20}) to a standard, typically 2,3,7,8-TCDD. (Another compound could be used as a standard as long as it is dioxin-like.) (The dose end point should be from the steep part of the dose-response curve.) The standard is assigned a TEF value of 1.0 and the individual compounds or mixtures are assigned TEFs of less than 1.0.

The TEF approach for a mixture is usually additive, and therefore, does not take into account nonadditive effects. As a result, the TEF approach can only be used to assess the risks of dioxin-like toxicity for compounds that act through the receptor; it cannot be used to assess the risks of nondioxin-like, PCB-induced responses.

The structure-activity relationships (SARs) which have been developed for PCBs are an important component of TEF development. The structural classes of PCBs which exhibit dioxin-like effects have been identified in several laboratories, and for several congeners their toxic potencies have been quantitatively determined relative to a standard toxin (e.g., TCDD).

The qualitative SARs are important for identifying the toxic dioxin-like congeners, and the quantitative SARs are important in TEF development. (In hindsight, if researchers had known how important the quantitative SARs would be in terms of regulation, a few more doses would have been added in these experiments to obtain more dose-response data.)

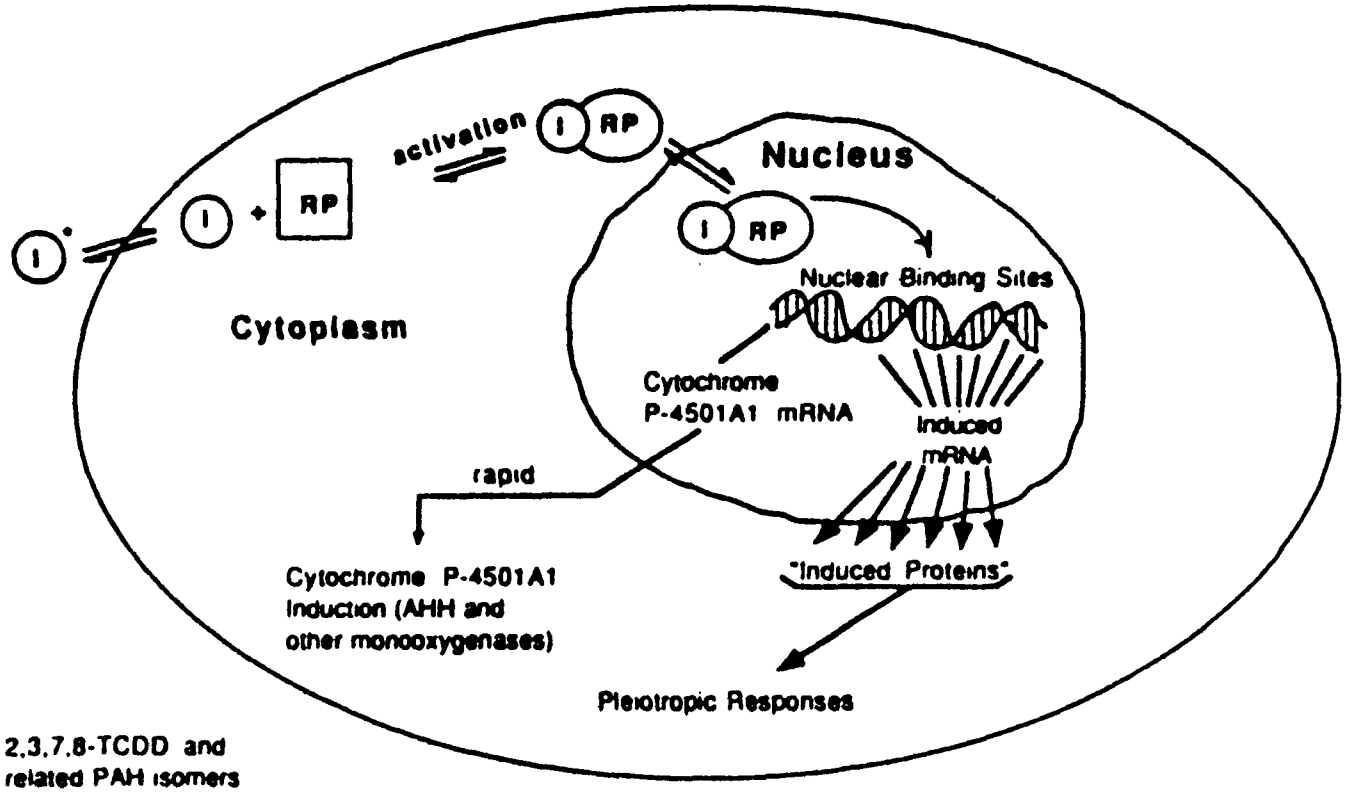

Figure 1. Proposed Mechanism for the Ah Receptor Mediated Responses.

Dioxin-like PCBs

At least four PCBs are dioxin-like in their coplanar conformation. These four coplanar PCBs (listed in Table 1) are approximate isostereomers of TCDD as indicated at the bottom of Figure 2, where the overlap of a coplanar PCB with TCDD is shown.

If a chlorine is added to an ortho position in the four coplanar PCBs, eight different mono-ortho-coplanar PCBs result (Table 2). Some of these are important in commercial mixtures and in the environment. They appear less dioxin-like because the ortho substitute makes coplanar conformation more difficult. Nevertheless, these compounds bind to the Ah receptor and cause dioxin-like responses; however, their potency is much less than the coplanar PCBs.

Finally, diortho substitution of the four coplanar PCBs results in 13 possible diortho-coplanar PCBs (Table 3). With two diortho substitutes, little coplanarity is expected; however, at sufficiently high doses, many of these compounds exhibit weak dioxin-like effects. We probably do not need to worry much about the diortho substitute analogs, although some of these compounds are the PCB congeners that persist in environmental mixtures as well as being major components of the commercial Aroclors.

Structural Activity Relationships or Classifications

PCBs have recently been classified into six different classes as shown in Figure 3. Class I and Class II compounds are the coplanar and mono-ortho-coplanar PCBs, respectively. Class III are mono-ortho coplanars, which lack a chlorine in a para position. Class IV PCBs are the diortho-coplanar compounds. Classes I, II, and IV are dioxin-like in decreasing order of potency. The activity of Class III PCBs, lacking one of the important or lateral substitutes, is like the diortho-coplanar PCBs (i.e., relatively weak, but significantly dioxin-like). Class III PCBs are like Class IV in terms of potency and not worthy of much concern. Class V and Class VI, tri-and tetraortho PCBs, are not coplanar and do not elicit significant dioxin-like activity, and can therefore probably be ignored in terms of human toxicity.

TABLE 1

COPLANAR PCBs
ISOSTEREOMERS OF TCDD

3,3',4,4'-TeCB[a]	IUPAC #77
3,4,4',5-TeCB	IUPAC #81
3,3',4,4',5-PeCB[b]	IUPAC #126
3,3',4,4',5,5'-HCB[c]	IUPAC #169

[a]TeCB = tetraCB

[b]PeCB = pentaCB

[c]HCB = hexaCB

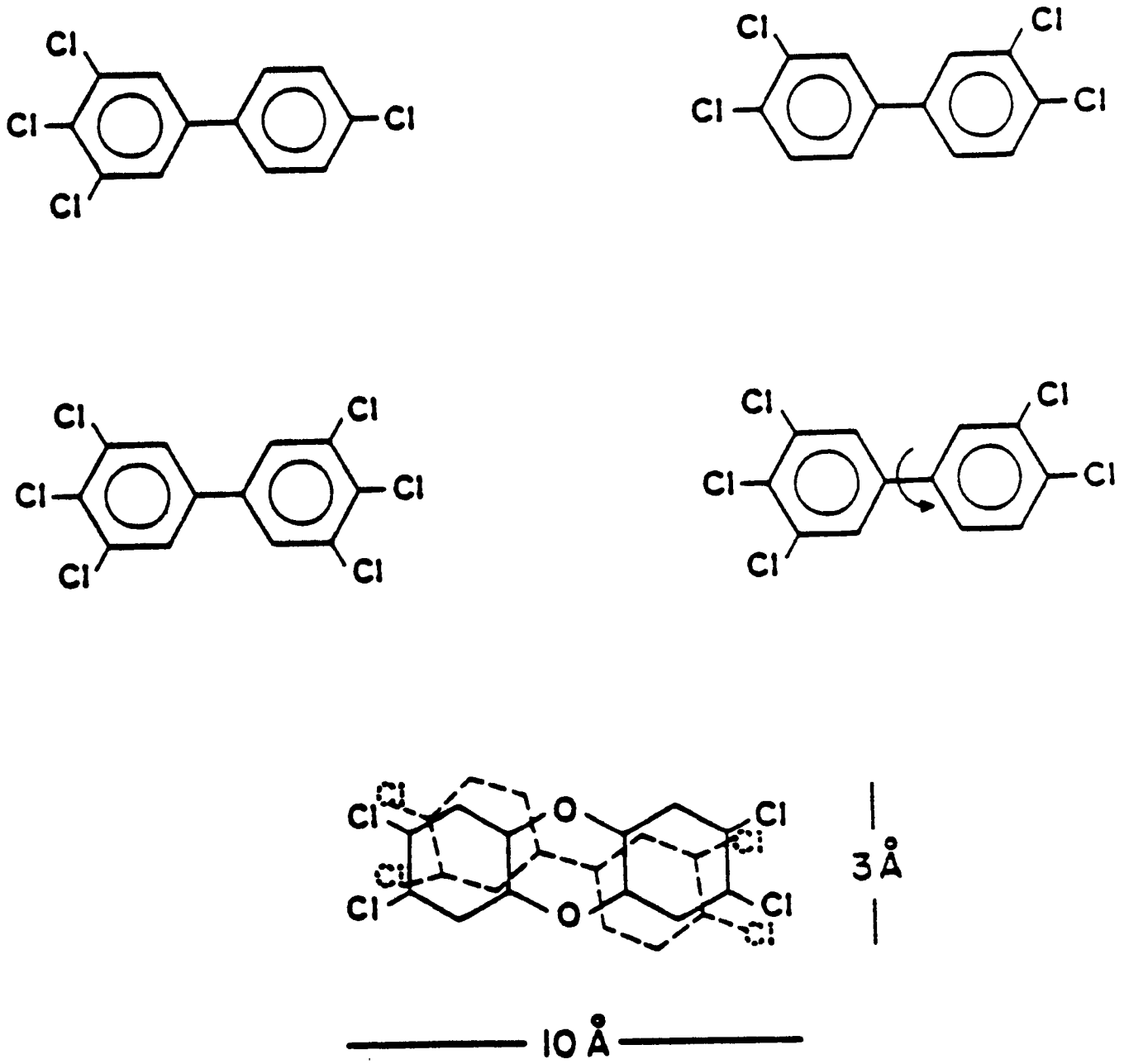

Figure 2. **Structures of the Coplanar PCB Congeners Substituted in Both Para and Two or More Meta Positions and Their Similarity in Shape to TCDD.**

TABLE 2

MONO-ORTHO COPLANAR PCBs

2,3,3',4,4'-PeCB	IUPAC #105
2,3,4,4',5-PeCB	IUPAC #114
2,3',4,4',5-PeCB	IUPAC #118
2',3,4,4',5-PeCB	IUPAC #123
2,3,3',4,4',5-HCB	IUPAC #156
2,3,3',4,4',5'-HCB	IUPAC #157
2,3',4,4',5,5'-HCB	IUPAC #167
2,3,3',4,4',5,5'-HCB	IUPAC #189

TABLE 3

DIORTHO COPLANAR PCBs

2,2',3,3',4,4'-HCB	IUPAC #128
2,2',3,4,4',5-HCB	IUPAC #137
2,2',3,4,4',5'-HCB	IUPAC #138
2,2',4,4',5,5'-HCB	IUPAC #153
2,3,3',4,4',6-HCB	IUPAC #158
2,3,4,4',5,6-HCB	IUPAC #166
2,3',4,4',5',6-HCB	IUPAC #168
2,2',3,3',4,4',5-HpCB[a]	IUPAC #170
2,2',3,4,4',5,5'-HpCB[a]	IUPAC #180
2,3,3',4,4',5,6-HpCB	IUPAC #190
2,3,3',4,4',5',6-HpCB	IUPAC #191
2,2',3,3',4,4',5,5'-octaCB	IUPAC #194
2,3,3',4,4',5,5',6-octaCB	IUPAC #205

