3. HEALTH EFFECTS

Contents of Chapter 3

3.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology and epidemiology of polychlorinated biphenyls (PCBs). It contains descriptions and evaluations of toxicological studies and epidemiological investigations, as well as toxicokinetic and other kinds of data pertinent to assessing the health effects of PCBs. Conclusions on the relevance of this information to public health, where possible, are discussed in Chapter 2 (Relevance to Public Health).

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

The health effects of PCBs have been extensively tested. Most studies investigated commercial PCBs mixtures that were produced in the United States before 1977 under Aroclor trade names. Health effects studies are also available for PCB mixtures produced in foreign countries. Among the most common tested foreign commercial PCB mixtures are Kanechlors, which were produced in Japan, and Clophens, which were produced in Germany. As in the United States, PCBs are no longer produced in Japan or

Germany. Foreign PCB mixtures differed from Aroclors mainly in percentages of individual chlorinated biphenyls, method of production, and level of contaminants. As discussed in Chapter 4, commercial PCB mixtures are comprised of various PCB congeners (there are 209 possible individual chlorinated biphenyls), as well as contaminants from the manufacturing process, particularly chlorinated dibenzofurans (CDFs). Information regarding the numbering system for PCBs and other chemical terms used to define the position of the chlorines on the biphenyl structure are provided in Chapter 4. The acronym PCBs is a general term used to refer to any commercial or other kind of mixture of congeners, such as environmental mixtures or animal tissue residues.

Evaluation of the health effects of PCB mixtures is complicated by numerous factors, particularly their congeneric composition, since ultimately, the toxicity of the mixture is due to the toxicity of the individual congeners, their interactions, and interactions with other structurally related chemicals such as CDFs and dioxins. For example, lot-to-lot differences in the congener distribution of commercial PCBs have been reported, which could contribute to some variations in toxicity observed among studies. The degree of CDF contamination is also a consideration in assessing the toxicity of commercial PCBs, because reported concentrations of CDFs varied among Aroclor formulations as well as with time period of manufacture. Concentrations of CDFs usually were higher in the Japanese and European PCBs than in Aroclors, and PCBs manufactured in the late 1970s had lower levels of contaminates than those produced earlier. In general, this profile is concerned with effects of PCBs in the presence of minimal CDF contamination. However, most health effects studies of PCB mixtures did not determine or report purity, or provide lot numbers that could be used to locate information on CDF contamination or congener distribution. Toxicological data for Kanechlors and Clophens are included in this chapter when these data provide information on effects that are not fully characterized for Aroclors because effects produced by Aroclors, Kanechlors, and Clophens are generally considered to be similar, at least for mixtures with equivalent percentages of chlorine (Kimbrough 1987). In addition, the lowest observed adverse effect levels for commercial PCB mixtures have been determined with Aroclors. Selected toxicity and mechanistic data on individual chlorinated biphenyl congeners also are included in this chapter because this information is potentially useful for assessing health effects and interactions of environmental mixtures of PCBs.

Using current health effects evaluation procedures, toxicity data for individual congeners may over- or underestimate the actual risk of PCB mixtures because the toxicity of congeners may be influenced by other congeners and chemicals in an additive, more than additive (synergistic), or less than additive (antagonistic) way. As discussed in Chapter 2 (Section 2.3), the current approach to assessing risks uses a

commercial mixture (Aroclor 1254) and an experimental mixture (a formulation representing the congeners found in breast milk) to develop health guidance values for environmental exposure to PCBs.

Information on health effects of PCBs in humans is available from studies of people exposed occupationally, by consumption of contaminated rice oil in Japan (the *Yusho* incident) and Taiwan (the *Yu-Cheng* incident), by consumption of contaminated fish and other food products of animal origin, and via general environmental exposures. As discussed in Chapter 6, people are environmentally exposed to PCBs that differ from commercial PCB mixtures due to changes in congener and impurity composition resulting from processes such as volatilization and other kinds of partitioning, chemical or biological transformation, and preferential bioaccumulation. Due to their stability and lipophilicity, PCBs usually accumulate in higher food-chain organisms and are stored in fatty tissues. Food consumption has been and continues to be the major source of body burden of PCBs in the general population. There is evidence that diets high in fish from PCB-contaminated waters, such as those in the Great Lakes and St. Lawrence River basins, can significantly increase a person's dietary intake of PCBs. Breast-fed infants of mothers who have diets high in contaminated fish may have a particularly increased risk for PCB exposure due to its presence in the milk.

