Stepping Backward to Improve Assessment of PCB Congener Toxicities

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Polychlorinated biphenyls (PCBs) are ubiquitous global contaminants that have been intensively investigated for three decades. They are broad-acting toxicants occurring in complex mixtures and accurate risk assessment has proven to be elusive. Focusing on a limited set of end points and emphasizing a fixed set of congeners have led to more streamlined data sets that are meant to expedite hazard characterization and risk assessment for the most potent congeners—aryl hydrocarbon receptor (AhR) agonists. Unfortunately, this has made it impossible to confirm or deny significant contributions from the more prevalent components of the mixtures. PCBs may be only coincidentally present, rather than causal, in some diseases. Still, attempts to determine associations with incomplete residue data may lead to erroneous conclusions and make accurate risk assessment even more elusive. Responses not mediated through the AhR are presented and emphasize large data gaps. Dissimilar analytical reports emphasize that selection of analytes is not consistent. Collectively, these data confirm that AhR-focused objectives unintentionally created the impression that nonplanar PCBs have little if any potential for hazards to humans and wildlife. Near steady-state exposure of healthy adults are probably of minor consequence except for emerging correlations with non-Hodgkin's lymphoma; however, pulses of exposure to more labile mixtures may contribute to developmental effects without leaving a residue record. More broadly based criteria are suggested and harmonization of data collection and presentation are desirable. A more comprehensive list of PCB congeners is proposed that would provide more adequate data upon which to base associations with adverse outcomes. — *Environ Health Perspect* 106(Suppl 1):171–189 (1998). *http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-1/171-189hansen/abstract.html*

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The recently proposed reclassification of individual polychlorinated biphenyl (PCB) congeners (*1*) is welcome, but is only a step in the right direction. The rationale was "to organize risk analysis along biologically plausible lines, thereby avoiding misclassification of exposure and strengthening the toxicologic associations" (*1*). The possible toxicologic associations must be more comprehensive. The next step toward enhancing the accuracy of risk analysis is to base the associations on more accurate concepts of exposure.

Because PCBs are found in nearly every organism and matrix examined, some of the associations with diseases may well be coincidental. These associations may also merely reflect physiological changes due to the disease that enhance accumulation of lipophilic chemicals. Even if PCBs do not prove to be guilty of all the adverse effects for which they are suspected, it is important to determine the real hazards. The tremendous database generated can also serve as a guide for evaluating similar mixtures such as toxaphene, other pesticide mixes, petroleum hydrocarbons, diesel emissions, polynuclear aromatic hydrocarbons, and water disinfection byproducts. The database also permits examination of basic physiological, biochemical, and molecular mechanisms with well-defined structural probes. This review does not claim to include all aspects of PCB toxicity and occurrence. Rather it presents select toxicities supported by specific information and suggests other criteria for consideration in compiling a more complete list of congeners of concern. The proposed list of congeners is also based on more comprehensive occurrence and exposure data than in previous lists.

If exposure data are incomplete, important associations may be overlooked or misassigned. Earlier PCB priority classification criteria and schemes (*2–5*) were premature (*6–8*) and overfocused on the single set of aryl hydrocarbon receptor (AhR)-mediated end points (*8–10*). In spite of suggestions to consider other toxicities (*7–11*), the limited breadth still dominates. Analytical limitations and complexities also favored selective screening (*3,5*). In fact, the complete characterization of some PCB mixtures released into the environment has only recently been accomplished (*12*).

The most important development for assessment of potential hazards and risks associated with the 209 possible chlorobiphenyl (CB) congeners and various mixtures has been the mechanistic linkage of certain configurations with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-like activity via the AhR (*7,13*). Much of the work was based on the observation that PCBs induced both phenobarbital (PB) and 3-methylcholanthrene (3-MC) types of cytochrome P450 activities.

It was readily shown that non-*ortho* and mono-*ortho* CBs with lateral chlorines were highly potent and equivalency to TCDD could be predicted based on enzyme induction and other parameters (*2–4,7,10,11,13*). The toxic equivalency (TEQ) of a mixture could be determined by summing the products of each component times its equivalence

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Abbreviations used: Ah, aryl hydrocarbon; AhR, Ah receptor; BROD, benzyloxyresorufin *O*-dearylase; CB, chlorobiphenyl (specific); ER, estrogen receptor; HO-CB, hydroxy chlorobiphenyl; LH, luteinizing hormone; 3-MC, 3-methylcholanthrene; MROD, methoxyresorufin *O*-dealkylase; PB, phenobarbital; PCB, polychlorinated biphenyl (mixture; generic); PCDD, polychlorinated dibenzo-*p*-dioxin; PCDF, polychlorinated dibenzofuran; PROD, pentoxyresorufin *O*-dealkylase; SAR, structure–activity relationship; T_4, thyroxine; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TEQ, toxic equivalent; TSH, thyroid-stimulating hormone; UDPGT, UDP-glucuronyltransferase.

to TCDD (*7*). TCDD equivalent is more accurate, as compounds with no equivalency to TCDD can still be toxic by other mechanisms. In spite of their relatively low environmental levels (*9,12,14*) the coplanar and potentially coplanar PCBs frequently contributed more TCDD equivalents than all dioxins, including TCDD (*15–17*); on the other hand, polychlorinated dibenzofurans (PCDFs) may contribute significantly greater TCDD equivalents than PCBs and/or polychlorinated dibenzo-*p*-dioxins (PCDDs), depending on the environmental source of the mixture (*17,18*). It was not the apparent intent of these efforts to determine TCDD equivalencies to exclude all other toxicities; however, the momentum provided for compiling more restricted lists of analytes to be reported has made it more difficult to detect or deny other associations.

Selection Criteria

Given the high potencies and low levels of coplanar CBs, analytical priorities became necessary for routine screening of large numbers of samples (*3,19,20*). In addition, large data sets that might include extraneous or less important congener values are difficult to interpret. Therefore, selective lists were developed based on "toxic potential and occurrence" (*5,19–21*) or other factors such as occurrence and metabolic clearance (*22*).

Regulatory decisions and analytical considerations drove some lists (*20*), but toxicological considerations were based mostly on AhR potencies. Only about a dozen CBs exhibit significant AhR potencies (*7,11,13*). The small proportion of the 209 congeners considered can be seen by comparing the proposed lists with the list of congeners generally accepted as being dominant in commercial preparations (*12*) (Table 1). Additional congeners of little environmental relevance are included for later consideration in structure–activity relationships (SARs).

The improved analytical capabilities and detailed characterizations of commercial mixtures (*12,19*) are complemented by a better understanding of the systematic changes in molecular properties of the individual CBs. These properties have been summarized in a single source (*23*) and this has facilitated modeling of environmental dispersion, toxicokinetics, and receptor interactions. Thus, we are now in a more favorable position to resolve many of the ambiguities in the risk assessment of PCBs. Even though a major concern of this article is stepping back to gain a more complete view of the PCB congeners present, some toxicological justifications for expanding the profile of congeners reported will be presented first.

Toxicities Independent of the Ah Receptor

PCBs are very broad-acting toxicants (*7–9*) and AhR-mediated responses represent a limited spectrum of toxicities. More subtle and, perhaps, more insidious responses to the remaining 190 congeners are poorly defined. More than half of these, including some very prevalent CBs, have not even been investigated. Other than enzyme induction, the largest database (about 35 CBs) was generated by Shain and colleagues for dopamine depletion (*24*). Kodavanti and colleagues determined the stimulation of phorbol ester binding (*25*) and inhibition of calcium sequestration (*26*) in the cerebellum for 24 congeners, most of which overlapped those of Seegal. Shorter lists have been investigated for activation of microsomal ryanodine-sensitive calcium channels (*27*), activation of neutrophils (*28,29*), and stimulation of insulin release (*30*). In all of these assays, the major theme has been that lower chlorinated and *ortho*-chlorinated congeners are the most potent, and coplanar congeners have essentially no activity. Derived relative potencies of various congeners toward neurochemical end points are presented in Table 2.

Responses Mediated by Multiple Mechanisms

Disruption of Thyroid Hormone Homeostasis

Nearly all CBs and PCB mixtures disrupt thyroid hormone homeostasis by a variety of mechanisms or combinations of mechanisms (*17,31–43*). However, most thyroid studies have focused on higher chlorinated and higher TCDD TEQ congeners and similar mixtures such as Aroclor 1254. Data are scant for lower chlorinated Aroclors and the 40 to 80 more common congeners that might contribute to the thyroid effects. Coplanar AhR agonists are usually more potent, but removal of these compounds from complex mixtures does not always attenuate thyroid effects (*37*). One of the earliest demonstrated mechanisms for declines in serum thyroxine (T_4) involved enhanced excretion following induction of T_4 conjugation through UDP-glucuronosyltransferases (UDPGTs) (*31,32*). This has been proposed as the major mode of TCDD disruption of thyroid hormone homeostasis (*36*); nevertheless, environmental PCB mixtures with distinctly different TCDD TEQs had similar potencies for T_4 depletion (*17*) and removal of AhR agonists from a mixture of primarily tri- and tetra-CBs did not affect acute T_4 depletion (*37*).

Other than binding of hydroxy-PCBs to prealbumin (transthyretin), there are limited data to derive SARs for PCB effects on thyroid hormones because most studies were conducted with Aroclors. Effects of specific congeners have been reported in isolated studies with major differences in age, gender, and dosing protocols; however, some insight into PCB mechanisms of thyroid hormone disruption can be gained by discussing reported effects for PCB mixtures and individual CBs.