[a]HpCB = heptaCB

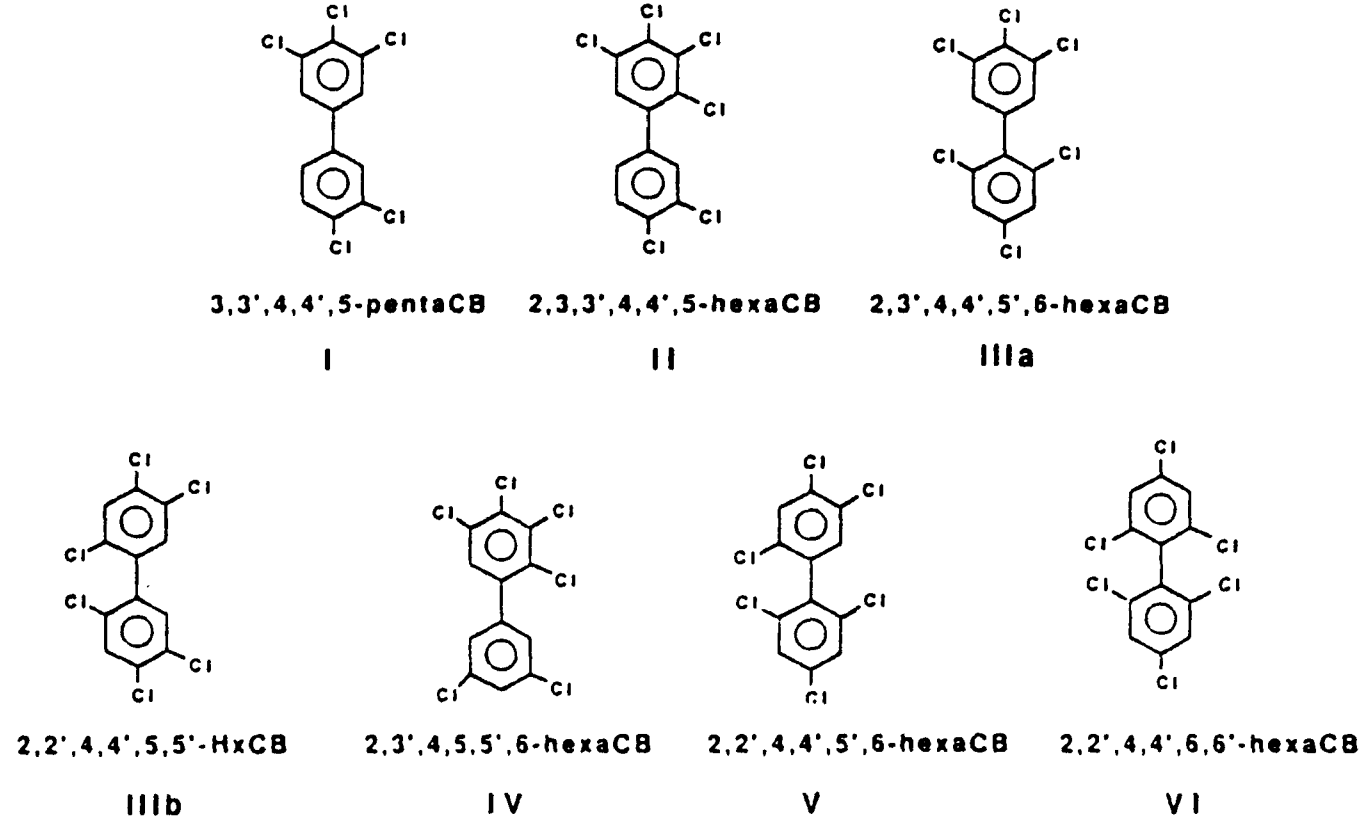

Figure 3. Structural Classes of PCBs Based on TCDD-like Activity.

In summary, the coplanar PCBs congeners are highly toxic, bind with high affinity to the Ah receptor, and are quite dioxin-like. The mono-ortho-coplanar PCBs exhibit moderate toxicity, interact with the Ah receptor with much lower affinity, but elicit the whole panoply of TCDD-mediated responses. The diortho-coplanar PCBs are so insoluble that receptor-binding activity cannot really be measured, although if a sufficiently high dose is administered to the appropriate animal, dioxin-like responses can be observed; the TEFs for these compounds are very low. The PCBs lacking a para-chloro group may also induce dioxin-like effects, but the responses are weak.

Calculation of TEFs

In calculating TEFs we will use TCDD as the standard. The initial step is to determine an EC_{50} or an ED_{50} value for TCDD. The corresponding EC_{50}(s) or ED_{50}(s) for the test compound(s) or mixture(s) are then determined. Next, we determine relative TCDD equivalents by comparing the EC_{50}/ED_{50} values for TCDD and the PCB compounds of interest. As illustrated in Figure 4, the concentration for the ED_{50} (or other end point such as ED_{70}) for the test can be derived from the dose-response curves, along with that of the reference material (i.e., TCDD). The test compound is presumed to be less toxic than TCDD, and therefore more of it is required to elicit the equivalent response (i.e., the higher the ED_{50}, the lower the potency). Therefore, the TEF equation is simply x/mx = 1/m. If the ED_{50} for the test mixture or compound is 100 mg/kg while the ED_{50} for TCDD is 10 mg/kg, the TEF for the test compound or mixture will be 0.1 (10/100). These are simple calculations where all TEFs are less than 1.

The dose-response effect of 3 coplanar PCBs in rats 14 days after a single intraperitoneal (IP) dose is summarized in Table 4. The responses included body weight loss, thymic atrophy, the induction of ethoxyresorufin O-deethylase (EROD) and AHH. The TEFs derived from this particular experiment were obtained by dividing the PCB ED_{50}s by the TCDD ED_{50}s. For each of the three compounds, one gets a range of TEFs, rather than a single TEF, reflecting the four different end points. The resultant TEFs are compared to TCDD, the standard, which is assigned a value of 1.0. For 3,3',4,4'-tetraCB (TeCB), the TEF is $8x10^{-6}$, so the relative toxicity is very, very low. In contrast, 3,3',4,4'5-pentaCB (PeCB), the most toxic of the PCBs, has TEFs ranging from 0.004-0.09. The 3,3',4,4'5,5'-hexaCB (HCB) has a TEF range of 0.003-0.01.

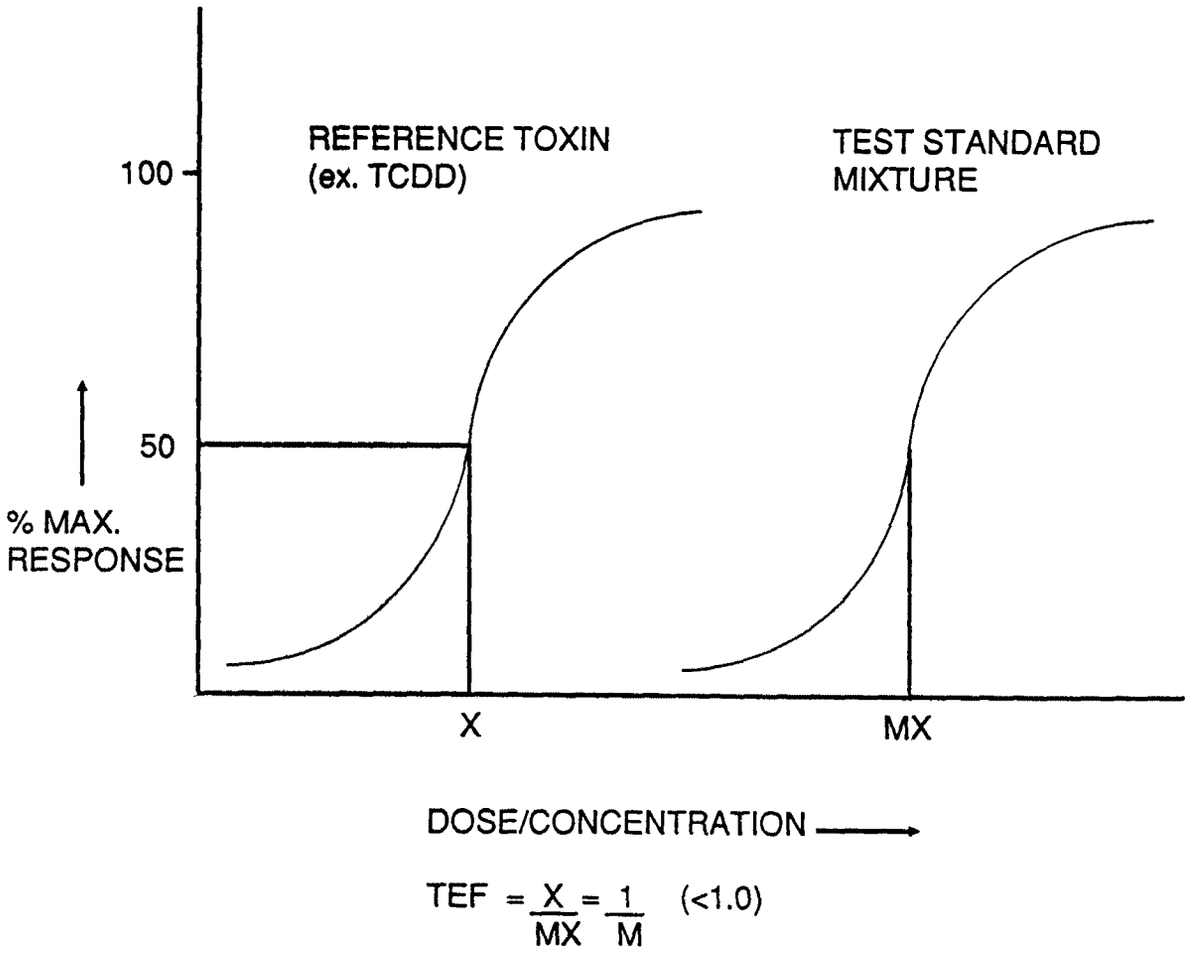

Figure 4. **Determinations of TEFs for Halogenated Aromatic Hydrocarbons or Aromatic Hydrocarbons.**

TABLE 4

DEVELOPMENT OF TEFs FOR COPLANAR PCBs - USE OF DATA FROM IN VIVO STUDIES[a]

Congener	ED50 Response (Rat) μmol/kg				
	Body Weight Loss	Thymic Atrophy	EROD Induction	AHH Induction	TEF Range
3,3',4,4'-TeCB[b]	>500	>500	~500	~500	$<8x10^{-6}$ - $1x10^{-4}$
3,3',4,4',5-PeCB	3.3	0.95	0.12	1.1	0.004-0.09
3,3',4,4',5,5'-HCB	15	8.9	0.65	0.5	0.003-0.01
2,3,7,8-TCDD	0.05	0.09	0.005	0.004	1.0

[a] 14 day dose-response study; single IP injection.

[b] Values are high due to in vivo metabolism.

Table 5 presents the results of the in vitro induction of AHH in the chick embryo hepatocytes and in rat hepatoma cells for the coplanar PCBs. The order of relative toxicity observed is similar to that presented in Table 4. A range of ED_{50}s was observed, depending on which cell system is used. This range of responses can be attributed to several factors, including solubility of the compound; uptake into the cells; and differential responsiveness, metabolism, and receptor-ligand activity related to structure-activity between species.

A similar TEF range is observed for EROD activities in the two cell lines (Table 6). It is noteworthy that 3,3'4,4',5-PeCB was the most toxic in the rat study and was also the most potent inducer of AHH and EROD activities and nearly as potent as TCDD in rat hepatoma cells. The other two coplanar congeners (i.e., 3,3',4,4'-TeCB and 3,3',4,4',4,4'-HCB) were less toxic.

Table 7 presents data for six mono-ortho-coplanar PCBs derived from in vivo rat studies similar to those conducted for the coplanar PCBs. Table 7 shows the range of ED_{50} values and the TEF range for four responses. For the six congeners, a very large range of ED_{50}s is observed due to several factors, including differential metabolism and pharmacokinetics. Not only is there a range for a single response, but there is a range among responses, which is the same pattern that has been observed for dioxin-related chemicals.

For one mono-ortho-coplanar compound, 2,3,3',4,4',5-HCB, considerable comparative data exist, including teratogenicity, immunotoxicity, toxicity, and enzyme induction data. The experimental TEF values for four responses in four species (Table 8) range from about 0.002 to 1.3×10^{-6}, about a thousand-fold variation. (Usually, the range is about a hundred-fold.) This wide range of responses to mono-ortho coplanars is due to differing rates of absorption and metabolism and differential binding to the receptor.

Table 9 summarizes Class I (coplanars) and Class II (mono-ortho-coplanar PCBs) comparative activity (ED_{50} values and EC_{50} values) for receptor binding, AHH induction, and body weight loss. As can be seen in the Table, TCDD binds well to the receptor whereas Class I PCBs do not bind as well. In addition, as the compounds increase in size, the potency for all responses decreases.