PCBs are 1 of 11 persistent toxic substances that have been identified as critical Great Lakes pollutants by the International Joint Commission Water Quality Board (GLWQB 1985). In 1990, Congress amended the Federal Water Pollution Control Act and mandated the Environmental Protection Agency (EPA), in consultation with the Agency for Toxic Substances and Disease Registry (ATSDR) and the Great Lakes states, to submit a research report on the adverse human health effects related to water pollutants in the Great Lakes. Since then, ATSDR has awarded research grants and established cooperative agreements to coordinate basin-wide human health effects research. The primary interests of ATSDR's Great Lakes Human Health Effects Research Program are to document and characterize the exposure, identify populations at higher risk, identify associations between the consumption of contaminated Great Lakes fish and short and long-term harmful health effects, identify the most sensitive end points, establish registries and surveillance cohorts, and identify ways to prevent or mitigate exposure and resulting health effects (Johnson and DeRosa 1999; Johnson et al. 1998, 1999, 2000). PCB-related findings from the Great Lakes Research Program, as well as results from a number of other studies on health effects associated with exposures to PCBs through fish consumption, are included in this chapter.

Health effects have been observed in humans who consumed rice oil contaminated with heat-degraded Kanechlors in the *Yusho* and *Yu-Cheng* poisoning incidents. There is a historical linkage between

Yusho/Yu-Cheng and PCBs, and some health assessment documents ascribe effects from these incidents to PCBs. Unlike usual PCB mixtures, the *Yusho* and *Yu-Cheng* Kanechlors were heated in thermal heat exchangers (before rice oil contamination occurred) and also during cooking, resulting in the production of relatively high concentrations of CDF and polychlorinated quarterphenyl (PCQ) impurities. The concentrations of PCBs and PCQs in the rice oils were 100- to 500-fold greater than the CDFs. CDFs are generally considered the main causal agent, based on the following evidence: comparisons with Japanese workers with higher PCB blood levels who had few or none of the symptoms present in the rice oil poisonings; decreasing serum levels of PCBs in victims with persistent health effects; induction of *Yusho* health effects in animals exposed to reconstituted mixtures of CDF congeners similar to those in *Yusho* oils, but not by exposure to PCBs or PCQs alone; and comparative toxicity evaluations of PCB and CDF congeners in the unheated source mixture, contaminated rice oil, and tissues of victims (Bandiera et al. 1984; Kunita et al. 1985; Ryan et al. 1990; Safe 1990; Tanabe et al. 1989). Although there is a general consensus that CDFs were main contributors to the health effects in the *Yusho* and *Yu-Cheng* victims, certain PCB congeners have the same mechanism of action as CDFs and polychlorinated dibenzo-*p*-dioxins (CDDs). Effects of *Yusho* and *Yu-Cheng* exposure, therefore, are indirectly relevant to assessing health effects of PCBs because they demonstrate the sensitivity of humans to dioxin-like toxicity and suggest that humans might respond to dioxin-like PCB congeners in a similar manner. Additionally, recent evidence indicates that some of the subtle effects can be attributed to non-dioxin-like PCB congeners (Guo et al. 1996; Soong and Ling 1997). Brief summaries of the effects from the *Yusho* and *Yu-Cheng* incidents are presented in this profile; a more complete discussion of the health effects associated with the *Yusho* and *Yu-Cheng* incidents can be found in the ATSDR toxicological profile on CDFs (ATSDR 1994) and CDDs (ATSDR 1998), and reviews by Hsu et al. (1994) and Masuda (1994).

Fires and other sources of high temperatures, such as hazardous waste incinerators and electrical transformer fires, also can greatly increase the toxicity of PCB mixtures by formation of CDFs (Rappe and Buser 1989). For example, in a transformer fire in the Binghamton (New York) State Office Building (BSOB), dielectric fluid composed of 65% Aroclor 1254 and 35% polychlorinated benzenes was pyrolyzed. The pyrolysis led to the formation of a fine, oily soot, which was distributed throughout the building via ventilation shafts. In addition to PCBs, the soot contained high levels of CDFs, CDDs, including 2,3,7,8-tetrachlorodibenzodioxin (TCDD), chlorinated biphenylenes, and other chemicals. Limited information is available on health effects in people who were exposed to this soot dermally, by inhalation, or by ingestion from eating with dirty hands. A discussion of the health effects associated with the BSOB incident can be found in the ATSDR toxicological profile for CDFs and reports by

Schecter (1983, 1986, 1987), Schecter and Tiernan (1985), Schecter et al. (1985a, 1985b), and Fitzgerald et al. (1986, 1989).