After acute (2-day) exposures, thyroid-stimulating hormone (TSH) levels may increase up to 5-fold (*38*) and the thyroid follicles show typical responses (*32,39*) to TSH-stimulated mobilization of T_4 (*39–41*). The follicular response (increased cell height and decreased colloid area) appears maximal at doses below those at which significant declines in serum T_4 are seen (*40,41*). Mobilization of stored T_4 from the thyroid follicles at doses lower than or times prior to those causing significant UDPGT induction (*17,38*) can cause elevations in serum T_4 (*17,42,43*); at higher doses, declines in serum T_4 are still seen at times prior to maximal UDPGT induction (*17,38,42,43*).

Modestly chlorinated congeners such as CB 47 and mixtures such as Aroclor 1242, as well as labile 2,3,6-substituted CBs, can acutely decrease serum T_4 to a greater extent (*17,37,38,40,42,43*) than more highly chlorinated congeners such as CBs 99 and 153 (*43,44*). Thus, mechanisms other than UDPGT induction, including direct effects on the thyroid gland (*32,33*), are also important and this makes additive and/or synergistic effects likely. Also, a strict TEQ approach will underestimate the thyroid hormone disruption potential of some environmental mixtures (*17*) as well as these congeners.

Long-term responses to Aroclor 1254 include direct thyroid effects (*32,33*) as well as pathological changes in liver, kidneys, and gastrointestinal tract (*7–9*). Collectively, these changes may be related to changes in the distribution of thyroid hormones, resulting in decreased serum levels that cannot be attributed to enhanced metabolism and clearance (*33*). TSH release from the

Table 1. Structures of chlorobipheyl congeners referred to in the text and inclusion in selective lists of congeners of importance. Designations of group or type in schemes B and C are as published and the same criteria may use different codes.

B & Z no.[a]	Chlorine		Scheme A[b]	Scheme B[c]	Scheme C[d]	Europe D[e]
	Ring A	Ring B				
1	2		++			
2	3		–			
3	4		+			
4	2	2	++			
5	23					
6	2	3	+			
8	2	4	++			(+)
10	26		+			
11	3	3	–			
14	35		–			
15	4	4	++			
16	23	2	++			
17	24	2	++			
18	25	2	++	3		(+)
19	26	2	++			
21	234		–			
22	23	4	+			
24	236					
25	24	3	+			
26	25	3	+			
28	24	4	++			++
30	246		–			
31	25	4	++		1A	[+]
32	26	4	+			
33	34	2	++			
37	34	4	++	4		
39	35	4				
40	23	23				
41	234	2				
42	23	24				
44	23	25	+	3	1A	[+]
45	236	2	+			
46	23	26	+			
47	24	24	++			[+]
48	245	2	++			
49	24	25	++			[+]
50	246	2				
51	24	26				
52	25	25	++	3	1A	++
53	25	26				
54	26	26				
56	23	34	++			[+]
60	234	4	++			
61	2345		–			
64	236	4				
66	24	34	++		2A	[+]
69	246	3				
70	25	34	++		1A	
71	26	34	+			
74	245	4	++	3	2A	[+]
75	246	4	–			
76	345	2	–			
77	34	34	++	1A	2A	
80	35	35	–			
81	345	4	–	4		
82	234	23	+			
84	236	23	+			
85	234	24	++			[+]
87	234	25	++	2		[+]
88	2346	2	–			
91	236	24	+			
92	235	25	+			
95	236	25	++			
97	245	23	+			[+]
99	245	24	++	2	3	[+]
100	246	24				
101	245	25	+	2	1B	++
103	246	25				
104	246	26				
105	234	34	++	1B	2A	[+]
110	236	34	+			[+]
114	2345	4	+	4		
118	245	34	++	1B	2A	++
119	246	34	–	4		
123	345	24		4		
126	345	34	++	1A	2A	
128	234	234	++	1B	2B	[+]
132	234	236				
133	235	235	–			
136	236	236	+			
138	234	245	++	1B	2B	++
141	2345	25	++			[+]
149	236	245	+			[+]
151	2356	25	+	3		[+]
153	245	245	++	2	3	++
155	246	246				
156	2345	34	++	1B	2A	[+]
157	234	345	+	4		
158	2346	34	+	4		
163	2356	34	+			
167	245	345	+	4	2A	
169	345	345		1A	2A	
170	2345	234	++	1B	2B	[+]
171	2346	234				
174	2345	236	+		1B	
177	2356	234	+	3	1B	
179	2356	236	+			
180	2345	245	++	2	3	++
181	23456	24	–			
182	2345	246	–			
183	2346	245	+	2	3	
187	2356	245	+	3	1B	[+]
189	2345	345	+	4		
190	23456	34				
194	2345	2345	+	2		[+]
196	2345	2346	+		3	
199(201)[a]	2345	2356	+	3	1B	
203	23456	245	+		3	
206	23456	2345	+			[+]
209	23456	23456				

[a]Numbering according to Ballschmiter and Zell (*14*) as revised (*12,19*). CB 199 (revised nomenclature) was referred to as CB 201 in the original designations and, thus, in earlier residue reports. [b]GM Frame, personal communication. Important congeners are indicated by (+) or, especially important, (++) based on coplanarity, occurrence in Aroclors, dechlorination products, and analytical considerations. Congeners safely ignored are indicated by (–). [c]Criteria for groups: 1A, the three pure MC-type inducers; 1B, the more potent and relatively abundant of the mixed inducers; 2, abundant known or predicted PB-type inducers; 3, abundant weak inducers which occur frequently in fish and invertebrates; 4, mixed inducers of low occurrence (*5*). [d]Criteria for groups: High occurrence in house dust and humans and/or demonstrated biological activity. 1A, weak PB inducers, estrogenic, not persistent; 1B, weak PB inducers, persistent; 2A, dioxinlike, moderately persistent; 2B, limited dioxinlike, persistent; 3, PB and 3-MC inducers, persistent (*1*). [e]The seven major congeners regulated, ++; Finland includes CBs 8 and 18, (+); Quality Assurance of Information for Marine Environmental Monitoring in Europe (QUASIMEME) additional congeners, [+] (*19,20*).

Table 2. Relative potencies of chlorobiphenyls in four different neurotoxicity bioassays. The 15 most abundant congeners and the reference potency for the most active congeners are indicated by bold numerals.

B & Z no.	Dopamine depletion[a]	Phorbol binding[b]	Brain calcium[c]	Brain calcium channels[d]
MonoCl				
1	0.35			
2	0.21			
3	0.19			
DiCl				
4	**1.00**	0.65	0.30	0.50
11	0.33	0.47	0.19	
14	0.29	0.38	0.14	
15	0.00	0.00		
TriCl				
18	0.78			
19		0.48	0.34	
21	0.32			
24	0.40			
25	0.28			
26	0.40			
28	0.33	< 0.3	0.35	
30	0.43			
31	0.36			
33	0.35			
39	0.21			
TetraCl				
40	0.11			
44	0.56			
47	0.56	0.31	0.41	
49	0.66			
50	0.90	0.68	0.33	
51		0.56	**1.00**	
52	0.74	**1.00**	0.49	0.33
54	0.00	0.00	0.00	0.00
66	0.25			0.00
69	0.82			
70				0.10
75	0.54			
77	0.00	0.00	0.00	
80			< 0.03	
PentaCl				
88				0.19
95				**1.00**
100	0.41			
103	0.41			0.34
104	0.69	0.74	0.44	0.11
105		0.29	0.45	
118		< 0.3	0.36	
126	0.00	0.00	< 0.03	0.00
HexaCl				
128		< 0.3	0.49	
133		< 0.3	0.47	
136		0.48	0.38	
153		< 0.3	0.36	0.10
155	0.41			
156		< 0.3	0.44	
169		0.00	0.00	
HeptaCl				
171	0.48			
180		0.00	0.50	
181	0.17			
183				

[a]Pheochromocytoma cells [(*24*); R Seegal, personal communication]. [b]Increased phorbol ester binding in cerebellar granule cells (*25*). [c]Inhibition of calcium uptake by cerebellar microsomes (*26*). [d]Activation of ryanodine binding to cerebellar microsomes [(*27*); I Pessah, personal communication].

pituitary does not appear to be impaired by Aroclor 1254 or Firemaster BP-6 (*33*). Yet, in contrast to Aroclor 1242 (*38,43*), TSH increases are very modest in response to Aroclor 1254 (*33,34*) and the thyroid response to TSH is decreased by Aroclor 1254 (*32,33*), further supporting mechanisms in addition to increased clearance.

One mechanism related to clearance is displacement of T_4 from prealbumin (transthyretin) by hydroxylated PCBs, PCDFs, and PCDDs; however, these compounds do not displace T_4 from thyroxine-binding globulin, which is a more important transport protein in humans than is prealbumin (*32,34,35*). Earlier work from Brouwer's group had shown that hydroxy-PCBs had profound effects on rat serum T_4 as well as retinol because the prealbumin-displaced T_4 was more readily metabolized by induced enzymes and more rapidly excreted. Prealbumin is thought to be important in translocation of T_4 into the brain so this mechanism may be related to neurotoxic effects of PCBs; however, the competitive binding effect on serum T_4 is probably much less important in man than in rodents (*35*).