TABLE 5

DEVELOPMENT OF TEFs FOR COPLANAR PCBs -
USE OF AHH EC50s DERIVED FROM IN VITRO STUDIES

	AHH EC50		
Congener	**CEH[a]**	**RHC[b]**	**TEF Range**
3,3',4,4'-TeCB	2.2×10^{-9}	3.5×10^{-8}	0.002 - 0.02
3,3',4,4',5-PeCB	2.0×10^{-9}	2.4×10^{-10}	0.01 - 0.76
3,3',4,4',4,4'-HCB	-	6.0×10^{-8}	0.001 - 0.008
2,3,7,8-TCDD	2.0×10^{-11}	7.2×10^{-11}	1.0

[a] Chick embryo hepatocytes in culture.

[b] Rat hepatoma H-4-IIE cells.

TABLE 6

DEVELOPMENT OF TEFs FOR COPLANAR PCBs -
USE OF EROD EC50s DERIVED FROM IN VITRO STUDIES

	EROD EC50		
Congener	**CEH[a]**	**RHC[b]**	**TEF Range**
3,3',4,4'-TeCB	2.5×10^{-8}	8.9×10^{-8}	0.002 - 0.02
3,3',4,4',5-PeCB	-	2.5×10^{-10}	0.01 - 0.76
3,3',4,4',4,4'-HCB	-	2.4×10^{-8}	0.001 - 0.008
2,3,7,8-TCDD	4.8×10^{-10}	1.9×10^{-10}	1.0

[a] Chick embryo hepatocytes in culture.

[b] Rat hepatoma H-4-IIE cells.

TABLE 7

DEVELOPMENT OF TEFs FOR MONO-ORTHO COPLANAR PCBs USING DATA DERIVED FROM IN VITRO RAT STUDIES[a]

Response	ED50 Values (μmol/kg)	TEF Range
Body Weight Loss	180 - 1120	$3.6x10^{-3}$ - $2.2x10^{-4}$
Thymic Atrophy	180 - 2790	$2.0x10^{-3}$ - $3.1x10^{-4}$
AHH Induction	6 - 130	$1.5x10^{-3}$ - $3.3x10^{-4}$
EROD Induction	7 - 220	$1.4x10^{-3}$ - $4.4x10^{-4}$

TEF Range: $1.5x10^{-3}$ - $4.4x10^{-4}$.

[a] 6 congeners included in the study.

TABLE 8

TEFs OF MONO-ORTHO COPLANAR PCBs (2,3,3',4,4',5-HCB) DERIVED FROM EXPERIMENTAL DATA

Species	Experimental TEFs			
	Toxicity	Immunotoxicity	Teratogenicity	Induction
Rat	$2\text{-}3\times10^{-3}$	-	-	$1.6 - 7.0\times10^{-4}$
Mouse	-	1.2×10^{-5}	3.5×10^{-4}	-
Rat Hep Cells	-	-	-	$3.4\times10^{-5} - 2\times10^{-4}$
CEH cells	-	-	-	$1.3\times10^{-6} - 3\times10^{-6}$

TEF Range: 1.3×10^{-6} to 2.0×10^{-3}

TABLE 9

COMPARATIVE ACTIVITIES OF COPLANAR (CLASS I)
AND MONO-CHLORO COPLANAR (CLASS II) PCBs

		ED50/EC50 Values (Rats)		
Congener	Class	Receptor Binding (nmol)	AHH Induction (μmol/kg)	Body Weight Loss (μmol/kg)
2,3,7,8-TCDD		10	0.004	0.05
3,3',4,4',5-PeCB	I	120	1.10	3.3
2,3',4,4',5-HCB	II	7100	6	220

Table 10 presents the range of in vitro and in vivo TEF values for the various classes of PCBs. The TEF range for 3,3',4,4'-tetrachlorobiphenyl varies considerably and there is also great variation in the TEF range for mono-ortho-coplanar PCBs. Quantitative data on diortho coplanars is available for only one of the 13 congeners; however, in the one study conducted, the TEF was estimated to be approximately 1×10^{-5}.

Recommended TEFs

Table 11 provides a summary of the potency ranges and recommended TEFs for the PCBs. The recommended values are very conservative. The TEF for 3,3',4,4',5,5'-HCB is probably closer to 0.01 than to the 0.05 shown in Table 11. In the environment, HCB is present at very low levels, so the uncertainty over the TEF value may not be important. There is considerable confidence in the TEF of 0.1 for 3,3,4,4',5-PeCB. The TEF of 0.01 for 3,3',4,4'-TeCB is fairly conservative for mammals. For mono-ortho-coplanar PCBs, the TEF of 0.001 is very conservative and perhaps should vary with congener structure. For the diortho-coplanar PCBs, the TEF, which is based on one piece of information, is about 0.00002. With few exceptions, the mono-ortho- and diortho-coplanar PCBs do not contribute a high percentage of the dioxin-like activity. In practice, we will focus on the penta-, hexa-, and tetra-CB coplanars. In terms of environmental exposure, the TEFs for 3,3',4,4',5-PeCB and 3,3',4,4'-TeCB (0.1 and 0.01, respectively) are the most important values.

Application of the TEFs to Risk Assessment

In applying the TEFs to estimate the risk from a mixture, we determine toxic equivalents (TEs). The toxic equivalents for a mixture can be determined directly from the analytical data by multiplying the congener concentration by its respective TEF value and adding the products of individual TCDD equivalents in a mixture to give the total TEs. For example, a congener in the mixture at 5 ppm and a TEF of 0.1 is equivalent to 0.5 ppm of TCDD. In this way, a complicated mixture for which a risk assessment is not usually possible, can be evaluated by converting each component into a TCDD equivalent.

TABLE 10

COMPARISON OF TEFs FOR VARIOUS TYPES OF PCBs
BASED ON IN VITRO AND IN VIVO DATA[a]

Congener Class	In Vitro	In Vivo
Coplanar	0.001 - 0.76	0.003 - 0.09
3,3',4,4'-TeCB	0.002 - 0.02	$8x10^{-6}$ - $1x10^{-4}$
Mono-ortho Coplanar	$5.5x10^{-6}$ - $8x10^{-4}$	0.00044 - 0.0014
Diortho Coplanar	-	~1 x 10^{-5} [a]
Others	-	?

[a] Immunotoxicity.

TABLE 11

RECOMMENDED TEFs FOR VARIOUS CONGENERS AND CLASSES OF PCBs

Congener Class	Relative Potency Range	Recommended TEFs
3,3',4,4'5-PeCB	0.3 - 0.0006	0.1
3,3',4,4',5,5'-HCB	0.1 - 0.0012	0.05
3,3',4,4'-TeCB	0.02 - 0.000006	0.01
Mono-ortho Coplanar PCBs	< 0.005	0.001
Diortho Coplanar PCBs	~ 0.00002	0.00002
Others	-	?

Source: Safe, S. CRC Crit. Rev. Toxicol. (In Press)

The obvious mixtures to examine initially are the Aroclors. In Table 12, the calculated fractional TCDD-like activity of Aroclors 1242 to 1260 is presented. The TCDD equivalents (labelled as fractional TCDD activity in the Table) were calculated by multiplying the TEFs times the concentrations of the coplanar PCBs for these mixtures. Compared to Aroclor 1260, the other Aroclors contain significantly higher TEs.

The application of the TEF approach to risk assessment involves three steps: (1) calculating the congener TE (PCB congener concentration X TEF); (2) summing all of the TEs to obtain the TEs for the entire mixture; and (3) calculating the ED_{50} values from the TEs, using the following relationship:

$$\frac{\text{observed } ED_{50} \text{ TCDD}}{\text{Aroclor TCDD equivalent activity (i.e., the TE)}} = \text{Aroclor } ED_{50}$$

For example, 1 g Aroclor 1260 contains 3 μg TEs and produces the response that 3 μg TCDD produces. The observed ED_{50} for immunotoxicity for TCDD is 0.77 μg/kg. To get the equivalent response from Aroclor 1260 would require a dose of 257 mg/kg of Aroclor 1260:

$$\frac{0.77\ \mu g/kg}{3.0\ \mu g/g} (1 \times 10^3\ mg/g) = 257\ mg/kg$$

Thus, the calculated ED_{50} (immunotoxicity) for Aroclor 1260 is 257 mg/kg, and this is equivalent in terms of response to 0.77 μg/kg TCDD.

Table 13 presents the calculated ED_{50}s for immunotoxicity and the values actually observed by testing 5 Aroclors. (The values are for half-maximum concentration required to inhibit the splenic, plaque-forming cell response to sheep red blood cells in mice.) For Aroclor 1254, the calculated ED_{50} was 70 mg/kg and the observed ED_{50} was 118 mg/kg; this is good correspondence. The calculated value for Aroclor 1260 overestimates the true value (observed) by about two-and-a-half-fold, which also is acceptable. Thus, for Aroclors 1254 and 1260, the TEF approach provides good correspondence between the calculated values and the observed values.

TABLE 12

TCDD ACTIVITY OF COMMERCIAL PCBs BASED ON TEF CALCULATIONS

	Fractional TCDD Activity (μg/g)			
Coplanar PCBs	**AR-1242**	**AR-1248**	**AR-1254**	**AR-1260**
3,3',4,4'-TeCB	52	61	6	2.6
3,3',4,4',5-PeCB	1.7	6.2	4.6	0.8
3,3',4,4',5,5'-HCB	~0	~0	~0	~0
Total	53.7	67	10.6	3.4
Relative Potency	1579	1970	310	100
	Increasing Chlorination ——————>			

AR = Aroclor

TABLE 13

LIMITATIONS OF THE TEF APPROACH FOR COMMERCIAL PCB MIXTURES - IMMUNOTOXICITY STUDIES IN MICE

Mixture	Calculated ED50 (mg/kg)	Observed ED50 (mg/kg)
Aroclor 1232	-	464
Aroclor 1242	14	391
Aroclor 1248	11	190
Aroclor 1254	70	118
Aroclor 1260	257	104

Using the TEF values, the TCDD-equivalents can be determined and the ED_{50} value calculated from the ED_{50} for TCDD (0.77 μg/kg).

Source: Davis and Safe, Toxicology Letters, 48:35 (1989)

The situation is different for the lower-chlorinated Aroclors. The calculated ED_{50}s are 14 and 11 mg/kg for Aroclor 1242 and 1248, respectively, based on the TEF values, the high resolution analysis of the commercial mixture, and TCDD as the baseline standard. The observed ED_{50}s are 28 and 17 times higher, respectively, for Aroclors 1242 and 1248. For these two lower-chlorinated Aroclors, therefore, the risk assessors would overestimate the toxicity. Although the TEF approach works poorly for these lower-chlorinated PCBs, it works well for the higher-chlorinated Aroclors found in the environment.

Figure 5 summarizes the dose-response induction of AHH activity in the rat for five different Aroclors. The doses are very large and the responses maximize at about the same level (i.e., 2000 pmol/mg/min). Using Probit analysis, the ED_{50}s for AHH induction in rats were calculated. The ED_{50} calculations and those observed in the animal studies are shown in Table 14. A good correspondence is observed for Aroclor 1254; there is a three- to four-fold difference, which slightly overestimates the toxicity. For Aroclor 1260, the correspondence is even better. For the lower-chlorinated Aroclors, as observed earlier for immunotoxicity in mice, the calculated and observed ED_{50}s for AHH induction in rats differ by over 10-fold. For these Aroclors, the risk managers would overestimate the toxicity based on what is actually observed.

Table 15 indicates the composition of a PCB mixture similar to the mixture identified in Yusho patients. Of the PCBs in the mixture, only one would be classified as a toxic PCB (i.e., 2,3,3',4,4',5-HCB). In this case, a toxic equivalent would be calculated only for this PCB congener. The TES was calculated to be 0.00018, so the mixture is 18/100,000 times as toxic as TCDD.

TEFs can be used to determine the toxic equivalence of a mixture relative to TCDD. Table 16 summarizes the ED_{50} (observed) and (calculated) for AHH induction and thymic atrophy for a PCB mixture in the rat. The TEs were calculated by multiplying the ED_{50} (observed) for the PCB mixture times 0.00018, the fractional activity relative to TCDD. The resultant calculated value (7.4 nmol/kg) for the TE is similar to the observed ED_{50} for AHH induction by TCDD (4 nmol/kg); similar results were obtained for thymic atrophy. Coupled with the previous data, this demonstrates that these TEFs are useful in hazard and risk assessment of the dioxin-like PCBs.