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by health effect (death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects), and then by human and animal studies subdivided by type of exposure (e.g., occupational, contaminated fish consumption, inhalation, oral, and dermal). These data are discussed in terms of three exposure periods: acute (14 days or less), intermediate (15–364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELS have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects may start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the Levels of Significant Exposure (LSE) tables and figures may differ depending on the user's perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAEL) or exposure levels at or below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

Levels of exposure associated with carcinogenic effects (Cancer Effect Levels, CELs) of polychlorinated biphenyls are indicated in Table 3-2 and Figure 3-2. Because cancer effects could occur at lower exposure levels, Figure 3-2 also shows a range for the upper bound of estimated excess risks, ranging from a risk of 1 in 10,000 to 1 in 10,000,000 (10^{-4} to 10^{-7}), as developed by EPA.

Estimates of exposure levels posing minimal risk to humans (Minimal Risk Levels or MRLs) have been made for PCBs as discussed in Chapter 2 (Section 2.3) . An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

A User's Guide has been provided at the end of this profile (see Appendix B). This guide should aid in the interpretation of the tables and figures for Levels of Significant Exposure and the MRLs.

Table 3-1. Levels of Significant Exposure to PCB Mixtures - Inhalation

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (mg/m3)	LOAEL Less serious (mg/m3)	LOAEL Serious (mg/m3)	Reference Chemical Form
INTERMEDIATE EXPOSURE							
Systemic							
1	Rat (Sprague-Dawley)	30 d 7 d/wk 23 h/d	Hepatic	0.009 M			Casey et al. 1999 1242
			Endocr		0.009 M (increased thyroid serum T3 and T4 hormones)		
			Bd Wt	0.009			
			Other		0.009 (epithelial hyperplasia in urinary bladder)		
2	Rat (NS)	213 d 5 d/wk 7 hr/d	Hepatic		1.5 (unspecified moderately severe degeneration)		Treon et al. 1956 1254
			Renal		1.5 (slight degeneration of renal tubules)		
			Bd Wt	1.5			
3	Rat (NS)	24 d 5 d/wk 7 hr/d	Hepatic	8.6			Treon et al. 1956 1242
			Bd Wt	8.6			
4	Mouse (NS)	24 d 5 d/wk 7 hr/d	Hepatic	8.6			Treon et al. 1956 1242
			Bd Wt	8.6			
5	Mouse (NS)	213 d 5 d/wk 7 hr/d	Hepatic		1.5 (unspecified slight degeneration)		Treon et al. 1956 1254
			Renal	1.5			
			Bd Wt	1.5			

Table 3-1. Levels of Significant Exposure to PCB Mixtures - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (mg/m3)	LOAEL Less serious (mg/m3)	LOAEL Serious (mg/m3)	Reference Chemical Form
6	Gn pig (NS)	121 d 5 d/wk 7 hr/d	Hemato	5.4			Treon et al. 1956 1254
			Hepatic	5.4			
			Bd Wt		5.4 (16% decreased body weight gain)		
7	Gn pig (NS)	213 d 5 d/wk 7 hr/d	Hepatic		1.5 (slight vacuolation)		Treon et al. 1956 1254
			Renal	1.5			
			Bd Wt			1.5 (22% reduced body weight gain)	
8	Gn pig (NS)	24 d 5 d/wk 7 hr/d	Hepatic	8.6			Treon et al. 1956 1242
			Bd Wt	8.6			
9	Rabbit (NS)	121 d 5 d/wk 7 hr/d	Hemato	5.4			Treon et al. 1956 1254
			Hepatic	5.4			
			Renal	5.4			
			Bd Wt	5.4			
10	Rabbit (NS)	24 d 5 d/wk 7 hr/d	Hepatic	8.6			Treon et al. 1956 1242
			Bd Wt	8.6			

Table 3-1. Levels of Significant Exposure to PCB Mixtures - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (mg/m3)	LOAEL Less serious (mg/m3)	LOAEL Serious (mg/m3)	Reference Chemical Form
11	Rabbit (NS)	213 d 5 d/wk 7 hr/d	Hepatic		1.5 (hydropic degeneration, fatty changes)		Treon et al. 1956 1254
			Renal	1.5			
			Bd Wt	1.5			

[a]The number corresponds to entries in Figure 3-1.