Although enhanced clearance of T_4 through AhR-mediated induction of UDPGTs may be the major mechanism for thyroid disruption by TCDD, additional mechanisms are obviously involved in thyroid disruption by the more broadly acting PCBs. Some direct effects on the thyroid gland include:

- Decreased iodine uptake and/or T_4 synthesis (*32*)
- Decreased mobilization (higher chlorinated) (*32*)
- Enhanced mobilization (lower chlorinated) (*40–43*); this is a classic TSH response (*32*), but still may not be TSH-mediated in these cases
- Inhibition of response to TSH (*33*)

The different responses to lower chlorinated congeners and Aroclor 1242 as compared to Aroclor 1254 may be related to the weak estrogenicity of these lower chlorinated PCBs (*44–48*), which may also be proestrogens (*49*). The more slowly metabolized Aroclor 1254 may yield metabolites that tend to be antiestrogens (*50*) and the parent mixture contains a greater proportion of antiestrogenic CBs than does Aroclor 1242 (*12,51,52*). Similar to estradiol, Aroclor 1242 sensitizes pituitary cells to gonadotropin-releasing hormone within a narrow dose range (*51*). Estradiol causes a similar sensitization at the thyrotrope level (*32*), so modest accumulation of estrogenic PCBs would enhance TSH release. It would be anticipated that greater accumulations (longer exposure and, especially, higher chlorinated and antiestrogenic Aroclor 1254) would inhibit release.

There are also effects on catabolism, transport, and storage of thyroid hormones

in addition to those related to UDPGT induction. De-iodinases can be increased and decreased by Aroclor 1254, depending on age and gender (*34*) and sulfatase conjugation of T_3 might also be increased (*32*). Binding to transport proteins is affected by PCB metabolites (*35*). The liver contains a greater reserve of extrathyroidal T_4 than does the serum (*32*), and hepatic enlargement by PCBs is well documented (*7,9,11,33*); thus, the effects of Aroclor 1254 and polychlorinated biphenyls on T_4 distribution may significantly influence serum levels of thyroid hormones without changing whole-body reserves (*32,33*).

All of these mechanisms have potential for interactive effects, so the effect of PCBs on thyroid hormones is probably much more complex than that of TCDD. Additional SARs for acute effects of low-dose CBs on thyroid hormones are needed before any consistent patterns can be elucidated. A more direct comparison of Aroclors 1242 and 1254 would also confirm or deny if thyroid responses were different or if the different responses reflected different experimental designs.

The consequences of transient hypothyroidism in healthy adults are probably not severe, so contributions from PCBs should be of minimal public health significance. Nevertheless, immature animals, including humans, are differentially sensitive to thyroid hormone disruption during critical windows of development. Therefore, exposure to pulses of labile CBs may result in manifestations of hypothyroidism (or even brief hyperthyroidism) not apparent until evidence of exposure (residues) no longer exists. Potential for these pulses will be discussed later, but the relevance to biologically plausible toxicologic associations is immediately obvious.

Estrogenic and Antiestrogenic Effects

PCBs have long been known to be estrogenic (*45–48*), but *in vitro* estrogen receptor (ER) binding is not strong (*48*) except for some lower chlorinated hydroxy metabolites (HO-CBs) (*49*). More persistent higher chlorinated HO-CBs have been reported to be antiestrogenic (*50*). Coplanar AhR agonists are also generally considered to be antiestrogenic (*51,52*), but coplanar CB 77 may act as an estrogen in some assays (*53*) and coplanar CB 126 is uterotropic at very low doses (*42,54*).

Uterotropic activity in immature female or ovariectomized adult female rodents has been the accepted standard of estrogenic activity, but it can be a relatively insensitive end point (*55–57*). Other reproductive tract changes and enhanced gene expressions can be used if the uterotropic effect is ambiguous (*55,56*), but these end points are not predictive of ER-dependent responses in other tissues (*57*). Likewise, *in vitro* ER-binding studies may not accurately predict relative *in vivo* estrogenic actions for different agents (*38,50,56,57*).

Thus, the many attempts to confirm or deny the significance of estrogenic and antiestrogenic actions of PCBs have failed to reach a consensus. Because of the magnitude of the impact of globally distributed persistent xenoestrogens, it is important to accurately determine the potential hazards. It is unlikely that the data generated to date is adequately reliable for accurate hazard assessment because of the many and variable actions of PCBs interacting with the many and variable responses of estrogen-sensitive targets. Some considerations include:

- Wide-ranging and systematic differences in the molecular properties of individual PCBs (*23*), including receptor-binding potencies (*58,59*)
- Presence of at least two distinct ERs with significant differences in the ligand-binding domain, resulting in different affinities for xenoestrogens (*60*)
- Cell-specific modulation of responses of even the same ER to different ligands (*57,61–64*), mainly because of receptor-associated proteins required for transcriptional activity by the entire complex
- Point 3 is compounded by extremely limited data on PCB interactions with ERs other than from uterus or breast tissue
- Attempts to assess relative potencies from tests conducted under nonphysiological conditions (e.g. protein binding, endogenous hormone levels, other hormone modulators, *in vitro* cytotoxicity)
- Reluctance to recognize nonmonotonic dose–response relationships in spite of the effects of PCBs on all of the interactive factors listed above, as well as on enzyme levels and toxicokinetics.

To assess relevant ER-associated hazards of PCBs, it is more important to determine a number of different tissue-specific responses in live animals rather than multiple responses in the same tissue or in similar *in vitro* systems (*38,56–58,61,64*). The reported estrogenic/antiestrogenic activities of PCBs are highly variable and response specific because of the natures of the assays employed. Perhaps some harmonization of the assays would be helpful in this assessment. In the meantime, it is necessary to attempt to normalize the data (as in Table 2) to permit some direct comparisons.

Table 3 compares recent demonstrations of *in vitro* ER binding and *in vivo* uterotropic activities for specific CB congeners. The attempt to normalize *in vitro* data to relative potencies was much less straightforward for Table 3 than for Table 2 and these values should not be directly compared except within the same study. Some unpublished data from our laboratories are included to increase the proportion of environmentally relevant congeners.

Uterotropic responses are also not strictly comparable because experimental designs differed. Ranges of doses for some CBs, including some that failed to cause a significant response, are presented to demonstrate that nonmonotonic responses may be common (*42,44,53,71,74*). For perspective, uterotropic response to the known xenoestrogen, *o,p*-DDT, was included under conditions similar to many of the other studies (*17,37,42,44,66–69,71*).

A few estrogenic lower chlorinated HO-CBs are also included for comparison. Mainly antiestrogenic activities have been reported for higher chlorinated HO-CBs (*50,75*), persistent in human blood and various environmental sources (*76*). However, these data were even more difficult to extract and normalize, except for relative binding to ER from human MCF-7 cells (*75*). In addition, some of the antiestrogenic activities were attributed to cytotoxicity (*75*) and the type of response varied with the added concentrations of estradiol (*75*). Finally, the very properties that favor retention of these metabolites in the blood would render them less available to tissues (*77*). Indeed, the more highly chlorinated mixtures (from which these metabolites arise) have weaker *in vivo* estrogenic potential than the lower chlorinated mixtures (*45–48,75*). Therefore, these data are not presented, but the distinction between lower chlorinated HO-CBs and persistent HO-CBs should be considered.

Table 3 demonstrates poor resolution among congeners when only estrogenic potency in MCF-7 cells is considered, and rather poor *in vivo* predictability of ER binding in some cases. Tight protein binding (e.g., to prealbumin) is considered to be partly responsible for retention of persistent HO-CBs in the blood (*35,76*) and endogenous estrogen is also mostly bound to other proteins in serum; *in vitro* assays that take this serum protein binding into account have

Table 3. Normalized measures of estrogenic activities for selected chlorobiphenyls and some 4-hydroxy (HO-*n*) metabolites. The 10 most environmentally relevant congeners in the table are indicated by bold numerals.

B & Z no.	MCF-7 relative potency[a]	ER-binding RBA,[b] %	Uterotropic[c] Dose(s), mg/kg	Uterotropic[c] Max % increase	Reference
1	<0.0001				(65)
HO-1		0.04			(49)
diOH-1		1.11			(49)
3	<0.0001				(65)
10	<0.0001				(65)
HO-10		0.26			(49)
18		<0.004[d]	16	30	(66)
			128	37	(66)
HO-18	0.001	0.15			(49,65)
		0.177[d]			
21	0.0001				(65)
24	<0.0001				(65)
HO-30	0.01	2.38	70	72	(49,65)
			10	42	(51)
47		0.003[d]	60	43	(67)
48	0.0001				(65)
52		<0.001[d]	26	70	(51)
54	0.0001		6	6 (NS)[e]	(68,69)
			20	24	(69)
			60	59	(69)
	0.0001				(70)
60	<0.0001				(65)
61	0.0001				(65)
75	0.0001				(65)
77	0.1	0.14	0.2[e]	207	(53)
			2.2	0	(53)
			22	400	(53)
95			2×16	12	(71)
			2×32	0	(71)
			4×8	20	(71)
99			2×4	22	(71)
			2×8	18	(71)
			2×16	6 (NS)	(71)
			4×8	6 (NS)	(71)
o,p-DDT	0.0001	0.1	50	41	(48,65)
			2×4	0	(71)
			4×8	25	(71)
101		<0.001[d]			
104	0.0001	0.91	16	18 (NS)	(70)
			202	64	(70)
110		0.002[d]	32	42	(42)
			48	20 (NS)	(42)
			96	33	(42)
126		<0.001[d]	0.002	22	(42)
			0.04	70	(54)
			0.40	110	(54)
136	0.0001				(65)
153		0.004[d]	22	14 (NS)	(44)
			50	63	(44)
			100	46	(44)
			118	0	(44)
155	+*[f]	0.28			(70)

[a]MCF-7 cell proliferation assays of various designs. Except for reference (*65*), relative potencies had to be estimated and are not strictly comparable. [b]Relative estrogen receptor-binding affinities (RBA) were also estimated from data provided. Competitive binding with estradiol in rat uterine ER preparations were generally used, except for MCF-7 ER in one case (*53*). [c]The increase (above control = 100%) in relative or absolute uterine weight in immature or ovariectomized rodents. Dose routes and times (2–4 days) varied. Vehicle was corn oil except for topical ethanol (*53*), which is a CYP2E1 and CYP3A inducer (*72*), and sesame oil (*70*), which contains CYP P450 inhibitors (*73*). Some doses resulting in nonsignificant (NS) changes are included to permit judgment regarding linearity. [d]K Carlson, J Katzenellenbogen et al., unpublished results from replicated studies at 0 and 25°C. Only the PCB 18-OH was active at 0°C. [e]Doses were published as absolute amounts and were converted to mg/kg assuming 25-day mice weighed 20 g. No effect was observed if dosing started at 21 days rather than 25 days (*53*). [f]Significant increase but not linearly related to dose (*70*).

greater predictability for *in vivo* responses to weak estrogens (*77*). Because of the profound effects of PCBs (*7,17,38*) as well as dosage vehicles (*72,73*) on biotransformation enzymes and the enhanced activities of some primary metabolites (*49*), toxicokinetic changes can readily influence the outcome of *in vivo* tests. In addition to protein binding considerations and dichotomous effects on ER-modulating thyroid hormones, these enzyme changes may also account for some of the nonlinearity in dose–response relationships (*38,44,67,74,77*).