In a practical application of TEFs to two well-known mixtures, Kanechlor and Yusho oil, one can estimate the contribution of the PCBs to their toxicity. The TEF values for the dioxins

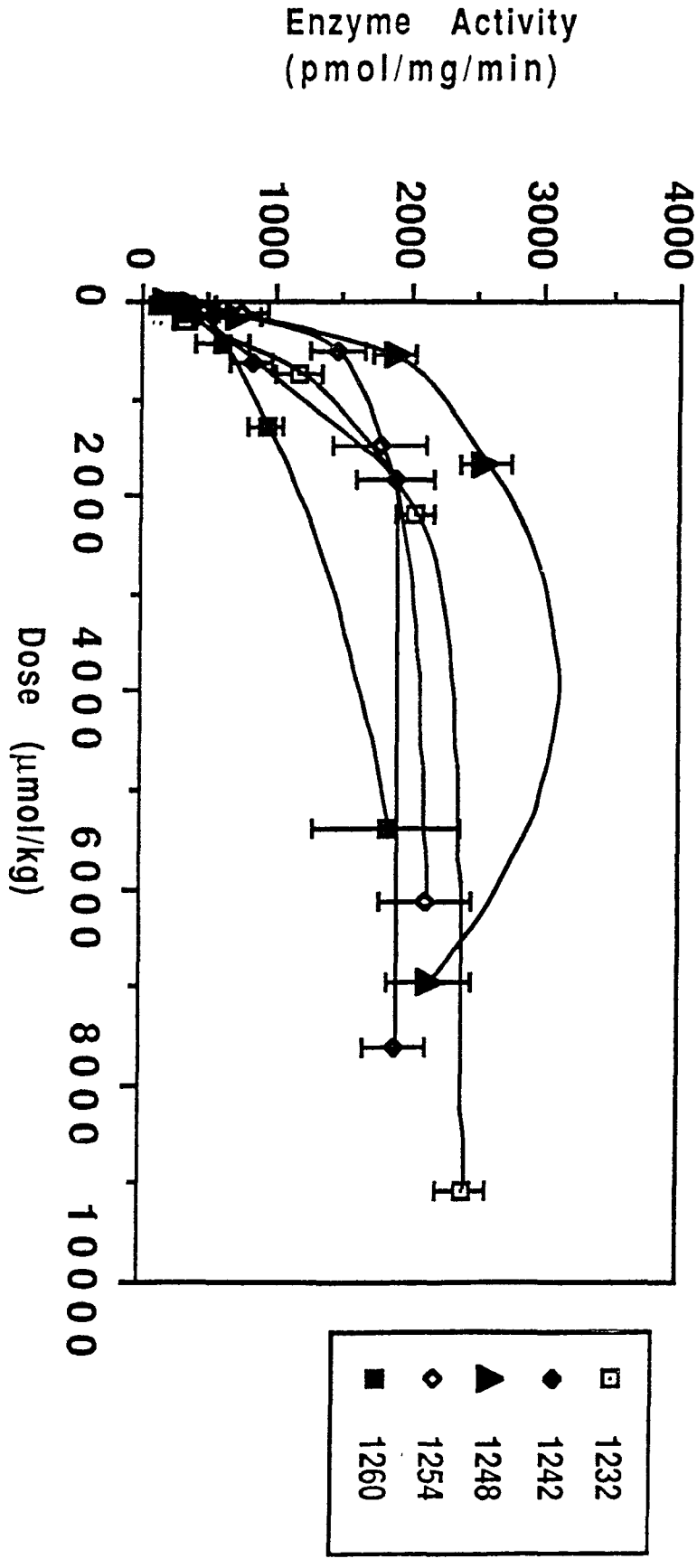

Figure 5. Induction of AHH by Various Aroclors in Wistar Rats.

TABLE 14

LIMITATIONS OF THE TEF APPROACH FOR COMMERCIAL PCB MIXTURES - BASED ON AHH INDUCTION IN RATS

Mixture	Calculated ED50 (mg/kg)	Observed ED50 (mg/kg)
Aroclor 1232	-	402
Aroclor 1242	23	450
Aroclor 1248	19	282
Aroclor 1254	116	440
Aroclor 1260	426	732

Source: Harris, Zacharewski, Safe (Unpublished)

TABLE 15

RECONSTITUTED PCB MIXTURE BASED ON LEVELS IN LIVERS OF YUSHO PATIENTS

Congener	Relative %
2,3',4,4'5-PCB[a]	5.7
2,2',4,4',5,5'-HCB	22.6
2,2',3,4,4',5'-HCB	28.2
2,3,3',4,4',5-HCB[a]	12.3
2,2',3,4,4',5,5'-HpCB[b]	19.1
2,2',3,3',4,4',5-HpCB	12.2

[a] Toxic.

[b] HeptaCB.

Source: Harris, Zacharewski, Safe (Unpublished)

TABLE 16

VALIDATION OF TEFs OF PCBs WITH RECONSTITUTED MIXTURES BASED ON IN VIVO RAT STUDIES OF AHH INDUCTION AND THYMIC ATROPHY

PCB Mixture	AHH Induction	Thymic Atrophy
ED_{50} (observed)[a]	41 μmol/kg	1100 μmol/kg
TE (calculated)[a]	7.4 nmol/kg	198 nmol/kg
ED_{50} TCDD (observed)	4 nmol/kg	90 nmol/kg

[a] Using TEF factors, the fractional activity (relative to TCDD) = 0.00018.

and furans present in Kanechlor 400, a commercial Japanese mixture, add up to about 0.6 ppm (Table 17). For the remainder of the mixture -- the coplanar PCBs and the mono-ortho coplanars -- the total TCDD TEs add up to 140. Therefore, the PCB component contributes about 140 times the TCDD equivalent contributed by the furans. The major dioxin-like components in this commercial mixture are in fact the PCBs, not the furans.

In contrast, in Yusho oil, the furans, as identified by high-resolution analysis, contribute almost 80 percent of the TCDD equivalents. Using the TEFs, the TEs were calculated for PCBs and dibenzofuran. In the Yusho oil, the dioxin/furan components contribute five times more to the TEs than do the PCBs. This finding confirms the experimental toxicological data on the relative contributions of dioxins and furans to these PCB mixtures.

Another example of a relevant PCB contribution to TEs relates to human fat samples where the PCB contribution can be added to that of dioxins and furans, using high-resolution analysis of human fat extracts. The coplanar PCBs have often been ignored in these analytical measurements. The TES for dioxins and furans are about 20 ppt, which is typical of most environmental samples. Multiplying the concentrations found in human fat by the TEFs yields TEs for coplanar PCBs of about 40 ppt (Table 18). In many environmental samples, the TEs for the PCBs are higher than those for the dioxin/furans.

In terms of using TEFs for PCB risk assessment, two factors must be kept in mind: the TEF approach accounts only for dioxin-like toxicity; and for some lower-chlorinated commercial mixtures, such as Aroclors 1242 and 1248, the TEF approach overestimates their toxicity. (This overestimation may be due to nonadditive, antagonistic interactions of toxic dioxin-like and nontoxic PCB congeners.)

Antagonist Screening

In antagonism studies, a dose of TCDD having an effect of 80 to 100 percent is administered as the baseline. To test for antagonism, PCBs are coadministered at a level which alone would not produce the effect (i.e., a subtoxic dose). Antagonism by the PCB would be demonstrated by a reduction in TCDD-induced effects such as AHH and EROD induction, thymic

TABLE 17

COMPARISON OF 2,3,7,8-TCDD EQUIVALENTS IN KANECHLOR 400 AND YUSHO OIL

		2,3,7,8-TCDD Equivalents (ppm)	
Congeners	**TEFs**	**Kanechlor 400**	**Yusho Oil**
PCDDs	1 - 0.001	~0	.0075
PCDFs	1 - 0.001	0.607	1.025
3,3',4,4',5-PeCB	0.1	8.9	.073
3,3',4,4',5,5'-HCB	0.05	.03	.0031
3,3',4,4'-TeCB	0.01	85	.12
Mono-ortho Coplanar PCBs	0.001	46.2	.072
Total PCB	-	140.13	0.268

Source: Tanabe, S., N. Kannan, T. Wakimoto, R. Tatsukawa, T. Okamota, and Y. Masuda. 1989. Isomer-specific determination and toxic evaluation of potentially hazardous coplanar PCBs, dibenzo-furans, and dioxins in the tissues of "Yusho" PCB poisoning victim and in the causal oil. *Toxicol. Environ. Chem.* 24, 215.

TABLE 18

2,3,7,8-TCDD EQUIVALENTS IN HUMAN FAT

Congeners	TEF	2,3,7,8-TCDD Equivalents (ppt)
PCDDs	1.0 - 0.001	12.01
PCDFs	1.0 - 0.001	8.38
3,3',4,4',5-PeCB	0.1	33.0
3,3',4,4',5,5'-HCB	0.05	4.5
3,3',4,4'-TeCB	0.01	3.5

Source: Tanabe, S., N. Kannan, T. Wakimoto, R. Tatsukawa, T. Okamota, and Y. Masuda. 1989. Isomer-specific determination and toxic evaluation of potentially hazardous coplanar PCBs, dibenzo-furans, and dioxins in the tissues of "Yusho" PCB poisoning victim and in the causal oil. *Toxicol. Environ. Chem.* 24, 215.

Note: Similar results observed for samples from fish and wildlife.

atrophy and immunotoxicity, body weight loss, teratogenicity, and porphyria in mice, rats, other animals, and cells in culture. A toxic member of the polyaromatic hydrocarbons (PAHs), namely TCDD, is typically used to examine the toxic response.

As illustrated in Figure 6, 20 μg/kg TCDD caused teratogenicity in C57BL/6 mice (about 60 percent incidence of cleft palate). Aroclor 1254 at a dose of 750 μmol/kg does not induce teratogenicity. When the two are coadministered, a significant inhibition or antagonism by Aroclor 1254 of the TCDD-mediated teratogenicity is observed.

Similarly, antagonism by Aroclor 1254 of other TCDD responses in mice has been observed. The interaction of TCDD, at a particular dose, with different doses of Aroclor 1254 was studied. Various types and levels of antagonism, have been determined ranging from 100 percent antagonism to no antagonism (see Table 19). There is a window for the antagonism, which is directly related to the relative concentrations of the antagonist/agonist. These antagonist/agonist interactions may be responsible for some of the lower toxicities that are observed for the lower-chlorinated Aroclors.

In summary, the TEF approach predicts the relative contributions of the PCDFs versus the PCBs in Yusho oil and in the commercial PCBs quite closely. For some PCB mixtures, the TEF approach can be used to estimate toxic equivalents. The TEF approach for halogenated aromatics suggests that for many environmental samples, the PCBs are the major contributors to the dioxin-like activity. And finally, several environmental studies show a correlation between the PCB toxic equivalents associated with contaminant residues and adverse effects in wildlife populations. On the whole, the TEF approach has utility as well as limitations. As with dioxins, it can also serve as an important tool for hazard and risk assessment of environmental mixtures.

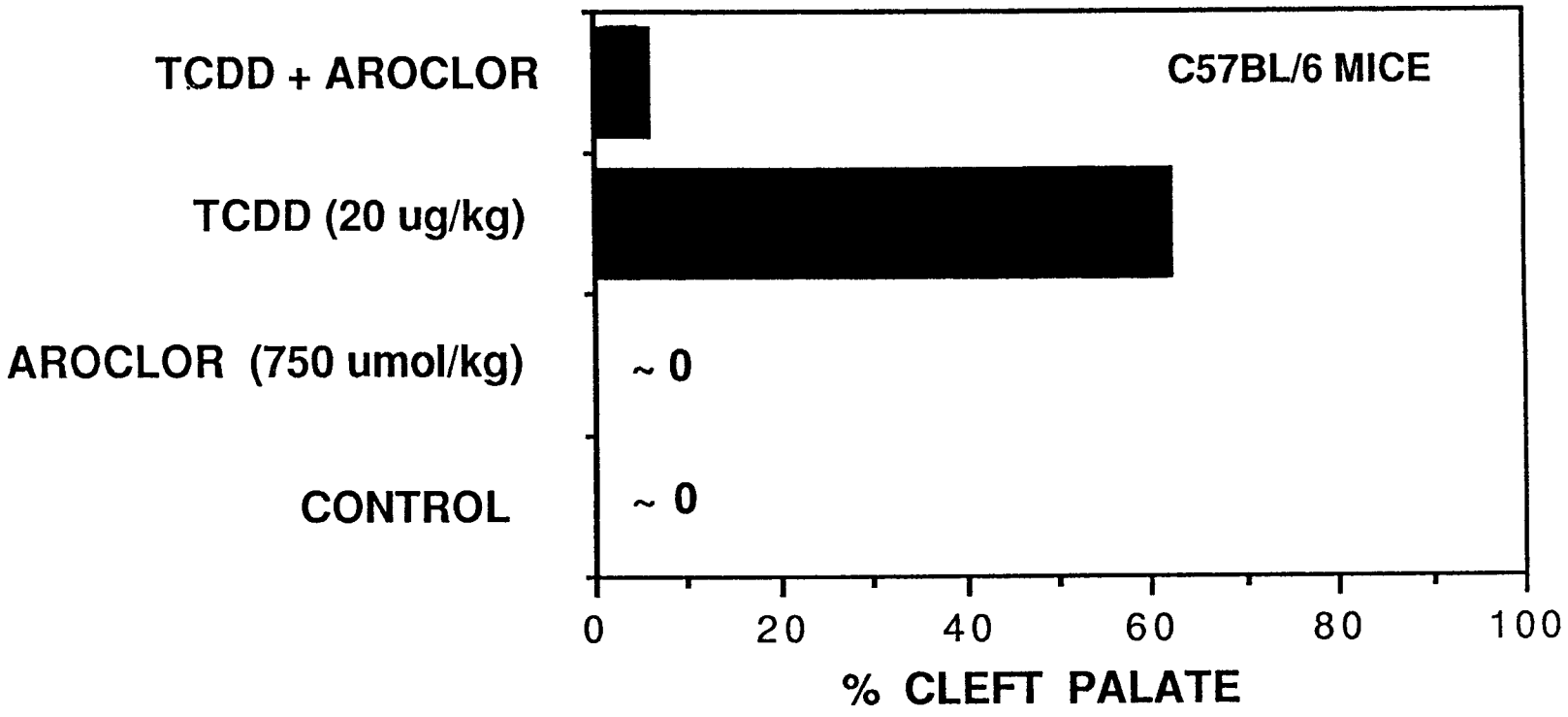

Figure 6. Teratogenicity of 2,3,7,8-TCDD - Antagonism by Aroclor 1254.