Bd Wt = body weight; d = day(s); Gn Pig = guinea pig; Hemato = hematological; hr = hour(s); LOAEL = lowest-observable-adverse-effect level; NOAEL = no-observable- adverse-effect level; NS = not specified; wk = week(s); 1242 = Aroclor 1242; 1254 = Aroclor 1254

Figure 3-1. Levels of Significant Exposure to PCB Mixtures - Inhalation

Intermediate (15-364 days)

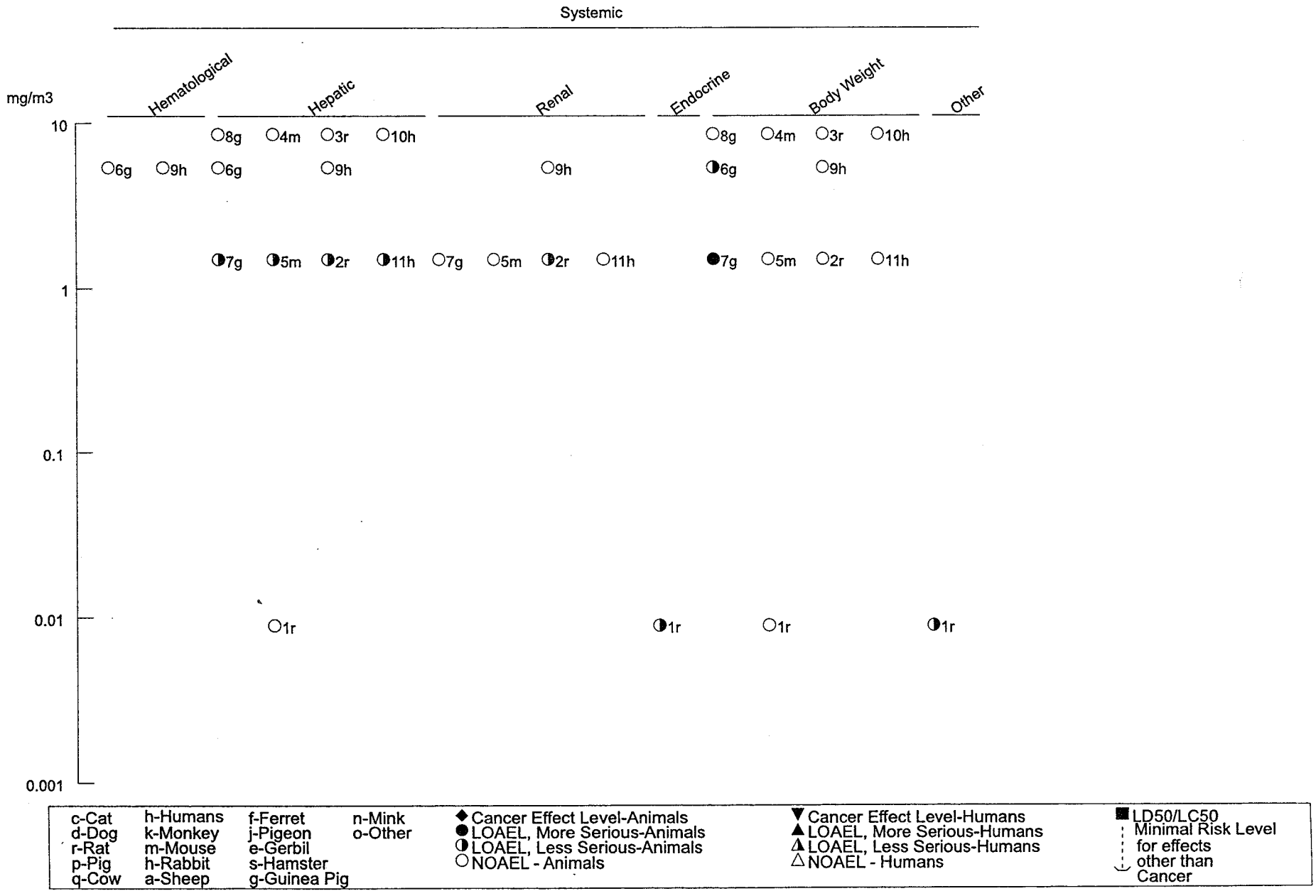


Table 3-2. Levels of Significant Exposure to PCB Mixtures - Oral

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
	ACUTE EXPOSURE						
	Death						
1	Rat (Sprague-Dawley)	once (GO)				4250 M (LD_{50})	Bruckner et al. 1973 1242
2	Rat (Osborne-Mendel)	once (GO)				1010 M (LD_{50})	Garthoff et al. 1981 1254
3	Rat (Sherman)	once (GO)				1295 M (LD_{50})	Linder et al. 1974 1254
4	Rat (Sherman)	once (GO)				1315 M (LD_{50})	Linder et al. 1974 1260
5	Mouse (ICR)	2 wk (F)				130 M (3/5 died)	Sanders et al. 1974 1254
6	Mink (NS)	once (G)				4000 (LD_{50})	Aulerich and Ringer 1977 1254
7	Mink (NS)	once (G)				750 (LD_{50})	Aulerich and Ringer 1977 1221
	Systemic						
8	Rat (Sprague-Dawley)	4 x (GO)	Bd Wt	25 F			Brown and Lamartiniere 1995 1221

Table 3-2. Levels of Significant Exposure to PCB Mixtures - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
9	Rat (Sprague-Dawley)	once (GO)	Resp	4000 M			Bruckner et al. 1973 1242
			Cardio	4000 M			
			Gastro	4000 M			
			Hemato		4000 M (crenated RBCs, increased PMNs)		
			Hepatic		4000 M (fatty vacuoles, necrotic foci)		