Although the uterotropic response is relatively insensitive, a significant effect at modest doses confirms at least the potential for weak estrogenic activity. Male (*74,78*) as well as female reproductive tissues and other tissues such as bone (*61*) and pituitary (*51,57,79–81*) are also sensitive to environmental estrogens as well as endogenous estradiol. In fact, the pituitary ryanodine receptor appears to be involved in GnRH-induced gonadotropin synthesis and secretion (*81*). Ryanodine-sensitive calcium channels are stimulated by di- and tri-*ortho* CBs (*27*) (Table 2); the weakly estrogenic Aroclor 1242 (*44–46,51,67*) also stimulates ryanodine-sensitive channels (I Pessah, personal communication) and enhances pituitary GnRH-mediated luteinizing hormone and follicle-stimulating hormone release (*51*). The PCB–pituitary interactions in thyroid hormone homeostasis were discussed in the previous section and T_4–estrogen interactions are well documented (*32,38*).

Table 3 demonstrates the breadth of congeners with some suggestion of weak estrogenic activity. About 50% of these (if CBs 47 and 48 are included) are environmentally relevant, so additivity is a definite possibility. Even though many of these relevant CBs (CBs 18, 52, 77, 95, and 110) are not persistent, pulses occur through various exposure routes (see "Sources and Identities of Intermittent Congeners"). However, as with thyroid disruption, these transient exposures may lead to biologically significant manifestations only in developing animals.

Table 3 also emphasizes the paucity of data for ER-related effects of environmentally relevant PCBs and it is hoped that additional *in vivo* data will be generated. The public health significance of PCB estrogenicity is far from established. At the same time, little advantage is gained by ignoring estrogen-like PCB actions until (or in case) a consensus is eventually reached. The larger disadvantage is that failure to

consider estrogenicity as a possible adverse effect of PCBs may result in further abridgement of effect and occurrence reports so that biologically plausible associations with adverse outcomes will be lost due to missing information. This also argues for multiple listings of congeners in different categories so that, for example, if the estrogenic action of CB 47 is concluded to be inconsequential, it can still be considered a thyroid hormone disruptor (*40*), neurotoxicant (Table 2), neutrophil activator (*28,29*), and inducer of CYPs 2B and 3A activities (see next section).

Enzyme Induction

PCBs influence the levels and activities of a wide spectrum of enzymes, especially CYP P450s and UDPGTs. Activities of biotransformation enzymes are important both as bioindicators of exposure, and as modulators of toxicokinetics and toxicity. Occupational exposure to PCBs induces hepatic enzymes in humans (*82*) and the distinct residue profiles of occupationally exposed humans, compared to less-exposed humans, can be accounted for by considering enzyme induction and the substrate preferences of the induced enzymes (*83*).

The more highly chlorinated of the former commercial PCB mixtures are generally the stronger inducers. Alvares and co-workers first reported that Aroclor 1254 induced both PB and 3-MC types of P450 activity. They later showed that the lower chlorinated Aroclor 1016, introduced as a substitute for Aroclor 1242, was mainly a PB-type inducer in rats (*82*).

Extensive work by Safe and co-workers (*4,7,84*) has firmly demonstrated that 3-MC-type induction is predictive of AhR potency. They also confirmed that mixed (PB + 3-MC) enzymes can be induced by some individual congeners as well as mixtures. The general rules developed by this group are used extensively in PCB classification schemes and are reasonably universally understood (*1,2,4,5,7,11,22,38,83–85*).

Table 4 includes relative CYP induction potencies, neurochemical potencies, and ER-related potencies for some of the more relevant CBs. Although there are no obvious relationships between induction and neurotoxicity/estrogenicity, all of the strong 3-MC inducers have some TCDD-like toxicities. The coplanar CBs 77, 126, and 169 have the greatest affinity for the AhR and are the most potent pure 3-MC inducers. Mono-*ortho* CBs with lateral chlorines (CBs 105, 114, 118, 123, 156, 157, 167, 189) induce both PB and 3-MC types of activities and have significant, but reduced, AhR affinities. Some di-*ortho* CBs (e.g., CBs 128, 138, 170) with lateral chlorines also induce both types of activities and have even more reduced affinities for the AhR. Di-*ortho* CBs 47, 99, 101, 110, 153, 163, 183, 187, 194, and 199 with 1 or 2 *para* chlorines are good PB inducers and have virtually no AhR-mediated toxicities. CBs 110 and 163 are unexpectedly strong PB-type inducers (*42,84*); the environmentally abundant and similar CB 149 should be assessed. Furthermore, although of doubtful environmental significance, other effects of CBs 64, 91, 163, and 164 should be compared to limited data on CBs 84 (*8,9*), 95 (*27*), and 110 (*42*) to enhance the understanding of this unique group of congeners.

CYP1A1 induction reaches maxima both *in vivo* and *in vitro* and decline at higher doses. CYP1A1 induction also interferes with PB-type induction by complex mixtures; e.g., if TCDD-like compounds are removed from a PCB-landfill extract, pentoxyresorufin-*O*-dealkylase (PROD) and benzyloxyresorufin-*O*-dearylase (BROD) induction is enhanced (*37*). The same phenomenon can be observed with single mixed inducers: the stronger mono-*ortho* mixed inducers (CBs 105, 118, 189) have rather flat PROD dose–response curves compared to pure PB-type inducers (*84*).

It is now known that 3-MC–TCDD-like induction increases aryl hydrocarbon hydrolase, CYP1A1, CYP1A2, and CYP1B1; PB-like induction increases CYP2B1, CYP2B2, CYP2A1, and CYP3A (*2,4,7,13, 17,22,38,82–85*). A number of additional phase 1 and phase 2 enzymes, especially UDPGTs, are also induced in concert (*85,86*) or with separate SARs (*4,7,31,34, 36–38,87–92*). CYPs of the 4A family are induced or suppressed, depending on congener and species (*89*). This database can be very useful for developing more complete associations with the AhR-dependent and AhR-independent actions of these compounds and for predicting biological activities of their mixtures. Building on and expanding this database is recommended.

The selective *O*-dealkylation and *O*-dearylation of resorufin ethers offers a sensitive *in vitro* estimation of the selective induction of critical P450s (*7,38,85, 87,93–96*). The following activities are differentiated by measuring resorufin production by oxidation of the indicated ethers in a sensitive fluorometric assay:

- CYP1A1 7-Ethoxyresorufin
- CYP1A2 7-Methoxyresorufin
- CYP2B1/2 PROD and BROD
- CYP3A23 BROD

Positions of testosterone hydroxylation can also differentiate activities (*91,96,97*). For noninvasive estimations, specific *N*-demethylations of caffeine can be used with [^{13}C]caffeine as breath tests for CYP1A2 and CYP2E1 (*96,98*). An erythromycin breath test or urinary excretion of 6β-hydroxycortisol are useful human biomarkers for CYP3A4 (*91*).

Unfortunately, most tests developed as bioindicators of environmental exposure have focused on CYP1A activities. PROD activity has been included in some surveys, but BROD appears to be a more sensitive indicator of exposure and may be more appropriate than PROD. Both basal activity and PCB inducibility is higher for BROD than for PROD in prepubertal rodents (*17,37,38,43,71,85,87*). Basal BROD activity is lower in adult female rats than in male rats and PCB induction of BROD activity in adult male Sprague-Dawley rats may be twice that of adult females (*99*). However, both basal and induced BROD activity in females is still higher than that of PROD.

Environmentally, ethoxyresorfin *O*-dealkylase activity is readily induced in fish whereas PROD activity is not (*100*). This may account for the higher proportion of normally labile congeners, metabolized by PB-induced enzymes, as compared to coplanar congeners which are metabolized by the coplanar-induced enzymes. CYP1A1 and CYP1A2 are not induced in aquatic/marine invertebrates (*100*), but CYP3A is induced in blue crabs (*101*); this may render fish/shellfish residue profiles distinct. BROD activity is higher than PROD in marine mammals (*102*). BROD may be less suitable for birds because rat CYP3A1 antibodies do not recognize the protein in embryonic chicken liver, even though erythromycin *N*-demethylation is induced by PB (*103*). In addition, PROD activity is induced to a greater extent than BROD by Aroclor 1260 in Japanese quail (*104*).