TABLE 19

ANTAGONISM OF 2,3,7,8-TCDD EFFECTS IN MICE BY AROCHLOR 1254

Response	% Antagonism (Maximum)	Antagonist/Agonist "Window"
AHH Induction	20	1667 - 10,000/1
EROD Induction	23	1667 - 10,000/1
Thymic Atrophy	0	No antagonism
Immunotoxicity	100	1340 - 20,160/1
Teratogenicity	>80	(±) 12,100/1

PLENARY SESSION

Following the presentations of Dr. Barnes and Dr. Safe, the meeting participants divided into two work groups: the Work Group on the Toxicity/Mechanism of Action Issues, chaired by Dr. Linda Birnbaum, and the Work Group on Exposure/Analytical Issues, chaired by Ms. Ann Alford-Stevens. These groups discussed the issues and questions listed in Appendix B for the remainder of the day. The next day, all the participants reconvened, and the work group chairs presented each group's findings for discussion.

Conclusions of the Toxicology/Mechanisms of Action Work Group
Linda Birnbaum

The Toxicology/Mechanisms of Action Work Group organized the discussion by asking four main questions:

- What are the toxic effects of PCBs?
- Can the PCBs be grouped in terms of structure activity relationships?
- Should the TEFs be developed for chemicals that don't fit into the dioxin group of PCBs?
- Are the TEFs the same for wildlife or for environmental concerns and for mammals?

The first task was to examine the different types of toxicity that have been reported as caused by PCBs and to prepare a matrix summarizing that database (see Figure 7). We couldn't completely fill in the matrix because data aren't available for many types of toxic effects. There are some compelling research needs before the toxicology of PCBs can be adequately understood.

In Figure 7, the major types of toxic effects that have been reported for exposures to complex mixtures of PCBs are listed along the horizontal axis of the matrix; it is certainly not an all-inclusive list. On the vertical axis, four structural classes of PCBs are listed. One class consists

STRUCTURAL CLASSES	TYPES OF TOXICITY	Neurotoxicity	Reproductive Toxicity	Respiratory Toxicity	Immunotoxicity	Dermal Toxicity	Genotoxicity	Carcinogenicity
	Dioxin-like PCBs	?	+	? -	+	+	-	+
	Ortho-substituted PCBs	+	+	?	?	?	? -	+
	Hydroxylated Metabolites		+	?	?	?	+ ?	+ ?
	Sulfonated Metabolites			+				

Figure 7. Matrix Comparing Toxicity of PCBs According to Structural Classses of PCBs.

of PCBs that have dioxin-like activities. The other three classes are hypothetical groupings of PCBs that have similar structural and functional properties, although we have little information as to the best methods and criteria for grouping them.

Dioxin-like PCBs

The first structural group is dioxin-like PCBs. We don't know whether the dioxin-like PCBs have neurological effects. There have been some reports that PCB exposures have caused neurological symptoms in people, although the PCBs responsible for the symptoms are unknown. A question mark was placed in the matrix.

PCBs clearly induce reproductive effects in mammals due to the dioxin-like PCBs. There are some data for PCBs in mink and monkeys, and I think there may be some data coming up in rodents as well, indicating reproductive effects. At high enough doses, reproductive failures are found, whereas at very low doses, definite reproductive deficits are observed (e.g., feminization of the male rodent offspring). There is also a very good correlation between the dioxin-like PCBs and reproductive failure in fish and wildlife. Thus, a plus sign was placed in the matrix.

Respiratory effects have been frequently reported in humans exposed to PCBs. It is unclear as to whether these effects are due to irritant properties or due to dioxin per se, although animal studies do not indicate that TCDD or the furans cause respiratory difficulties. Therefore, a question mark and a minus sign were entered in the matrix.

Dioxin-like PCBs are clearly immunotoxic for a number of different kinds of immunotoxic end points and some of them produce dermal effects. Therefore, plus signs were entered for both immunotoxicity and dermal toxicity.

Genotoxicity is probably a misnomer; direct-acting mutagenic should have been listed instead. Dioxin-like PCBs are not direct-acting mutagens and are probably not indirect mutagens,

either. They may have an epigenetic mechanism. A minus sign was entered in the matrix for genotoxicity.

Clearly, complex PCB mixtures are carcinogenic; however, very few studies of complete carcinogenicity have been conducted with individual congeners. Based on initiation-promotion studies, PCBs appear to be strong promoters. Whether they're also weak initiators is unclear at this time. Considering some of the initiating properties of some of the PCB congeners, it appears that the dioxin-like PCBs probably have promoting activity, like dioxin. Thus, a plus sign is warranted for carcinogenicity.

Ortho-substituted PCBs

Ortho-substituted PCBs are the second structural class that are dioxin-like in activity. Some ortho PCBs, however, may have nondioxin effects in addition to dioxin-like effects. This class is not well defined yet, although scientists who are trying to develop structure activity relationships for some ortho-substituted PCBs, have found that these ortho-substituted PCBs have neurotoxic potential. Some ortho-substituted PCBs appear to accumulate in the brains of nonhuman primates and depress levels of dopamine. In certain tissue culture models, these compounds appear to block dopamine synthesis in the brain. A definite mechanism for this effect is unknown, although there are several hypotheses under discussion. So this class appears to exert neurotoxic effects, although not through a dioxin-like or a dioxin receptor-based mechanism, so a plus sign was entered into the matrix.

There's a suggestion that some of the ortho-substituted PCBs (that may or may not be in the same class) have reproductive effects. A recent report indicates that some of the ortho-substituted PCBs may potentially cause decreased sperm motility, and thus male reproductive effects. We entered a plus into the matrix.

There's no information available regarding respiratory effects, so a question mark was entered in the matrix. Some of these ortho-substituted PCBs (i.e., the nondioxin, receptor-

mediated ones) don't cause the same kind of immunotoxic effects as dioxin-like PCBs; other kinds of immunotoxicities have not been evaluated. Thus, a question mark is assigned to the matrix for this effect.

There is little evidence for dermal effects, but again, no one has clearly looked for them. A question mark was entered for dermal toxicity. In terms of genotoxicity, we wouldn't expect the ortho-substituted PCBs to be direct-acting mutagens, but there is a possibility that some of the lower-chlorinated ortho PCBs might be metabolized to active intermediates, which are direct-acting mutagens. So we entered a question mark and a minus in the matrix.

Based on the results of initiation-promotion studies, some of the ortho-substituted PCBs appear to be potent promoters, so we entered a plus sign in the carcinogenicity column.

Hydroxylated Metabolites

Hydroxylated metabolites, the components of the third structural class, are not actually PCBs but hydroxylated metabolites of some of the lower-chlorinated PCBs. Reports indicate that some of the hydroxylated metabolites may be toxic. Reproductive effects have been reported and these hydroxylated metabolites appear to be estrogenic in nature. There may be multiple endocrine effects, including antithyroid effects, antivitamin A effects, and estrogenic effects, and while the structure activities for those are not clear, it appears that at least some of the antivitamin A effects, as well as some of the estrogenic effects, may involve a hydroxylated metabolite. So we entered a plus sign for reproductive toxicity.

We don't know whether there are respiratory, immunotoxic, or dermal effects, so we entered question marks for all three columns. We might expect that hydroxylated metabolites are produced through arene oxide intermediates, which would be reactive and therefore might have potential for genotoxicity and carcinogenicity. There's been no research in these areas, so we entered both a plus and a question mark for these two columns.

Sulfonated Metabolites

Sulfonated metabolites are the fourth and final structural class. Some PCBs can be metabolized to sulfonated metabolites, which appear to concentrate in certain lung cells where they bind to a specific protein. It has been hypothesized that the persistence of the metabolites in the cells interferes with some of the cells' secretory properties. It has also been hypothesized that the metabolites play a role in the respiratory effects that have been reported following PCB exposures. Another question must be considered: If PCB-exposed people are reporting elevated incidences of cough and bronchitis, is it due to PCB irritation of the lung, or is it due to a suppressed immune system, resulting in sensitivity to viral and bacterial infections? We don't know the answers yet. We entered a plus for respiratory toxicity. The sulfonated metabolites haven't been examined for other types of toxicity.

We don't know if there are potentially other classes of PCBs which may have toxicities that have not been defined. But there are clearly PCB-like effects which are not due to the dioxin-like PCBs; we know virtually nothing about these nondioxin-like effects.

Critical Effects

Several issues and questions relate to the most sensitive end points or toxic effects. For example, what might be the "most critical" effects and can we develop assays that would integrate all of these effects? Is there a "most critical effect" for which an assay can be used? Should the human TEFs be the same as wildlife TEFs? Should the dioxin-like TEFs for PCBs be added to the total equivalents that have been developed for the dioxins and furans?

Environmental PCB Mixtures/Testing

An absolutely critical need is to determine which PCBs actually exist in the environment, and which PCBs actually persist in people. It was agreed that we need to test mixtures to which people are exposed rather than commercial mixtures.

Research Needs

We have a good understanding of the dioxin-like effects and need to focus on the nondioxin-like effects. In this regard, neurotoxicity, which appears to be a very sensitive end point, is of paramount concern. Indeed, neurotoxicity may be orders of magnitude more sensitive than most other end points. The neurotoxicity appears to be developmental neurotoxicity resulting in IQ and behavioral deficits; you might expect that these effects are occurring in offspring of exposed people.

Clearly, the dioxin-like PCBs cause cancer. It is also clear that some of the nondioxin-like PCBs also cause cancer. The dioxin-like PCBs work through dioxin-based, receptor-mediated mechanisms. The mechanism for nondioxin-like PCBs is less clear, but it definitely does not work through a dioxin-based, receptor-mediated mechanism. So, there are at least two mechanisms involved in the induction of cancer by PCBs. To understand cancer resulting from a mixed exposure, we need to identify the cause (agents) and mechanisms involved. Research is needed to define other nondioxin, PCB-induced toxicity (e.g., male reproductive effects, immunotoxicity, and respiratory toxicity).

A critical need exists to define the other classes of PCB chemicals. If we can define other classes that have certain structural and functional similarities, then a prototype for these classes should be developed, just like TCDD is the prototype for dioxins, an entire large class of chemicals. It would be advantageous if there was a single ortho-substituted PCB that was not dioxin-like, but was neurotoxic and maybe reprotoxic. Then we could focus on understanding the cause and effects, rather than evaluating every chemical in that class.

Dioxin-like TEFs

The only TEF scheme that we currently have in which there are a number of different end points that can be used to develop the TEFs is the scheme for the dioxin-like PCBs. Some end points involve in vitro assays and some are derived from in vivo assays. The in vitro assays do not incorporate any of the metabolic or pharmacokinetic characteristics of the organism, which may be very important, especially in mammalian systems. Some of the in vitro TEF assays may be very useful in a hazard identification phase. They may identify a potential problem and, in that case, one can be very conservative. When we switch into a predictive model to define the types of toxicities, we may want to focus more on the in vivo TEFs. There certainly is a need for more kinds of chronic exposure or longer-term tests on which to base TEFs; these tests would essentially give an integrated measure of dose, including the pharmacokinetic characteristics.