			Renal		4000 M (vacuolated, fatty tubular cells; protein casts)		
			Endocr	4000 M			
			Dermal	4000 M			
			Bd Wt	4000 M			
10	Rat (Fischer-344)	4 d (F)	Hepatic	0.5 M	1.0 M (increased serum cholesterol)		Carter 1984 1254
			Bd Wt	3.9 M			
11	Rat (Fischer-344)	4 d (F)	Hepatic	0.5 M	1.0 M (increased relative liver weight; increased serum cholesterol)		Carter 1985 1254
			Bd Wt	1.9 M			
12	Rat (Fischer-344)	2 wk (F)	Hepatic		1.9 M (increased serum cholesterol)		Carter and Koo 1984 1254
			Bd Wt	1.9 M			
13	Rat (Sprague-Dawley)	7 d (F)	Endocr		2.3 M (decreased thyroid serum T_4 hormone)		Hood et al. 1999 1254

Table 3-2. Levels of Significant Exposure to PCB Mixtures - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
14	Rat (Wistar)	6 d (F)	Hepatic		50 M (increased serum cholesterol and liver weight)		Kato and Yoshida 1980 1248
15	Rat (Wistar)	14 d (F)	Hepatic		50 F (vacuolar degeneration)		Kling et al. 1978 1254
			Bd Wt			50 F (30% decrease in body weight gain)	
16	Rat (Wistar)	7 d (F)	Hepatic		2.5 M (increased relative liver weight; decrease glucose 6-phosphatase in liver)		Price et al. 1988 1254
			Endocr		2.5 M (increased colloid droplets in thyroid; reduced serum T_4 hormone)		
17	Mouse (ICR)	2 wk (F)	Hepatic	130 M			Sanders et al. 1974 1254
			Endocr		130 M (10-fold increase in serum corticosterone; 2-fold increase in relative adrenal weight)		
18	Pig (NS)	11 d 1 x/d (G)	Gastro			100 (gastric ulceration)	Hansen et al. 1976 1254