Failure to discriminate between CYP2B and CYP3A activities with BROD as a substrate is not especially critical as CYP2A1, CYP2B1/2, and CYP3A are regulated in concert by an endocrine-sensitive process (*85,86,105*). As endocrine disruption is a major consideration in associating PCB actions with bioindicators, the more inclusive BROD would be preferred because all four of these monooxygenases are important in the catabolism of steroids, especially testosterone (*85,86,91,96,97,105*). CYP3A is also active in the oxidative metabolism of

Table 4. Relative enzyme induction types and potencies for chlorobiphenyls of potential environmental relevance (*4,7,42,43,84*).

B & Z no.	Env rel[a]	3-MC[b]	PB[c]	Neurochemical[d]	Estrogenic[e]	Antiestrogenic[e]
1	++	0	0	+++	+	
3	+	0	0	++		
4	++	0	0	+++++		
6	+	0	0			
8	++	0	0			
10	+	0	0		+	
15	++			0		
16	++					
17	++					
18	++	0	+/–	++++	++	0
19	++			+++		
22	+					
24				+++	(o)	
25	+			++		
26	+			+++		
28	++		+	+++		
31	++			+++		
32	+					
33	++			+++		
37	++	+	+			
40				+		
42			+			
44	+			++++		
45	+					
46	+					
47	++	0	+++	+++	++	
48	++				+	
49	++		+	++++		
51				+++++		
52	++	0	+	+++++	++	0
54				0	++	
56	++					
60	++	++			0	
66	++		+	0,++		
70	++			+		
71	+					
74	++					
77	++	++	0	0	+++	++
81	–	+	++			
82	+					
84	+		(–)			
85	++					
87	++					
91	+					
92	+					
95	++	0	+	++++	+	
97	+					
99	++	0	+++		++	
100				++		
101	+	0	+++		(o)	
104				++	++	
105	++	++	+	+++		++
110	+	0	+++		++	
114	+	++	+			
118	++	+++	++	+		++
119	–	+	+			
123		++	+			
126	++	++++	+	0	+++	++
128	++	+	++	++		
132						
136	+		(–)	+++	+	
138	++	++	+++			
141	++					
149	+					
151	+					
153	++	0	+++	++	+++	0
155			+	++	++	
156	++	++	++	++		
157	+	++	0			
158	+	+	++			
163	+		++++			
167	+	+	++			
169		+++	(+)	0		++
170	++	+	++			
171				+++		
174	+					
177	+					
179	+					
180	++		+++	0,+++		
183	+		+++			
187	+		+++			
189	+	++	++			
194	+		+++			
196	+					
199 (201)[d]	+		+++			
203	+					
206	+					

Env rel, environmental relevance. [a]Environmental relevance is based on Frame's suggestions (Table 1). [b]3-MC, TCDD- or 3-methylcholanthrene-type induction of CYPs1A1, 1A2 and/or 1B1. 0, no demonstrated induction; +, relatively weak inducer through ++++; ++++, the most potent of the PCBs. [c]PB, phenobarbitol-type induction of CYPs 2A, 2B, and 3A; (–), evidence of suppressed activity; 0, no demonstrated induction; +/–, weak activity at high dose; +, weak activity through ++++; ++++, most potent of the assayed PCBs. [d]Neurochemical potencies (Table 2) are adequately resolved to permit a 5-unit designation, compared to the cruder estimates for other effects. [e]Table 3 and/or effects of major metabolites (*49,50,75*). 0, probable lack of activity; (0), lack of demonstrated binding, but activity suggested by SARs; coplanar CBs 77 and 126 are listed as more potent estrogens than antiestrogens because the estrogenic activity occurs at lower doses. [f]Designation 201 in earlier studies is actually CB 199.

chlorinated aromatics (*92*), even though CYP1A and CYP2B have been considered prototypes (*7*).

In summary, there is a firm link between enzyme induction and AhR effects. There are developing associations between induction and patterns of PCB body burdens; enzyme patterns and species/food chain differences in residues; metabolites and thyroid and ER-mediated effects. There is a large database describing enzyme induction by PCBs that has rather firm SARs for different enzymes. With induction/effects information on a few additional environmentally relevant congeners and some harmonization of other SARs, the CYP P450 induction type and potency seems to be the obvious index for categorizing associations among the various congener types and biological effects. It is still important to monitor a profile of biotransformation enzymes when comparing congeners and/or mixtures, when investigating responses mediated by metabolites, or when using enzyme activities as bioindicators (*38,87,106*).

Concepts of PCB Occurrence

With only the considerations in Table 4, it might seem obvious that the list of priority congeners (*5*) should be modified. Comprehensive reports of human burdens (*83*) and dietary exposures (*107*) confirm the importance of many congeners not routinely analyzed (Table 1). Nevertheless, these congeners recently shown to have biological activities not mediated through the AhR have already been omitted from several analytical studies and reports. Therefore, it is not unusual for newly

reported activities to be discounted because the CBs tested do not appear to be detected (*1*) in important matrices. It must be recognized that not analyzed/not reported is not synonymous with not detected; in addition, not detected at a point in time does not confirm lack of exposure to labile congeners.

Recent comprehensive characterizations of Aroclor mixtures, especially those by Frame and colleagues (*12,108*), have established a set of occurrence values that deviate from earlier studies (*14,19,109–112*) when the standards available were severely limited. Because of the better resolution on multiple systems, availability of all standards and close agreement among collaborating laboratories, these values are probably the most accurate published. It was suggested that the concentrations of CBs 91 and 95 might have been reversed in a classic Aroclor composition study (*109*) due to a typographical error. The senior author felt that the error was due to theoretical retention times almost too close to differentiate; furthermore, he expressed amazement that more cross-checking was not practiced before this 16-year-old report (*109*) was published intact in more recent documents (P Albro, personal communication).

Residue determinations conducted for environmental degradation and distribution studies still tend to include comprehensive lists of congeners more in line with Aroclor contents (*113–115*). Recent relatively unabridged reports, cross-referenced with the most recent reports of Aroclor compositions, are the most useful for developing concepts of actual exposure profiles. Even in these studies, important pairs of unresolved congeners might be reported as a single congener or include co-eluting congeners in the standards that are not present in the sample. Mass spectrometric differentiation of nonisomeric co-eluting congeners (e.g., 37/42, 66/95, 77/110) is important, so the method(s) of analysis should be noted before examining data tables.

The most universal exposure and global transport route is atmospheric, and the profile of congeners (Table 5) differs markedly from that normally associated with food chains and human residues (*1,7–9,21,107*). Part of this difference is due to the greater volatility (high in air) and more efficient metabolism (low in humans) of the lower chlorinated and tri-*ortho* penta- and hexaCBs (*23,83*). Another source of variation is the target analytes of the specific study. In addition to the mass spectrometric differentiation of nonisomeric co-eluting congeners in the Sangamo analyses (*116*), there are differences in the airborne congeners reported by the two closely related studies (Table 5) and by a separate laboratory analyzing the same extracts (*116*). These seemingly minor differences are not discrepancies. They merely illustrate how inaccurate concepts of residue profiles can originate and be perpetuated from considering a limited spectrum of even high quality comprehensive residue reports.

Even within these closely related data sets, the Green Bay data might be interpreted as showing that the very significant congeners 22 and 41 (Sangamo data set) were not present (Table 5). Likewise, the Sangamo data set did not include CBs 24, 26, and 63 reported at significant levels in the Green Bay study. This is more likely due to the analytes targeted rather than major distinctions in the two sets of samples.

A totally different perception of airborne PCBs would be formed from the plant accumulation data (Table 5). Peaks containing CBs 17, 47/48, 56/60, and 101 were consistently found at 2 to 3% of the airborne PCBs (Table 5). However, it might be assumed that the important CB 17 (Table 1) is absent from plants and CBs 47/48 and 56/60 are found only in vascular plants. Again, the differences are due mainly to choice of analytes, which is sometimes influenced by interferents peculiar to the matrix being analyzed.

The CB 77/110 peak dominates the plant samples even though it contributes only a modest percentage of the airborne PCBs (Table 5), so one or both CBs may have specific plant accumulation potential. This might be due to the greater number of congeners reported for the air samples (which would decrease the relative contribution of individual values). On the other hand, this peak was also the most consistently dominant one in lettuce, potato, and tomato samples collected either near the New Bedford, Massachusetts, superfund site or from nonlocal markets (*119*), which may account for high levels in the Italian national diet (*107*). As with the lichens (*117*), confirmed airborne CBs 17, 47/48, and 56/60 were not target analytes in the New Bedford study (*119*).

Although these differences in reported compositions may seem trivial and possibly irrelevant, they serve to emphasize that exclusion from an analytical report may simply mean that the congener was not a targeted analyte. Such exclusions build a base of historic data that influence future congeners selected for analysis, further distorting concepts of actual PCB profiles. The residues are adequately complex so that abridged reports of major congeners are preferred by many over more complete and detailed characterizations, further entrenching concepts of false absence.

Human Body Burdens

Recent comprehensive residue determination studies in humans are rare because of the selection of high priority congeners for quantitation. In older studies with limited standards available (*120,121*), there may be poor agreement among the congeners reported (*19*). There is, however, general agreement that CBs 138, 153, and 180 are the most consistently detected and quantitatively dominant congeners in human adipose tissue and breast milk (*120–131*). CBs 28, 118, and 170 also make frequent large contributions; the coplanar congeners 77, 126, and, less often, 169, make toxicologically important contributions. Together, CBs 138 and 153 frequently account for 40 to 60% of the reported PCB congeners (Tables 6, 7). Concentrations of CBs 138, 153, and 180 are highly correlated with total PCB in breast milk and can be used to determine important geographical and temporal trends (*123,127–131*). When corrected for background levels, it was also determined that these CBs were 96 to 98% absorbed by an infant from mother's milk (*131*). Therefore, they serve as useful, readily obtained values for general concepts of infant exposure. The result, however, has been that many attempts to associate disease with chlorinated aromatics have used highly abridged data consisting primarily of these three congeners and total dioxin TEQs. Possible associations with other congeners can be neither suggested nor denied.