Use of Dioxin TEFs for PCBs

The dioxin-like PCBs are the only group of PCBs for which we have a proposed set of TEFs. The question at hand is: Can these be used as a "first cut" in defining the toxicity of an environmental mixture and situation? The question could also be phrased as: Are the dioxin-like responses the most sensitive end points? In other words, for a mixture of PCBs, if we protect against the dioxin-like effects, would this protect against everything else? We don't have the answers to these questions yet. We need much more dose-response information for the nondioxin-like responses so that we can compare them to the dioxin-like ones. As stated previously, we need to know whether these nondioxin-like effects are occurring at higher doses than the dioxin-like effects.

It is important to note that, in terms of ecological situations, the dioxin-like TEFs appear to very adequately predict the reproductive failure and the birth defects in fish and wildlife, or at

least in a subset of fish and wildlife with a high correlation. Whether there are other end points is unknown.

We know that in experimental animals PCBs cause cancer. The epidemiology studies are really inadequate to determine whether PCBs have or are causing cancer in people. If they do, the effect is far less than that of vinyl chloride or benzene, and is probably a weak increase in risk. In animals, PCBs clearly cause cancer. But it is unclear which congeners induce cancer. For example, for Aroclor 1260, the dioxin-like TEFs account for only a small percentage of the total cancers observed. It appears, therefore, that for Aroclor 1260, the nondioxin-like PCBs may be responsible for much of the carcinogenicity observed in animal studies.

On the other hand, in assessing the toxicity of an environmental mixture of dioxins, furans, and PCBs, the dioxin-like PCBs must be included in that assessment. However, as stated earlier, to ignore the nondioxin PCBs will result in an underestimation of the health effects of environmental mixtures.

TEF Criteria

A short version of Donald Barnes' criteria for using the TEF approach is as follows:

- A need
- Well-defined group
- A broad database of information
- Consistency across end points
- Additivity for the effects
- A common mechanism
- Consensus on the TEFs

Have we met these criteria, or should we try? As regards need, there is an obvious regulatory need and it's not so much a scientific question. Regulators need some means to improve their ability to regulate PCBs. The answer as to whether there is a well-defined group of chemicals is both yes and no. There is a well-defined group in terms of the class of PCBs and maybe even polybrominated biphenyls. But we know that it is not a single uniform group with a single mechanism of action. It is a complex group with many mechanisms of action and potentially many different structural subclasses.

The database is not very broad. We have lots of data, but it's not necessarily focused to address the questions for which we need answers. We need more information in terms of specific kinds of toxicities that can be linked to the subgroups other than the dioxin-like group.

By consistency across end points, we mean: Do we get the same TEF if different kinds of toxicities are used? As Steve Safe has demonstrated, even for the dioxin TEFs, there is a range for each effect. If the effects are grouped into in vitro TEFs versus in vivo, the divergence is narrowed. In terms of consistency across end points, we have multiple classes and there is no reason to expect consistency across classes.

Additivity is a criterion and a toxic equivalency scheme (which assumes additivity) can be developed for chemicals that have the same mechanism of action. Clearly, the dioxin-like PCBs have a dioxin-like mechanism of action and can be expressed in an additive manner. The other groups cannot be added to the dioxin-like grouping.

The issue of a common mechanism is basically the same issue as additivity. We need to find out if there are other classes of PCBs that have common mechanisms of action. The consensus that our work group reached was that there is a potential for multiple groups, and that the dioxin-like ones can be considered as dioxin equivalents while other groups cannot.

In summary, the TEF approach is useful when the criteria are met. For PCBs as a class, the criteria <u>are not</u> met. While it may be possible in the future to develop TEFs for the different classes of PCBs, we are quite a way from that right now.

In response to a question as to whether humans are different from other mammalian wildlife, Dr. Birnbaum responded that people are animals in one sense or another. She noted that at the Banbury meeting (October 1990), which looked at opportunities to move forward in the risk assessment of dioxin, consensus was reached on three issues. First, it was agreed that people in fact are animals, and most animals respond in a similar dose-response range for most end points. There are outliers for any given response, but an organism which is an outlier for one response is not necessarily an outlier for another response. The doses needed to cause dioxin-related responses in people appear to be very similar to the doses that cause those responses in several different experimental animal species. Second, dioxin effects are clearly receptor-mediated, and therefore, there is the potential to develop a regulatory scheme based upon receptor-mediated mechanisms. The third consensus was that the reason people are not dying from dioxin-like effects is that exposure has been below the point where human health effects occur.

Conclusions of the Exposure and Analytical Issues Work Group
Ann Alford-Stevens

For purposes of this discussion, the work group defined environmental media as anything analyzed to determine its PCB composition. The group had no difficulty making the general statement that environmental samples do not usually resemble Aroclors, and noted that is a problem because the traditional approach to determining PCBs in environmental samples has been to relate the sample composition to that of an Aroclor mixture or a mixture of Aroclor mixtures. Unfortunately, the composition of the PCB residues changes dramatically in the environment as the PCBs move from environmental media such as soil, sediment, or waste to a biological system. This chemical transformation also makes it more difficult to analyze the sample because the longer the PCB remains in the environment, the longer it is subjected to transformation processes, and the less similar it becomes to an Aroclor standard. Therefore, data related to the Aroclor standard become less valid. We certainly agree with the Toxicity/Mechanisms of Action Work Group that, in many cases, reliance on the traditional determination of environmental PCBs as Aroclors produces misleading data.

Transformation Processes

One of our assigned questions was: What are the most important transformation processes? In short, we don't know. These processes depend on many factors, such as the media. For example, if PCBs are on soil exposed to sunlight, photolysis and evaporation might occur. However, if the same group of compounds is in sediment, little photolysis or evaporation will occur, although dissolution of particular congeners might occur. The bottom line is that there is no overall, most important transformation process in these environmental samples. We identified transformation processes for which we lack sufficient information: bioaccumulation, selective metabolism, and bioavailability.

Analytical Processes

Different analytical approaches yield different results. This is an important consideration when trying to decide how to use data that have been acquired and how to acquire future data. I'd like to give a simplistic example to illustrate. The sample in question is an extract of a soil sediment from a Superfund hazardous waste site. Let's take three hypothetical analyses. The first analyst who attacked this problem thinks that the sample might consist of Aroclor 1242 and 1254. But he/she doesn't have a mixture of those standards, so he/she assigns the first half of the sample to 1242 and the remainder to 1254, chooses a couple of gas chromatograph (GC) peaks for the first half of the sample and uses them to quantify Aroclor 1242, and uses a similar process to quantify Aroclor 1254. Let's say analyst number one comes up with 20 μg.

Then analyst number two comes along and uses a different approach. He/she uses the same extracts and assumes the sample is a mixture of Aroclor 1242 and 1254, but takes a more holistic approach and measures every peak. He/she also has a very good computer system, which is used to measure the areas of all the peaks, both in the sample and in the standard, leading to an overall measurement of 60 μg.

Analyst number three uses a gas chromatograph/mass spectrometer (GC/MS) system to measure the mass spectra as well as retention times of all sample components. He/she finds that many of the electron capture detector peaks were produced by chlorinated naphthalenes, not PCBs. This analyst does a more rigorous determination of which peaks are really PCBs and which are really chlorinated naphthalenes and determines that there are 30 μg of PCBs and 30 μg of chlorinated naphthalenes. The reported sample results are 20, 60, and 30 ppm PCBs. Does the sample exceed 50 ppm PCBs? This is not a simple issue for people who have to regulate PCBs in the environment.

To determine which analytical approach is appropriate, the data ultimately needed must be determined at the very beginning. If you really need to know whether a sample has something other than PCBs in it, you either have to go through a rigorous cleanup procedure to remove the extraneous peaks that you don't want to measure as PCBs, or you have to try to identify and

measure each individual compound. The latter approach is required if these data will be used for TE factors.

Our group strongly agreed that we must use capillary columns in order to provide congener-specific data. Although some labs are still using packed columns, particularly for determination of PCBs as Aroclors, capillary columns are widely used. (Some of the original problems with using them have long since been solved.) Unfortunately, for the determination of PCBs as Aroclors, using a capillary column magnifies the identification problem. Identification is based on peak pattern recognition, and it is much easier to recognize peak patterns when you only have 15 or 20 peaks rather than possibly 150 peaks, particularly when samples vary from one to another. One of the major problems with identification of PCBs, particularly PCBs as Aroclors, has been that the standardized methods give almost no criteria for what constitutes positive identification. A very vague statement is usually made directing the analyst to compare the pattern of the sample to that of the standard and determine whether it is the same or not. But what one person perceives to be the same is not going to be the same as what another person perceives. And how similar do the sample and standard have to be?

Our group agreed that if TEF data are required for a specific group of congeners, we can provide it. The analytical procedures are available, although they are not standardized. These procedures are not widely used right now, and many laboratories do not have the expertise and the capability to go through the rather rigorous determinations that are required for valid identification and measurement of the individual congeners, but it can be done. The cost, as a rule of thumb, increases as the number of congeners increases. For example, we can identify the 11 or 12 PCB congeners that have been discussed in this meeting relatively inexpensively and without great difficulty. But if we decide that we must have information on all 209 possible PCBs, the analytical problem is going to be difficult and expensive. (For starters, the standards are going to be very expensive.) Also, if information is required for all PCB congeners in environmental samples containing Aroclor residues, procedures are available to obtain this information.

Markers

Another of our questions concerned markers (i.e., indicators). For example, if I am interested in our 11 or 12 specific congeners, could I possibly measure 1 or 2 of those in every sample and relate that to the presence of the other 9 or 10? This would only be feasible for a situation in which all 11 or 12 congeners always occur together in a sample type, which would be rather unusual. The use of marker congeners would have to be designed for each particular scenario and the model would have to be tailored to a specific exposure system going from point a to point b through a particular process that almost invariably behaves the same way. Unfortunately, that's an ideal situation, but I believe one of the people in our group did have a particular situation where he thought this was possible. A marker system may be possible for certain situations to simplify the analytical data gathering, but for many cases, this is not probable.

Based on the overall body of knowledge about PCBs, the available congener-specific information is minimal. Standardized procedures to develop congener-specific information are essentially nonexistent. No one in our group had any information about interlab studies, which are needed to determine the accuracy and precision of results obtained with standardized procedures. Consequently, we really don't have any way to determine the quality of the congener-specific data that are produced.

Biological Assays

Biological assay was another one of our issues. Three or four people in our group had some information about various approaches for using biological assays to determine which samples were of highest priority, most significance, and needed the most attention. Right now, however, few data are available. When a particular end point in a particular assay is reached, a lot of data are needed to correlate specific sample components with an end point. Although data are being generated, time is required to develop biological assays to accomplish the goal. In addition, little is known about the effect of individual PCB congeners other than the 11 or 12 congeners that

we've been discussing. Other sample components, like the chlorinated naphthalenes in the sample mentioned previously, could possibly be producing effects that are currently attributed to PCBs.

Needs

We identified a number of needs. One big need is for standardized calibration solutions. A solution containing all 209 congeners would be very expensive ($4,000 to $5,000), and standards are not yet commercially available for all congeners. Once the standards are obtained, a solution containing a known amount of each one must be prepared. You can appreciate how unlikely it is that one would be able to work with 209 standards and not make any mistakes.

The approach that has been used in procedures to identify and measure the Aroclor congeners is to use a mixture of Aroclor mixtures. Because the composition of each of these mixtures has been relatively well defined, one can develop credible information based on those solutions. Because no standardized mixture of mixtures has been developed and used consistently, different people have selected different compositions. In addition, different labs may think they are using the same method of congener-specific identification, but slight variations in congener retention times can produce drastic differences in results. Therefore, we need a standardized set of calibration solutions and written procedures.

Another problem that must be faced is developing feasible and well-defined detection limits. This may be easier to achieve with congener-specific data than with data expressed as Aroclors.

Isotopically labelled standards will be necessary if PCB congener data are to be compared with dioxin data. For example, one approach that could be used to obtain data for the 11 or 12 PCB dioxin-like congeners would be to piggy-back the determination of these compounds onto dioxin determinations. The procedures currently routinely used to determine dioxins and furans require an isotopically labelled standard to determine its unlabelled analog. This provides what we refer to as "data corrected for recovery." To obtain analogous data for the PCBs, one would have to have isotopically labelled PCB congeners. Right now, very few exist, and as with isotopically

labelled dioxins, they are expensive. If an isotopically labelled standard is required for every compound to be determined, the analysis becomes more and more difficult and more and more expensive.