Table 3-2. Levels of Significant Exposure to PCB Mixtures - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL				Reference Chemical Form
					Less Serious (mg/kg/day)		Serious (mg/kg/day)		
Neurological									
19	Rat (Sprague-Dawley)	once (GO)		1000 M	2500 M	(diminished exploratory behavior, decreased response to pain stimuli, unusual gait)	6000 M	(ataxia, coma)	Bruckner et al. 1973 1242
20	Rat (Fischer- 344)	10 d Gd 6-15 1 x/d (GO)		2			4	(behavioral alterations; impaired swimming performance and acquisition of one-way avoidance response)	Pantaleoni et al. 1988 1242
21	Rat (Wistar)	once (GO)			500 M	(decreased dopamine in caudate nucleus)			Seegal et al. 1986b 1254
Reproductive									
22	Rat (Holtzman)	5d Ld 1, 3, 5, 7, 9 1x/d (GO)		8 M			32 M	(decreased fertility in male offspring; 52% decreased number of fetuses)	Sager 1983 1254
23	Rat (Holtzman)	5d Ld 1, 3, 5, 7, 9 1 x/d (GO)			8 F	(reduced uterine weight and mating rate in female offspring)	64 F	(reduced implantation rate and increased post-implantation loss in female offspring)	Sager and Girard 1994 1254

Table 3-2. Levels of Significant Exposure to PCB Mixtures - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
24	Rat (Holtzman)	5 d Ld 1, 3, 5, 7, 9 1x/d (GO)				8 M (decreased fertility in male offspring; 21% decreased implants, 29% decreased embryos)	Sager et al. 1987 1254
25	Rat (Holtzman)	5 d Ld 1, 3, 5, 7, 9 1x/d (GO)		8 M		16 M (decreased fertility in male offspring)	Sager et al. 1991 1254
	Developmental						
26	Rat (Sherman)	9 d Gd 7-15 1 x/d (GO)		50		100 (60% decreased survival at weaning)	Linder et al. 1974 1254
27	Rat (Wistar)	7 d Gd 10-16 1x/d (GO)			5 (decreased thyroid plasma T_4 hormone in fetuses and 5-day-old pups)		Morse et al. 1996c 1254
28	Rat (Wistar)	10 d Gd 10-20 1x/d (GO)			25 (decreased thyroid serum T_4 hormone in pups)		Schuur et al. 1998a 1254
29	Rat (Sprague-Dawley)	10 d Gd 6-15 (F)		2.5	5 (12% decreased fetal weight)	15 (65% decreased fetal survival)	Spencer 1982 1254

Table 3-2. Levels of Significant Exposure to PCB Mixtures - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL			Reference Chemical Form
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
30	Rat (Wistar)	10 d Gd 6-15 1 x/d (GO)		100				Villeneuve et al. 1971 1254
31	Mouse (C57BL/ 6N)	once Gd 9 (GO)				244	(hydronephrosis)	Haake et al. 1987 1254
32	Mouse (ICR)	12 d Gd 6-18 (F)		12.5				Welsch 1985 1254

Table 3-2. Levels of Significant Exposure to PCB Mixtures - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	INTERMEDIATE EXPOSURE						
	Death						
33	Monkey (Rhesus)	2-3 mo (F)				4 M (nearly 100% mortality)	Allen 1975; Allen and Norback 1976 1248
34	Rat (Osborne-Mendel)	2.5 wk 2 x/wk (GO)				1530 M (LD_{50})	Garthoff et al. 1981 1254
35	Rat (Sherman)	8 mo (F)				72.4 F (8/10 died)	Kimbrough et al. 1972 1260
36	Mouse (BALB/c)	6 mo (F)				48.8 M (17/25 died)	Koller 1977 1254
37	Mink (NS)	4 mo (F)				2.8 (4/12 died)	Aulerich and Ringer 1977 1254
38	Mink (NS)	247 d (F)				1.9 (death in 2/3 males and 8/10 females)	Bleavins et al. 1980 1242
39	Mink (NS)	28 d (F)				1.2 (1 in 5 died)	Hornshaw et al. 1986 1254

Table 3-2. Levels of Significant Exposure to PCB Mixtures - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
	Systemic						
40	Monkey (Rhesus)	2-3 mo (F)	Gastro			4 M (hyperplasia, ulceration)	Allen 1975; Allen and Norback 1976 1248
			Hemato		4 M (unquantified anemia, increased macrophages, decreased WBCs)		
			Hepatic		4 M (hypertrophy, decreased serum cholesterol)		
			Dermal		4 M (alopecia, acne)		
			Ocular		4 M (excessive lacrimation, congestion of the conjunctiva)		
			Bd Wt			12 M (25% weight loss)	
41	Monkey (Rhesus)	3 mo (F)	Cardio			12 M (pericardial edema)	Allen and Norback 1973; Allen et al. 1973 1248
			Gastro			12 M (ulceration of gastric mucosa)	
			Hemato		12 M (moderate anemia; 18% decreased Hgb and Hct)		
			Dermal		12 M (alopecia, facial edema)		
			Ocular		12 M (eye discharge)		
			Bd Wt			12 M (26% weight loss)	