The lack of testing associations among unreported congeners and diseases is basically unnoticed because of misconceptions regarding occurrence. The same potential misconceptions resulting from the choices of congeners reported (as discussed for airborne PCBs in Table 5) can be noted for breast milk PCBs (Tables 6, 7). For example, the Inuit (*122*), Dutch (*123*), and Norwegian (*126*) studies did not report peak 77/110 but analyzed CB 77 separately; the other three studies (*112,124,125*) reported peak 77/110 collectively. Variations in the reported contributions from CBs 74, 101, 128, 138, 153, and 180 might well be explained by population/geographical differences or the numbers of congeners considered in the percentage contribution calculations. Examples of

Table 5. Percent congener composition of airborne PCBs over an NPL landfill in southern Illinois, over two areas near Green Bay, Wisconsin (Lake Michigan), and in plant tissues.

B & Z no.[a]	Sangamo[b] landfill	North[c] Green Bay	UnivWisc[c] Green Bay	Lichens[d]	Vascular[e] plants	B & Z no.[a]	Sangamo[b] landfill	North[c] Green Bay	UnivWisc[c] Green Bay	Lichens[d]	Vascular[e] plants
4 + 10[f]		0.64	1.36			100		0.40	0.26		
5 + 8	0.21			2.1		101	2.59	2.84	3.02	2.0	11.0
6		1.66	1.23			105 (w 132,153)	0.18				7.0
7	0.05					107 IUPAC 109[a]		0.03	0.13		
8 w 5						110 (+ 77)	1.89	1.74	1.75	16.0	13.0
10 w 4						114 (w 134)	0.01				0.1
12 + 13	0.05	1.07	0.37			118	0.59	0.83	0.69	2.0	8.5
13 w 12						119	0.00	0.07	0.08		
15	0.91				0.1	123	0.00				
16 + 32	7.40	6.63	5.75			126	0.02				
17	2.18	2.85	2.74			128	0.05	0.13	0.15		1.5
18	7.38	5.75	6.48	1.0	0.1	129 (w 178)	0.02				0.5
22	5.64					131		0.10	0.12		
24 + 27		1.09	1.67			132 (w 105)	0.11				
25	0.29					134 (+ 114)	0.05	0.36	0.44		
26		1.58	2.20			135 + 124	0.00	0.36	0.51		1.5
27 w 24						136	0.26	0.00	0.00		
28 (+ 31)	19.89	14.07	13.49	3.0	0.1	137 (w 176)					0.5
29		0.27	0.60			138 (+ 163)	0.41	1.34	1.36	11.0	8.0
31 (w 28)					0.1	141	0.07	0.28	0.27		1.0
32 w 16						146	0.08	0.23	0.43	1.0	
33	1.37	4.15	4.41	0.5		149	0.59	0.90	1.02	8.0	5.0
37 (+ 42)	1.52	2.30	2.54			151	0.15	0.39	0.56	1.0	1.0
40	1.22	1.22	1.54		0.1	153 (+105,132)	0.41	2.36	2.64	10.0	5.0
41	4.48					156 + 171		0.23	0.30		0.1
42 (w 37)	2.90					157 + 200		0.00	0.14		
44	4.66	4.91	3.38		3.0	158	0.00	0.00	0.17		
45	1.92	0.85	1.70			163 (w 138)					
46		0.41	0.39			169	0.00	0.00	0.04		
47 + 48	2.87	3.15	2.37		4.0	170 + 190		0.25	0.83	0.5	0.5
48 w 47						171 w 156					
49	4.18	2.71	2.47			172 + 197		0.03	0.06		
51		0.52	0.42			173		0.00	0.02		0.1
52	6.42	5.96	5.20		2.0	174		0.34	0.56	0.5	0.3
56 + 60	2.22	2.52	2.36		2.5	175		0.00	0.06		
60 w 56						176 + 137	0.00	0.03	0.26		
63		0.80	2.27			177		0.09	0.33	0.3	
64 (+ 71)	3.53					178 (+ 129)	0.02	0.13	0.28	2.0	
66 (+ 95)	2.85	4.98	3.88	4.0	9.0	180		0.65	0.74	5.0	0.6
70 (+ 76)	3.35	4.68	3.93	3.5		182 (w 187)					
71 (w 64)						183		0.28	0.53	0.3	0.2
74	1.83	1.78	1.52		0.5	185		0.02	0.08		0.1
76 (w 70)						187 (+ 182)		0.60	0.23	2.5	0.9
77 (w 110)	0.23					189		0.00	0.06		
81	0.01					190 w 170					
82	0.22	0.35	0.25			191		0.00	0.01		0.1
83		0.10	0.12			193		0.00	0.08		
84 + 92	0.98	1.99	1.02			194		0.07	0.06		0.1
85		0.47	0.31			195 + 208		0.60	0.31		0.1
87 (+ 81)	1.04	1.38	1.16	+	6.0	196 + 203		0.27	0.95		0.1
91		0.70	0.42			199 IUPAC[a]		0.02	0.03		0.1
92 (w 84)	0.50					200 w 157[a]					
95 (w 66)	1.85					201 IUPAC[a]		0.33	0.41		
97	0.65	0.86	0.97		3.0	203 w 196					
99	0.87	1.34	0.84	+		208 w 195					
						Total	NA[g]	328 pg/m^3	339 pg/m^3	ca 8 ng/g	ca 5 ng/g

UnivWisc, University of Wisconsin. [a]Numbering according to Ballschmiter (*113*) as revised (*12,19*). The revised Ballschmiter convention differs in that congeners 107, 108, and 109 are now referred to as numbers 109, 107, and 108, respectively. Congeners 199, 200, and 201 are now numbers 200, 201, and 199, respectively. [b]High-volume collection on XAD-2 resin after filtering dust (*116*). [c]Over water (North Green Bay) and over land (University of Wisconsin-Green Bay) (*115*). [d]Estimated from detailed figure (*117*). Great Lakes region in Southeast Ontario. [e]Estimated from detailed figure (*118*). Canadian Arctic near military radar site. [f]Unresolved congeners are reported as X + Y for one and Y w (with) X for the co-eluting one(s). If one or more laboratories achieved resolution, co-elution is presented parenthetically; the Sangamo samples were analyzed by gas chromatography–mass spectrometry so nonisomeric co-eluting congeners (e.g., 66/95 and 77/110) are resolved. [g]Not available. Pooled extracts from eighteen 24-hr high-volume collections for bioassay.

Table 6. PCB congeners reported in human breast milk samples (mean nanogram per gram milk fat).

Congener	Quebec[a] Inuit n = 35–109 1989–1990 1st 3 days[c]	Quebec[a] Rural n = 16×6 1988–1989 14–21 days	Dutch[b] n = 19 1990–1992 7–14 days
28			12.1
52			2.6
66			11.6
70			18.5
77	0.025	0.008	0.019
99			19.7
101			1.5
105		4.4	9.4
118	58.7	17.4	35.5
126	0.209	0.08	0.152
128			4.0
137			16.8
138	230.6	38.9	129.9
141			1.1
151			0.9
153	394.8	37.4	174.7
156		6.2	21
169	0.221	0.033	0.084
170	45.9	10.3	37.1
177			6.3
180	191.2	20.2	76.8
183			12.2
187			20.0
194			8.6
195			2.9
202			0.9
Total	922	135	608

[a]Data from Dewailly et al. (*122*). [b]Data from Koopman–Esseboom et al. (*123*). [c]Postpartum time of samples.

congeners consistently found in most studies, but simply not included as analytes in other studies, are CBs 141, 157, 183, 187, and 206 (Table 7).

Experimental, occupational, and ambient exposures are to rather consistent levels of similar composition mixtures. If nearly all the PCBs ever detected in human tissues are considered, lifetime accumulation factors from Aroclor 1260 can be determined from very ambitious correlations with other constants (*83*). Equilibrium proportions of congeners in human tissues are generally achieved under rather constant exposure conditions within a few years, but these proportions will differ between highly exposed persons and those with normal levels (*83*). In addition, intermittent changes in lifestyle (e.g., refuse worker in Bloomington, Indiana, to sport fishing in Green Bay, Wisconsin) can distort the equilibrium residue composition. Total PCB residues may stay constant.

Although there have been suggested relationships, total serum PCB levels do not correlate especially well with specific disease conditions such as breast cancer (*132,133*). Better biologically plausible associations have been found with more specific and more direct measurements. In actual breast tissue, CB 99 (weakly estrogenic in Table 5) and DDE correlated strongly with ER-positive breast cancer whereas mirex and the mono-*ortho* CB 118 (coplanar, probable antiestrogen) correlated strongly with ER-negative breast cancer (*134*). In another recent study, the association between AhR agonists and non-Hodgkin's lymphoma (NHL) was reasonably discounted, but a positive association between adipose levels of higher chlorinated non-coplanar PCBs and the disease was found (*135*). A separate study has reported a highly significant correlation between total serum PCB and NHL (*136*).

In order to determine if these correlations are of public health significance (*132–136*) or coincidental (*83,137*), more reliable PCB analyses must be conducted (*138*) and more complete descriptions of unabridged residues must be compared. No biologically plausible associations can be found for PCBs not investigated because they are infrequently reported and/or not reported because they are virtually uninvestigated. For the more labile congeners, especially in developing animals and humans, pulses of exposure may result in developmental changes not apparent until later in life when all evidence of the exposure (residues) are erased. Thus, it is important to consider congeners that are found intermittently (low frequencies) but at significant levels.