We must have some way to ensure that congener-specific data are of sufficient quality to be used for what we want. Right now, we have no reference materials or quality control materials that are appropriate for congener-specific PCB determinations.

To determine a specific class of compounds, particularly the dioxin-like compounds, one needs to use special procedures that have been devised to enrich those particular compounds with respect to other sample components and to get rid of potentially interfering components one isn't interested in seeing. This requires rather laborious and tedious cleanup procedures, which need to be improved. We particularly need to automate them, so that they become a feasible, routine, cost-effective mechanism in the laboratory. All of these needs make the situation sound terrible, but this is where we were with dioxins not so many years ago, and we've come a long way in a rapid fashion.

Another consideration is the availability of analytical labs to provide the data. Right now, congener-specific determinations are being performed in a research lab mode. These procedures are not being routinely used by service analytical labs, and requiring a congener-specific determination for all PCBs in all samples would to be a "cultural shock" for many of these labs.

Reservations

Our group had quite a number of areas of consensus, and we agreed with the Toxicity/Mechanisms of Action Work Group on several issues. We also, however, had significant reservations. One thing that bothered us was that this TEF approach is supposed to be a dynamic approach. We're supposed to be changing, we're supposed to be considering improving it and improving better ways to assess the data that we acquire. If this is the case, it may be a mistake to develop procedures that only measure a small group of selected congeners, because we would

be spending all of our resources to develop numbers, albeit good numbers, for only those particular compounds. After additional toxicity data are available for the effects of the nondioxin-like PCB congeners, we may wish that we had data for those compounds, too. It is possible to get it now, and although data acquired now could be retained in raw form (i.e., not processed or manipulated), it is frequently very difficult to go back and treat previously acquired data to get a valid answer to current questions.

Additional Questions

Our group added three questions to the list of questions given to us, but we didn't reach any consensus answers to these questions. Our questions were: Do we really need this TEF approach? Is this approach better than the Aroclor toxicity data we have now? Can we logically test only for dioxin-like PCB congeners and ignore all the rest? To this last question, we as a group had difficulty accepting a positive response.

PLENARY SESSION DISCUSSION

Research at General Electric and the Swedish Institute of Physiology

Dr. Barnes asked Steve Hamilton, from General Electric, and Thure Svensson, from the Swedish Institute of Physiology, to describe their research.

General Electric has been very involved in PCB research activities over the last 10 years. Initially, the company looked at worker health and PCB exposure levels and participated in cooperative efforts with various organizations to conduct mortality studies. More recently, GE has turned to animal studies, which are supervised and planned by a panel of toxicologists. GE is contemplating further work on Aroclor mixtures. Dr. Hamilton stated, however, that as a result of this workshop, GE may need to reconsider their research plans. Dr. Hamilton added that he would be interested in talking to people with ideas on how to clarify the toxicological implications of both the dioxin-like and nondioxin-like congeners.

GE has conducted research on the fate of PCBs in the environment, particularly in river sediments. GE found that PCBs in certain river or lake bottom sediments undergo a natural anaerobic dechlorination, which tends to remove the meta- and parachlorines preferentially, affecting the dioxin-like congeners. Unfortunately, the process does not completely destroy the congeners, rather dechlorination produces a mixture that has a much lower degree of chlorination and a higher percentage of ortho chlorines than meta- and parachlorines. The implications of this process are unclear. Certainly in terms of the cancer data in which mixtures with high levels of chlorination are positive in carcinogenicity assays, this anaerobic dechlorination would appear to reduce the risk.

GE has also sponsored research designed to develop a much simpler but equipment-intensive approach to analyzing for toxic congeners. The method is extremely sensitive and it may possibly represent a gold standard for determination of these congeners, although the method is

not likely to be used in many analytical laboratories across the country because of the costs involved.

Dr. Svensson explained that Sweden, other Scandinavian countries, and most countries in Europe have shifted their focus from CDDs/CDFs to PCBs. These countries acknowledge that the PCB problem is a greater problem than CDDs/CDFs. There are many labs in Germany and the Scandinavian countries determining specific congeners in various environmental samples (e.g., mothers' milk, etc.) The Swedish EPA is conducting a study to compare herring oils at two different levels with five synthetic PCBs; about 26 different labs are participating in the studies, including two U.S. and two Canadian laboratories. In this comparison study, each lab uses the same sample and same reference solutions, but its own procedure. Sweden hopes to obtain an international consensus regarding which PCB congeners should be determined and the TEF values to be used so that the international community can avoid having many different methods to calculate the toxicity equivalents, as was the case with dioxins.

Analytical Issues

One participant wondered to what extent chlorinated naphthalene contamination affects quantitation of PCBs given the mutual interference with an electron capture detector. The interference problem is highly variable and is of special concern in Aroclor determinations. (Another participant noted that selective decomposition and disappearance of some of the PCB congeners is as significant as most of these interferences.)

One person reported that the European Community (EC) does not consider the toxicity end point but uses the major capillary GC peaks of six selected PCB congeners as a surrogate for a total PCB analysis. These six PCBs, which fall into the coplanar group or have dioxin-like toxicities, constitute the major PCB congeners that are frequently found in environmental samples. The EC regulates on the basis of ceilings for those six compounds rather than on ceilings for a total Aroclor.

Can we deal only with dioxin-like chemicals, asked one participant? It is clear that the nondioxin-like congeners have inherent toxicities of their own, distinct and different from the toxicity of the dioxin-like congeners. Analysis should not focus on the "dirty dozen" chemicals that have dioxin-like effects. EPA clearly needs information on the others, she stressed.

Members of the analytical group explained their reasoning in choosing to concentrate on the dioxin-like congeners. They explained that, once the methodology for analyzing the "dirty dozen" is developed, the techniques are available to measure any of the Aroclor-derived congeners, since those 12 are the most difficult to measure.

In addition, if the laboratory currently determines only mono-ortho chloro PCB compounds, then the others are ignored for the time being but could be measured later. The data sets will be in the computer, so it will be possible to pull out any fraction in the future. Of course, the chemist must analyze all sample extract fractions rather than discarding them.

What are the Costs Involved?

Several participants wondered what time and financial resources are required to do congener-specific analyses.

Few laboratories that currently provide "PCB data" provide any congener-specific data. Instead, they rely on the traditional approach of comparing sample component GC profiles to those produced by Aroclor standard(s). The cost of changing to a congener-specific analysis is significant, although in terms of sample preparation and extraction, there is little procedural difference between current Aroclor and the congener-specific methods. Congener-specific methods would involve one additional carbon cleanup step to separate the mono orthos and the mono-ortho chlorinated congeners, analysis of additional extract fractions, and perhaps two-column GC verification.

Any significant change in the method of analysis of PCBs (e.g., requiring congener-specific analyses or adopting a bioassay method) would have an impact on laboratories responsible for providing data on PCBs in the environment, in the food supply, and elsewhere. For example, the

U.S. Department of Agriculture (USDA) will conduct about 5,000 PCB analyses next year on agricultural commodities and food products. A shift to a congener-specific procedure or a bioassay approach would significantly affect their operation and pose questions about the comparability of the new data with data already collected.

Within FDA, specific congener analysis for future sampling has not been discussed. If FDA did adopt congener-specific analysis, it would take a year or more before it could be put into place because training, method development, and validation would be necessary.

The primary factor at this point is standardizing valid laboratory procedures for congener-specific determinations. To obtain data of known quality, standardized analytical procedures and interlaboratory testing are necessary, developments that would take time and large financial resources, as ASTM requires 8 labs on an interlab validation. One participant suggested that the estimated cost for laboratory validation is over a million dollars.

One participant reported that Josh Mess has started an interlaboratory study of some PCB congeners in samples in regulatory laboratories, mostly in Canada. There are very few labs in the United States doing this work, however. The laboratories did not have standard written procedures; rather each laboratory used the same standards with different procedures. The results were difficult to evaluate and were, as a first effort, quite poor. A second round to follow up on preliminary results may be conducted.

Another participant reported that the New York State Environmental Conservation Department, collaborating with EPA Region 2, is conducting a similar study for analyzing PCBs in water. Two consultant laboratories and an EPA laboratory are participating. The Conservation Department has supplied the laboratories with a written protocol.

In sum, it appears that the procedures exist to obtain congener-specific data, but these procedures have not undergone interlaboratory validation. Theoretically, congener-specific data could be provided, but considerable time and expense would be involved with development and implementation.

Exposure: PCB Levels in the Environment and in Humans

One participant noted that EPA had tried to estimate the occurrence of dioxin-like congeners of PCBs in the environment and found it highly variable. Most of the effort used environmental monitoring of matrices that were not directly associated with Aroclor accidents. Of the total composition of PCBs in those samples, only 1 percent or less was dioxin-like, coplanar PCBs.

However, argued another person, if the assessment is based strictly on percent occurrence, ignoring toxic potency, the wrong congeners may be measured. Recent data from the Great Lakes on lake trout and the eggs of fish-eating birds (either colonial or bald eagle), show that the non-ortho-coplanar compounds range from 0.1 to 0.2 percent of total PCBs. If just three mono-ortho compounds are also determined, measured PCB congeners may constitute as much as 10 percent of total PCBs in the sample. What congeners you measure depends on whether you're trying to determine the most toxic congeners or the congeners contributing the largest amount to the total PCBs in the sample.

Using market basket surveys, FDA has found that on a total PCB basis (the analysis was not congener-specific), the total intake of PCB concentrations has decreased at least 5-fold over the last 10 years. The only two foods in which FDA is finding PCBs are fish and some meat products. Apparently, the single largest factor responsible for the decrease is the control of PCBs in recycled paperboard. Formerly, cereals and other foodstuffs became contaminated with Aroclors or PCBs, which the food extracted from the paperboard. The FDA is no longer finding Aroclor or PCB residues in these samples.

Rather than measuring PCBs in food, a better way to measure human exposure, countered another participant, would be to measure PCB concentrations in human adipose tissue or blood. Then, if one wants to determine the source(s) of the PCBs, one could look at the contribution from food. Some data for PCB levels in adipose tissue are available, although validated methodologies were not used. There have been surveys of adipose tissues in Canada, for instance, carried out within the last two or three years, to determine which congeners are present in adipose

tissue. Larry Needham and coworkers also measured PCBs in serum and adipose tissue. Coplanar compounds constituted less than 1 percent of the total PCBs. The size of the peak for the same PCB differed between serum and adipose tissue, probably due to metabolic differences. Another person argued that, for some of the PCBs that may exert their toxicity via metabolites, looking at the adipose tissue will not provide information about the metabolites.

Mono-ortho coplanar compounds contribute 0.1 percent of the total PCB body burden and about 0.1 percent of the total PCBs in environmental samples. Essentially the exposure in terms of concentration is the same.

When considering TEFs, the persistence of the compound must be taken into account, added another participant. There are data, albeit only for rats. Dr. Shayne, at the New York State Department of Health, has conducted rat studies to determine which congeners partition where, and which congeners increase or decrease with levels in the dam's food. In most mammalian systems, there are 3 to 8 PCB congeners that persist (e.g., 2,4,5,2',4',5' and 2,3,4,2',4',5, etc.). Although these congeners have relatively small dioxin TEFs, they are present at much higher levels than the dioxin-like PCBs. Moreover, they are very stable and persist for about 90 percent of a rat lifetime. Dr. Shayne demonstrated that para-substitution on both rings of the PCBs conferred resistance to degradation. This is another piece of information that should be worked into the TEF formula, concluded one participant.

General Electric conducted some research regarding PCB congeners' persistence in humans. These were longitudinal studies of capacitor workers exposed mainly to Aroclors 1254 and 1242 over 15 years. GE located some unpublished studies on transformer workers exposed to 1254 and 1260 that indicate that the mono-ortho congeners that have some dioxin-like activities, namely numbers 105 and 118, are intermediate in persistence in the human. In the capacitor workers, GE observed metabolic halflives of 4 to 6 years for those congeners. Congener 77 (3,4,3',4') is very rapidly metabolized; no data are available on congener 126 (3,4,5,3',4'), although the researchers will follow up and reanalyze the samples. This is in contrast to some of the more heavily chlorinated, frequently observed congeners. In the study population, congener 153 (2,4,5,2',4',5') has a halflife of about 13 years. Finally, some of the more heavily chlorinated compounds (e.g.,

congeners 180 and 170, which are heptachloro biphenyls, and one or two of the octa-chlorinated compounds), show no evidence of being cleared. In contrast, the lower congeners are cleared so rapidly that the researchers cannot track them in this type of group.