Table 3-2. Levels of Significant Exposure to PCB Mixtures - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
42	Monkey (Rhesus)	2 mo (F)	Gastro	0.8 F	1.3 F (gastric ulceration)		Allen et al. 1974a 1248
			Hemato	0.8 F	1.3 F (anemia)		
			Hepatic	0.8 F	1.3 F (focal necrosis)		
			Renal	1.3 F			
			Dermal		0.8 F (facial edema, alopecia)		
			Ocular		1.3 F (edema of the eyelids)		
43	Monkey (Rhesus)	2 mo (F)	Dermal		0.1 F (acne, alopecia)		Barsotti et al. 1976 1248
			Ocular		0.1 F (swelling of the eyelids)		
44	Monkey (Rhesus)	8 mo (F)	Hepatic		0.1 (lipid accumulation, focal necrosis, increased serum SGPT, decreased albumin/globulin ratio)		Barsotti et al. 1976 1248
45	Monkey (Rhesus)	2 mo (F)	Gastro		0.12 M (cysts formation in gastric submucosa)		Becker et al. 1979 1242
			Dermal		0.12 M (facial edema)		
			Ocular		0.12 M (reddening of eyelids)		
			Bd Wt		0.12 M (no weight gain)		
46	Rat (Fischer- 344)	10-15 wk 1 x/d (GO)	Musc/skel		0.1 M (increased femur density)		Andrews 1989 1254
			Endocr	25 M			

Table 3-2. Levels of Significant Exposure to PCB Mixtures - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL			Reference Chemical Form
					Less Serious (mg/kg/day)		Serious (mg/kg/day)	
47	Rat (Fischer- 344)	5 wk 1 x/d (GO)	Hepatic	1	10 M	(increased relative liver weight and serum cholesterol)		Andrews 1989 1254
			Renal	1	10 M	(increased relative kidney weight; 3-fold increase in urinary LDH; increase in protein in urine)		
			Bd Wt	10	25 M	(12-15% body weight loss)		
48	Rat (Sprague-Dawley)	3 wk 3 d/wk (GO)	Resp	100 M				Bruckner et al. 1973 1242
			Cardio	100 M				
			Gastro	100 M				
			Hepatic		100 M	(necrotic foci; increased SGOT)		
			Renal		100 M	(lipid vacuoles and protein casts in tubular epithelium)		
			Endocr	100 M				
			Dermal	100 M				

Table 3-2. Levels of Significant Exposure to PCB Mixtures - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
49	Rat (Sprague-Dawley)	2-6 mo (F)	Hemato	1.5 M			Bruckner et al. 1974 1242
			Hepatic		0.3 M (increased relative liver weight, lipid content and increased urinary coproporphyrin)		
			Renal	1.5 M			
			Endocr	1.5 M			
			Bd Wt	1.5 M			
50	Rat (Sprague-Dawley)	35 d (F)	Hepatic	0.25 M	1.25 M (increased relative liver weight and liver triglycerides)		Bruckner et al. 1977 1254
			Bd Wt	1.25 M			
51	Rat (Sprague-Dawley)	5 mo (F)	Endocr		0.09 F (decreased thyroid serum T_3 and T_4 hormones)		Byrne et al. 1987 1254
			Bd Wt	4.3 F			
52	Rat (Sprague-Dawley)	5-7 mo (F)	Hepatic	2.5 F			Byrne et al. 1988 1254
			Endocr	0.05 F	0.25 F (decreased adrenal serum corticosterone, DHEA and DHS hormones)		
53	Rat (Osborne-Mendel)	4 wk (F)	Endocr		0.25 M (altered thyroid follicular ultrastructure, increased serum T3 hormone)		Collins and Capen 1980a 1254