Sources and Identities of Intermittent Congeners

Another criterion for excluding some congeners from already complex analytical profiles is inconsistent or low frequency occurrence. Some means of data reduction is necessary and this can be accomplished by reporting ranges and/or mean concentrations and/or median concentrations. For intermittently present congeners, measures of central tendency are decreased when used for the entire data set. Occasionally, frequencies of detection above the limits of quantitation are also included but few readers have the time or inclination to take these into consideration or ponder over the implications. It is the responsibility of the analyst to ensure that the data and notations are not repeatedly abridged, thereby creating false impressions even if unintentional (*19*). Even if this responsibility is met (*124–126*), it is the reader's responsibility to make note of these exceptions when forming and relating concepts of occurrence.

Many of the congeners most often considered absent from food chains are not targeted in human samples, but are present at high levels in the environment (Table 5 vs Tables 6 and 7). These include many lightly chlorinated (diCB and triCB) congeners such as 4, 8, 16, 17, 18, 31, and 33 as well as the tetraCBs 47, 48, 49, 56, 60, and 70. TriCB 28 and tetraCBs 44, 52, 66, 74, and 77 are more frequently reported. Many of the infrequently reported lightly chlorinated congeners are active (or share characteristics with active but environmentally irrelevant congeners) in neurotoxicity (Table 2) or estrogenicity (Table 3) assays.

Confirmation of temporary exposure to lower chlorinated CBs was presented as early as 1981; currently exposed and occasionally exposed capacitor workers had higher proportions of lower chlorinated CBs than did past-exposed workers (*139,140*). After a capacitor accident, several CBs including 18, 28, 33, and 66 were greatly elevated in the serum and adipose tissue of exposed workers (*141*). One month later, adipose levels of CB 18 were 10-fold lower, but still 4- to 10-fold higher than in the general population. Concentrations of CBs 153 and 156 remained very similar during the 30-day period, while CBs 101, 171, and 183 declined along with the lower chlorinated congeners (*141*). Should such accidental (or intense airborne) exposures occur in a nursing mother, the record of exposure would be erased before potential developmental deficits were realized.

Surprisingly, serum levels of CB 74 increased 4-fold and adipose levels doubled during this same period (*141*). It is possible that the CB 74 was mobilized from an unsampled adipose compartment (*142*), but the other congeners remained stable or declined. Thus, it adds credibility to previous suggestions (*83,120*) that increases in human body burdens of the virtually unstudied CB 74 might be due to dechlorination of higher chlorinated congeners in humans.

As with many of the lower chlorinated congeners, the 2,3,6-substitution pattern is very susceptible to metabolism by mammals (*7–9*) and most of these congeners are not considered important components of food chains. Hydroxy metabolites may be relevant, but this 2,3,6-substitution pattern is also the most susceptible to formation of methyl sulfonyl products through epoxidation and the intestinal β-lyase pathway

Table 7. Selected samples of additional studies reporting PCB congeners in human breast milk (% total quantified congeners based on mean nanogram per gram milk fat). Co-eluting congeners are designated as X+Y for the major congener and Yw (with) X for the minor cogenor as in Table 5.

Congener (sample *n*)	Michigan[a] (*1*)	Canada[b] (*412*)	Wales[c] (*32*)	Norway[d] (*28*)	Congener (sample *n*)	Michigan[a] (*1*)	Canada[b] (*412*)	Wales[c] (*32*)	Norway[d] (*28*)
15			9.4		136		Rare		
18			4.3		137	0.9	Low, rare		
22	0.6				138 (163;164)	10	6.65	10.1	23.33
28	8.8	1.87	4.7	2.10	141	0.3	0.21		0.05
31					146	1.9			
33	2.2	5.20			149			1.8	
37	2.9	3.33	Omit[e]		151	0.6	0.19		
41	1.3	0.83			153	12	38.67	12.7	30.75
44	0.8	Omit	1.8		156 (+202)	4.9	1.46	2.5	3.12
46	0.25				157	0.5	0.42		0.43
47 + 48	0.4				158	0.6			
48 w 47					163 (w 138)				
49	0.7	Omit			164 (w 138)				
52	1.9	1.87	3.9	Omit	167 (w 128)	0.8			
56 (w 60)					169				0.05
60 (+ 56)	0.7	2.91			170 (+ 190)	5.3	2.29	3.5	6.64
66 (+ 95)	NR[f]	1.66	3.3		171 + 202	0.4			
70 (+ 76)	0.6				172 + 192	0.8			
74	11	3.74	3.7	3.39	174	0.4			
76 (w 70)					177	0.6			
77 (w 110)				0.01	180	5.3	4.57	11.1	13.60
82 (w 151)					182 (w 187)				
87 (+ 115)	0.8	0.62			183	1.4	2.54	1.2	
95	NR		NR		185	0.1			
99	4.8	5.82	4	3.63	187 (+ 182)	1.5	1.87	6.3	
101	1	1.04	2.2	0.30	188			1.8*[h]	
105 (+ 132)		Low, rare[g]	2.8	2.07	189	2.4	0.21		
107 (new 109)	0.3				190 (w 170)				
110 (+77)	1	1.04	1.3		191	0.9	0.42		
114 (+ 134)	0.3			1.08	192 w 172				
115 (w 87)					193	0.2	1.04		
118	6.5	4.16	4.2	7.04	194	0.5	0.83	0.7	1.29
119	0.1		1.9		195	0.3			
122	0.5				196 w (203)	0.2			
126			1.1*[h]	0.04	201 (new 199)	0.8	1.25	1.8	
128 + 167	0.3	1.04	0.8	0.99	202 (w 171)				
129		0.62			203 + (196)	0.8	0.83		
130	0.6				205	0.1			
132 (w 105)					206	0.2	0.21		0.11
134 (w 114)					209	0.1	0.62		
135	0.5				Total %	103.65	100.02	100.00	100.03

[a]Data from Safe et al. (*112*). [b]Data from Mes et al. (*124*). [c]Data from Duarte-Davidson et al. (*125*). [d]Data from Johansen et al. (*126*). [e]Omit. The congener was detected, but omitted because of low frequency, erratic levels, interference, or some other inconsistency. [f]NR, not reported. The congener was not reported, but published chromatograms provided strong evidence of its presence. [g]Low, rare. The congener occurred infrequently above detection limits so that mean and median values were below detection. [h]Probable interference.

(*143*). These metabolites are persistent, bioactive (*143,144*), and probably responsible for selective accumulation in lung and liver (*145*).

Accumulation of parent CB 110 in produce has been described previously (*119*). The 2,3,6-chlorinated CBs 84, 91, 136, and especially 95, 110, and 149 are significant components of Aroclors (*12,108*) environmental residues (Table 5), and some tend to concentrate to greater extents than some of the more prevalent congeners in pelagic fish such as tuna (*146*). Although co-eluting CB 66 is generally about 50% higher than CB 95 in Aroclors (*12*) and environmental samples (*116*), they are present in about equal proportions in National Institute of Standards and Technology marine reference materials (*147*). CB 149, although initially included for quality assurance/quality control confirmation of CB 118 resolution (*19*), is found at significant levels in most marine samples (*148*). CBs 101, 110, and 118/149 dominate profiles in some Siberian lakes and fishes (*149*).

Fish have readily inducible CYP 1A activity and moderate to low CYP 2B/3A activities (*100*); therefore, coplanar and mono-*ortho* congeners would be depleted relative to lower chlorinated as well as tetraCBs, pentaCBs, and hexaCBs with 2 and 3 *ortho* chlorines. This is further suggested by comparing diverse fish residue studies in order to provide a more complete profile of congeners detected through the different profiles of congeners selected for analysis (Table 8). These profiles have little resemblance to the profiles reported for human breast milk (Tables 6,7). Even the readily metabolized (by mammals) CB 4 was found in small lake trout and whitefish, but was below detection levels in medium and large fish (*150*). CBs 66/95, 101, and 110 were found in 100% of the samples of 98 fish comprising 15 different species from various waters in Wisconsin (*153*) (Table 9).

A more recent study (*154*) that quantitated 91 peaks representing well over 100 CBs in Lake Superior whitefish, trout, and walleye as well as carp and Pacific salmon (control) further distinguished fish residue

Table 8. Partial compositions of PCB residues in fish as reported by various authors.

B & Z no.	Hudson Estuary[a] Striped bass	Lake Superior[b] Large lake trout	Lake Ontario[c] Lake trout	Lake Ontario[c] Coho salmon	Deep N. Atlantic[d] *C. armatus* (Rattail)
4	++[a]	0.00[b]			
8		11.43			
10	++				
15		12.57			
18	++	2.40			
28 + 31	1.4		0.72	1.12	0.22
31	1.9				
33		8.29			
40		1.71			
42	2.8				
44	2.3				0.84
47	4		1.40	1.42	
49	4.7	3.43			0.99
52	4.4	3.71	1.49	1.58	1.72
56+60			1.85	1.78	
59	3.1				
60					0.33
64	4.1				
66	2.4		3.54	4.27	
70	2.4				0.66
70+76			3.21	4.17	
74	1.6				
77			0.18	0.41	
81			0.90	1.32	
82	3.8				
84	2.4		6.38	5.49	
85	1.9				
86					0.99
87					0.59
87+97			3.91	6.86	
95		3.71	1.71	2.13	4.51
97	1.5				
99	2.8				
101	3	6.29	6.11	6.50	7.33
105	0.8		2.54	2.44	4.73
110			4.77	5.79	
118	2.5	6.00	6.36	5.08	
128	1.1		1.74	1.52	1.61
129					2.49
136	2.4				
137		4.29			1.68
138	4.5	16.00	6.03	5.49	21.83
141			1.97	1.68	0.55
149	1.6	3.14	3.28	4.93	
151	1.3				
153	7		10.84	7.77	20.88
156					1.98
170	1.2				
170+190			2.07	1.63	
180	3.1	7.14	4.73	3.96	12.89
182+187			2.86	2.64	
183	1		1.65	1.42	4.40
187	1.7	5.14			
190					
194	0.6	1.60	0.56	0.46	2.16
195					2.20
196+203			1.25	1.02	
199 (201)		3.14	1.10	0.91	
203					
206		0.00	0.24	0.05	1.94
207					0.37
209			0.16	0.00	0.00
% Sum			83.544	83.841	97.875
No. CBs	32/74	19/22	37/92	37/92	24/??