The New York State Department of Health has published data on 40 milk samples and 100 mothers' blood and fetal cord blood samples. Results from analysis of 150 Mohawk Indian women's milk samples (using a DB-5 capillary GC column, the Mullin-type of analysis, which provides congener-specific information) will be published by the middle of 1991. The Department is monitoring ongoing exposure using urine and has found that with appropriate analytical sensitivity, one can determine the amount of PCB in urine. This has also been observed with Seegal's monkeys, where the urine concentrations reflect ongoing exposure. (Exposure to Aroclor 1016 shows up in urine, but not in serum, because the liver metabolizes most of the congeners very rapidly.) Hopefully, within a year, New York may be in a position to provide much more information regarding human exposure.

One or two years ago, reported one participant, WHO funded an analysis of human milk to measure dioxins and furans. The study found that, on average, a breast-feeding child was exposed to over 30 to 40 pg/kg/day (on a TCDD equivalence basis), which is higher than the ADI that most countries have advocated. One participant reported that WHO concluded that the TEF approach, which is used to evaluate chronic toxicity, should not be used for human milk, because there is not lifetime exposure to breast milk.

Toxicity Issues

A paper by Tilson, Rogan, and Jacobson suggested that neurobehavioral effects in human infants were several orders of magnitude more sensitive than some of the other measures that have been used to assess dioxin-like congeners. These neurobehavioral effects may be due to the coplanars as well as some of the ortho-substituted monoplanar congeners. Perinatally exposed nonhuman primates show similar behavior dysfunctions, even with exposure only to Aroclor 1016. The human infant is at risk, has an immature blood/brain barrier, and has an immature liver that

is initially unable to metabolize congeners as well as an adult. We should consider whether it is the dioxins in the milk or the ortho-substituted congeners from which the infants are at greater risk for neurobehavioral effects. These effects (e.g., deficits in intelligence and inability to respond in the environment) pose long-term consequences.

Because of the differences in development between the human infant and the rat, one person argued that the suckling rat may not be a good model for human response. The suckling rat is much more immature at birth. For example, in the early weeks rats do not have much adipose tissue. This effect has been observed in for other contaminants, for example, lead and manganese. One participant recommended that if animal models are used to predict human responses, then developmental stages and their relative physiology should be compared as well.

Recent work using a rat model appears to indicate fairly significant male fertility problems associated with exposure to Aroclor 1254 via the milk only, but it is difficult to determine what the results mean in terms of which class of PCB congeners poses the problem. Lifetime exposure is one consideration, but effects on development in young organisms is an extremely important alternative hypothesis.

Can Congener Toxicity Be Related to Degree of Chlorination?

One person wondered if the Toxicology/Mechanism of Action Work Group had considered using percent chlorination to scale toxicity. Dr. Birnbaum explained that the group did not discuss that issue, but explained that toxicologically, that approach does not work. For example, two very distinct hexachlorinated biphenyls have totally different types of toxicities and different types of mechanisms of action. The dose responses for those toxicities may be orders of magnitude apart as well.

Should a Bioassay be Used?

A participant asked: If a bioassay approach is used, could there be a question as to whether or not the response seen is in fact due to the PCBs?

From a toxicological point of view, the response is the critical factor, answered one participant. Whether the effect is caused by a dioxin-like PCB, a dioxin or furan, a halogenated naphthalene, or an azoxybenzene, from a health perspective, does not make much difference. But it does hark back to the issue of whether common responses are elicited by a common mechanism of action. Nonetheless, some toxicologists see the bioassay approach as the better method, because it integrates all the additivity and synergistic effects that might be occurring.

The Use of TEFs for PCBs

Dr. Birnbaum addressed the three questions where consensus was not reached in the Exposure/Analytical Issues Work Group. First, do we need the TEF approach? If only the specific congeners are measured and not the toxicity potency of those chemicals, then information will be lacking. Second, is the TEF approach better than using Aroclor toxicity data? The Toxicity/Mechanisms of Action Work Group would say that yes, potentially the TEF approach would be more useful. Third, can we deal only with dioxin-like chemicals? The Toxicity/Mechanisms of Action Work Group would adamantly say no. It is clear that the nondioxin-like congeners have inherent toxicity of their own, distinct and different from the toxicity of the dioxin-like congeners.

In response to the statement that TEFs do not pertain to short-term exposure, Dr. Birnbaum stated that the TEF approach is important not only for chronic toxicity but is potentially important for acute or short-term toxicities as well. TEF numbers, even the in vivo numbers, are based upon short-term exposures. It would be nice to use TEFs for chronic effects, but that use is not validated.

Workshop Summary
Donald Barnes, Workshop Chair

Dr. Barnes, the workshop chair, stated that the workshop had met the goal of stimulating thinking on TEFs for PCBs. He concluded that the major conclusions and recommendations were the following:

- The application of TEFs to PCBs is not as straightforward as it is in the case of CDDs/CDFs.
- TEFs for dioxin-like congeners appear to be feasible. The resulting dioxin-like TEs should be added to the TEs for CDDs/CDFs to estimate the total dioxin-like risk.
- Other toxic end points would not be accounted for, however, by considering only dioxin-like TEs in a risk assessment.
- The nondioxin-like toxicities could be significant (e.g., neurotoxicity in animals [Ref Bush] and neurobehavioral development in humans [Ref Jacobson and Tillson]), and may be suited for a separate SAR-based TEF scheme of their own, although additional mechanism-of-action information is needed.
- Current dioxin-like TEFs appear to be useful in assessing traditional measures of wildlife toxicity.
- A TEF scheme for PCBs should be seen as an interim procedure. Efforts with promising bioassay approaches should be pursued vigorously.
- Additional testing of commercial mixtures is not likely to add significantly to our understanding of PCBs. Testing should focus on individual congeners and/or the mixtures of PCBs as they are found in the environment.
- Capillary column gas chromatography is capable of generating congener-specific information on mixtures of PCBs.
- However, analytical methods for congener-specific PCB determinations are at a stage of development comparable to the methods for CDDs/CDFs more than a decade ago. The Dioxin Implementation Program (DIP) should be reviewed as a model for the kind of effort that could lead to the development of a standard, congener-specific method for PCBs.
- To the degree that different governmental groups (both inside and outside the U.S.) and private groups are contemplating research on PCBs, this work should be coordinated in order to maximize its impact and effectiveness (similar to the

public/private partnership in the successful DIP endeavor).

APPENDIX A

AGENDA

U.S. Environmental Protection Agency

WORKSHOP ON TOXICITY EQUIVALENCY FACTORS FOR POLYCHLORINATED BIPHENYL CONGENERS

Holiday Inn Capitol
Washington, DC

December 11-12, 1990

AGENDA

TUESDAY, DECEMBER 11

8:00AM	Registration
9:00AM	Opening Remarks--Donald Barnes, Director, U.S. Environmental Protection Agency Science Advisory Board
9:30AM	A Perspective on Toxicity Equivalency Factors for PCBs--Stephen Safe, Texas A & M University
10:00AM	Discussion
10:15AM	Break
10:30AM	Work Groups Convene
12:00PM	Lunch
1:15PM	Work Groups Continue
5:00PM	Adjourn

WEDNESDAY, DECEMBER 12

9:00AM	Plenary Session-- Discussion of Work Group Conclusions
10:15AM	Break
11:45AM	Workshop Summary--Donald Barnes

APPENDIX B

DISCUSSION INITIATION ISSUES

U.S. Environmental Protection Agency

WORKSHOP ON TOXICITY EQUIVALENCY FACTORS FOR POLYCHLORINATED BIPHENYL CONGENERS

Holiday Inn Capitol
Washington, DC

December 11-12, 1990

DISCUSSION INITIATION ISSUES

Exposure Issues

Issue:

When subjected to environmental conditions, commercial PCB mixtures can undergo differential photolytic, enzymatic, and other degradation processes which can alter the original congener distribution. Current reporting of PCBs in environmental samples generally involves expressing the concentrations as total PCBs or as Aroclor equivalents with the underlying assumption that the degree of similarity to commercial formulations is sufficient to warrant expressing environmental concentrations in terms of Aroclors.

Some have expressed concern that reporting as total PCBs or as Aroclor equivalents provides little information about the potential biological significance of the particular mixture in the sample.

Questions:

Is the extent of environmental transformation of PCB mixtures significant? Would treating them as commercial mixture equivalents (i.e., not taking environmental transformation into account) under- or overestimate the hazard associated with these residues? If so, what are the most important transformation reactions, under what conditions, and what components of the commercial formulations would be expected to be transformed and to what?

Issue:

PCB congeners can be selectively metabolized and bioconcentrated through the food chain, thereby altering the congener distribution to which humans and other species are exposed.

Questions:

What is the relationship between concentrations of specific congeners in commercial mixtures, environmental media, and in fish, wildlife, and humans?

What congeners have been identified as being present in soils/sediments, water, particulate matter, and other ambient samples?

Are some congeners consistently seen in environmental samples; if so, could these congeners serve as markers for all PCBs?

Issue:

There are 209 possible PCB congeners. Subsets of these possible congeners have been suggested (e.g., Environmental Health Perspectives, Vol. 81, pp 225-239, 1989; Marine Pollution Bulletin, Vol. 19, pp 19-25, 1988) as important for purposes of concern about environmental exposure.

Questions:

What criteria should be employed in developing a subset of congeners?

Based on these criteria, which PCB congeners should be the focus of environmental sampling?

Analytical Issues

Issue:

Routine identification and measurement of specific PCB congeners would be necessary for any TEF scheme to be useful for risk assessment and risk management purposes. Availability of laboratories capable of performing these analyses, associated costs, and accuracy of data obtained are critical considerations in deciding whether moving to congener-specific analyses is warranted.

Questions:

What techniques are readily available, for which sample matrices, and what are the detection limits?

What are the problems with quantifying individual congeners? How comparable are results from different laboratories?

What are the implications of moving to congener-specific analyses, i.e., costs, availability of labs, analytical standards, and protocols?

Issue:

Short-term, biological assays, which more directly provide a measure of the mixtures potential toxicity, are an alternative approach to chemical analyses.

Questions:

What is the state of development of these techniques? Are they ready for use?

Have any of these techniques been validated for specific endpoints?

What is known about interferences, antagonists, and synergists?

Toxicity/Mechanisms of Action

Issue:

For purposes of TEF scheme development, a broad base of toxicity data on individual members of the family of chemicals should be available.

Questions:

For the PCBs, which endpoints are most important to intelligently compare toxicity of PCB congeners in different species and to design a weighting scheme?

Because physical/chemical properties of each PCB congener ultimately determine its toxicity, would it be useful to develop different TEF schemes based on the various possible PCB reactivity patterns that we already know something about, e.g., the so-called coplanar PCBs with dioxin-like toxic potential and promotion potential, the ortho-substituted but resistant PCBs with neurotoxic potential as well as hepatic tumor potential, the ortho-substituted PCBs metabolizable to hydroxy derivatives with estrogenic potential, etc.?

Issue:

Data obtained on PCB congener toxicity in the course of in vivo experiments differ with respect to species or strain used and dosing regime.

Question:

In light of these variations, are the current in vivo data sufficient to draw conclusions regarding relative potencies of PCB congeners and to support TEF scheme development applicable to humans and other species?

Issue:

The relative toxicity of individual isomers should be consistent across endpoints for purposes of TEF development.

Question:

How consistent are the relative potencies of PCB congeners across endpoints both for the in vivo and in vitro systems studied?

Issue:

There should be a demonstrated relationship between in vitro and in vivo test results.

Questions:

There has been a reliance on enzymatic data in developing proposed TEF schemes for the PCB congeners. How well do effects on liver enzymes correlate with other effects in different species (e.g., reproduction, development, immune function, neurotoxicity, and chloracnegenic potential)?

For the PCBs studied, is potency as a toxicant proportional to binding affinity? If so, for what effects?

Issue:

Fish and aquatic invertebrates typically have enzyme systems that are similar but not identical to some of those of mammals and birds.

Question:

How can the in vivo data on fish and aquatic invertebrates be utilized in TEF development?

Issue:

TEF scheme development requires an index chemical whose toxicity has been relatively well characterized.

Question:

Which PCB congener(s) should be used as an index chemical in developing a scheme and why?

Issue:

In developing a TEF scheme, a general additivity (or modest antagonism) but not synergism of toxicity among PCB congeners should be demonstrated.

Question:

To what extent has general additivity been shown in in vivo or in in vitro systems?

APPENDIX C

LIST OF PARTICIPATING SCIENTISTS

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WORKSHOP ON TOXICITY EQUIVALENCY FACTORS FOR POLYCHLORINATED BIPHENYL CONGENERS

Holiday Inn Capitol
Washington, DC
December 11-12, 1990

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December 11-12, 1990

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