[a] n=60. Other areas were sampled. Early eluting peaks (CBs 4, 10, 18, 19) were at high levels upstream but marginal in the estuary (*152*). [b] n=6. Siskiwit Lake on Isle Royale. Also includes different sizes of trout and whitefish. CB 4 contributed to residues in small trout and whitefish, but was below quantitation in larger fish (*150*). [c] n=10. Only the 37 most common of 92 congeners measured were reported (*6*). [d] n=5. From Hudson Canyon off New York. Area more contaminated than comparable collection site off Newfoundland. Primary objective was to confirm CYP1A induction (*151*).

profiles from those normally claimed for the food chain. The only peaks detected consistently above limits of quantitation in all five species were congener pairs 5/8, 16/32, 66/95, 70/76, and 77/110 (*154*). Of the 91 peaks, the 6 most dominant peaks in the carp were CBs 28/31 < 49 < 66/95 < 41/64/71 < 52 < 16/32.

Human Implications

Serum total PCBs in Native Americans consuming the well-characterized fish above (*154*) were more related to age than to fish consumption (*155*). Correlations with specific congeners have not yet been tested. Even then, it is unlikely that strong correlations would be found because most of these congeners are quite labile in humans. Nevertheless, pulses of these labile and unexpected congeners would be anticipated following every fish meal, although the intermittent levels would not be as high and persistent as with the accidentally exposed workers (*141*). In fact, such pulses of total PCB after fish meals have been recorded with maximum serum levels being reached in about 10 hr and declining rapidly to near ambient by 48 hr (*156*).

The major conclusion, however, must be that humans and other animals are intermittently exposed to profiles of PCBs that leave sparse records of their presence. Although the persistent congeners 118, 138, 153, and 180 remain dominant in adult human residues, young fish (*153*) and young children (*130,157*) may retain congeners infrequently encountered and/or reported in adults (Table 9).

There are plausible physical–chemical and toxicokinetic reasons for the distinctly different profiles of congeners in various matrices (*23*). The associations of PCBs and

Table 9. Congeners found to be prevalent in fish and children (mean % of total PCB).

Congener or pair	Fish residues (*153*) Mean	Fish residues (*153*) % Frequency	High-residue children (*157*) Female	High-residue children (*157*) Male
5 + 8			3.5	0.5
18			1.8	1.8
28 + 31	7.9	53	2.7	2.7
66 + 95	7.4	100		
70 + 76	4.5	72		
99			2.9	2.7
101	6.0	100		
105	2.6	78		
110	6.8	100		
118	2.7	92	4.6	6.5
138	8.1	90	17.0	14.0
146	3.4	83		
149	4.3	90		
153 + 132	11.0	92	19.6	15.8
170			4.6	3.9
180	3.7	87	8.1	6.8
182 + 187			2.7	2.0

Table 10. Proposed list of congeners which merit monitoring presented with estimates of relative exposures.

B & Z no.[a]	Substitution[b]	Frame[c]	Air[d]	Fish[e]	Meat, milk[f]	Produce[g]	B & Z no.[a]	Substitution[b]	Frame[c]	Air[d]	Fish[e]	Meat, milk[f]	Produce[g]
1	2	++	+				87	234-25	++	++	+	+	+++
3	4	+	+				91	236-24	+	+/–			
4	2-2	++	++	+			92	235-25	+	+/–			
6	2-3	+	++			+	95,66	236-25	++	++	+++	++	+++
8	2-4	++	+	+++		++	97	245-23	+	+	+		+++
10	26	+	+	+			99	245-24	++	+	+	+	++
15	4-4	++	+	+++	+	+	101	245-25	+	+++	++	++	++++
16,32	23-2	++	++++			++	105,153	234-34	++	+/–	+	+	++
17	24-2	++	+++				110,77	236-34	+	++	++	+	+++++
18	25-2	++	++++	+	+	++	114,134	2345-4	+	T		o	+
19	26-2	++					118	245-34	++	+	++	++	+++
22	23-4	+	++++			+	119	246-34	–	T			
24	236		++				126	345-34	+	T		T	
25	24-3	+	+/–[h]				128	234-234	++	+	+	+	++
26	25-3	+	++				136	236-236	+	+/–	+	+	+/–
28,31	24-4	++	+++++	+	++	++	138	234-245	++	+	+++	+++	+++
31,28	25-4	++	++	+	?	++	141	2345-25	++	+/–	+	+	+
32,16	26-4	+	?[h]			?	149	236-245	+	+	+++	++	+++
33	34-2	++	+++	++		+	151	2356-25	+	+/–	+	+	+
37	34-4	++	+++		+	++	153	245-245	++	++	+++	+++	+++
40	23-23		++	+		+	156	2345-34	++	+/–	+	+	+
42	23-24		++	+			157	234-345	+	T		T	+
44	23-25	+	+++	+	+	++	163,138	2356-34	+				
45	236-2	+	++				167	245-345	+				
46	23-26	+	+/–				169	345-345				T	
47,48	24-24	++	++	+	+/–	+++	170	2345-234	++	+	+	++	++
48,47	245-2	++	?	?	?	?	171	2346-234				+/–	
49	24-25	++	+++	++	+	++	174	2345-236	+	+		+	+
52	25-25	++	++++	++	++	++	177	2356-234	+	+/–		T	
56,60	23-34	++	++	+		++	179	2356-236	+				
60,56	234-4	++	?	?	+	?	180	2345-245	++	+	++	++	++
66,95	24-34	++	+++	++	+	++	183	2346-245	+	+	+	+	+
70	25-34	++	+++	++	+		187	2356-245	+	+	++	++	+
71	26-34	+					189	2345-345	+	T			
74	245-4	++	++			++	194	2345-2345	+	T	+	+	+
77,110	34-34	+	+/–	+	T		196,203	2345-2346	+	+	+		+
81	345-4	–	T[h]	(+)[h]			199(201)	2345-2356	+	T	++	++	+
82	234-23	+	+/–	++			203,196	23456-245	+	?	?		?
84	236-23	+	+	+++		+	206	23456-2345	+		+		+
85	234-24	++	+/–	+									

[a]Important co-eluting congeners are indicated after the primary congener to emphasize possible ambiguity; note that CB 199 was referred to as CB 201 in some studies. [b]Abreviated structures in format commonly presented. [c]Based on Aroclor occurrence (*12*), analytical compatibility, and potential dechlorination processes. [d]Mainly from Table 5. [e]Tables 8 and 9 and references (*146–154*). [f]Products from warm-blooded animals such as meat, milk, eggs, and cheese. Derived from the general literature and influenced by references (*8,9,12,107*). [g]From Table 5, especially modified according to Cullen et al. (*119*) and influenced by Turrio-Baldassarri et al. (*107*). [h]Other than obvious 1+ to 5+ semiquantitative estimates, +/– indicates very low and T indicates trace or extremely low. Because of frequent lack of resolution, some congeners (?) could not be estimated.

other persistent lipophilic substances with disease conditions may frequently be coincidental because of ubiquitous occurrence and selective retention based on physiological conditions associated with the disease rather than vice versa. The associations may also be causal. Some effort is necessary to expand the database to determine whether PCBs are guilty of many adverse health effects, as suspected. PCBs are broad-acting toxicants and disrupt neuroendocrine and biotransformation systems experimentally with a tremendous potential for interactions. The significance of these disruptions for public health and wildlife has not been adequately defined or even firmly established.

Some, but not all, factors have been suggested for consideration to develop a strategy to efficiently and accurately determine whether medical surveillance and/or intervention need to be increased or if resources should be focused on other problems. The knowledge and experience gained in defining the high-profile problem (or nonproblem) of PCBs will assist in the evaluation of other mixtures.

This review is not comprehensive and some additional complicating factors such as metabolites and kinetic interactions have been mostly neglected. For example, perhaps the persistent metabolites detected in human serum (*76*) are "just there," but the active metabolites have bound to receptors or other important cellular constituents. Certainly, little attention has been paid to the early observation (*145*) that 2,3,6-chlorinated PCBs and/or metabolites bind detectably to lung cells but are not found at significant levels elsewhere in the body. Insects, which comprise the greatest terrestrial animal biomass, may play a unique role in dispersion and biodegradation. Their unique susceptibilities (*158*) and potential for congener profile changes (*159,160*) have also not been addressed. New information regarding the dissociation of hepatic effects and other AhR effects in mice deficient in the protooncogene C-SRC (*161*) must also be considered.

If we step backward and look at the broader picture of PCB toxicity/occurrence and recall information previously reported, we will be in a better position to accurately assess the risks of environmental PCBs. Table 10 proposes an expanded list (based on this review) of PCBs that merit monitoring to encourage

a broader, more accurate, and more critical evaluation of the immense body of PCB information available. It is hoped that criticisms, comments, addenda, and most importantly, interest will be stimulated so that plausible toxicological associations can be confirmed or denied. Then we can move on to other problems with knowledge and confidence.

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