Table 3-2. Levels of Significant Exposure to PCB Mixtures - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
54	Rat (Osborne-Mendel)	4 wk (F)	Endocr		2.5 M (vacuolated thyroid follicular cells, decreased serum T_4 hormone)		Collins et al. 1977 1254
55	Rat (Sherman)	2 mo (F)	Hepatic		5 F (25% increase in relative liver weight, porphyria)		Goldstein et al. 1974 1254
			Bd Wt	5 F			
56	Rat (Fischer- 344)	15 wk 7 d/wk (GO)	Hepatic	0.1 M	1.0 M (increase liver weight; hypertrophy and vacuolar degeneration)		Gray et al. 1993 1254
			Renal	0.1 M	1.0 M (cortical tubular protein casts)		
			Endocr		0.1 M (reduced serum thyroxine levels)		
			Bd Wt	1.0 M	10 M (13% reduced weight gain)	25 M (55% reduced weight gain)	
57	Rat (Holtzman)	5 wk (F)	Endocr	0.025 M	0.25 M (altered thyroid follicular ultrastructure)		Kasza et al. 1978 1254
58	Rat (Wistar)	20 d (F)	Hepatic		15 M (increased liver weight and serum cholesterol)		Kato et al. 1982a 1248
			Endocr	15 M			
59	Rat (Sherman)	8 mo (F)	Hepatic	1.4 M	6.5 M (increased relative and absolute liver weight; cytoplasmic vacuolation)		Kimbrough et al. 1972 1260
			Bd Wt	7.2 F	32.8 M (12% reduced weight gain)	38.2 F (27% reduced weight gain)	

Table 3-2. Levels of Significant Exposure to PCB Mixtures - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
60	Rat (Sherman)	8 mo (F)	Hepatic	1.6 F	6.8 M (increase in relative liver weight; cytoplasmic vacuolation)		Kimbrough et al. 1972 1254
			Bd Wt	7.5 F		36.4 M (35% reduction in weight gain)	
61	Rat (Wistar)	30 d (F)	Hepatic			50 (severe vacuolar degeneration, lipid accumulation and necrosis)	Kling et al. 1978 1254
			Bd Wt			50 (72% decreased body weight gain)	
62	Rat (Osborne-Mendel)	4 wk (F)	Hepatic	2.5 M	25 M (increased liver triglycerides)		Litterst et al. 1972 1242
63	Rat (Wistar)	120 d 7 d/wk (F)	Endocr		7.1 M (degenerative changes in adrenal medulla)	14.3 M (increased severity of the adrenal changes)	Rao and Banerji 1993 1260
64	Rat (Sprague-Dawley)	35 d (gd 0-pnd 15) (F)	Endocr		12.5 F (increased relative thyroid weight; depressed T_4 levels)		Seo and Meserve 1995 1254
			Bd Wt	12.5 F		25 F (27% decreased body weight)	
65	Rat (NS)	10 wk (W)	Endocr	35 M			Wassermann et al. 1973 1221
66	Mouse (Swiss-Webster)	23 wk (F)	Dermal		26 F (hyperkeratosis, erythema, cysts)		Bell 1983 1254

Table 3-2. Levels of Significant Exposure to PCB Mixtures - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
67	Mouse (BALB/c)	6 or 11 mo (F)	Hepatic			49.8 M (liver necrosis, adenofibrosis, increased liver weight)	Kimbrough and Linder 1974 1254
			Bd Wt	49.8 M			
68	Mouse (BALB/c)	6 mo (F)	Hepatic	0.5 M	4.9 M (mild degeneration and necrosis of hepatocytes, increased absolute liver weight)	48.8 M (severe liver necrosis)	Koller 1977 1254
69	Mouse (BALB/c)	6 wk (F)	Resp	22 M			Loose et al. 1978a 1242
			Hepatic	22 M			
70	Gn pig (NS)	8 wk (F)	Hemato	4.0 F			Vos and de Roij 1972 1260
			Hepatic	4.0 F			
			Renal	4.0 F			
			Endocr	4.0 F			
			Dermal	4.0 F			
			Bd Wt	4.0 F			
71	Rabbit (New Zealand)	8 wk (F)	Hemato	6.5 M			Street and Sharma 1975 1254
			Hepatic	6.5 M			
			Renal	6.5 M			
			Endocr	6.5 M			
			Bd Wt	6.5 M			

Table 3-2. Levels of Significant Exposure to PCB Mixtures - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
72	Pig (NS)	91 d (F)	Gastro		9.2 (gastric erosions)		Hansen et al. 1976 1242
			Endocr		9.2 (increased relative adrenal weight)		
			Bd Wt			9.2 (55-62% decreased weight gain)	
73	Mink (NS)	39 wk (F)	Hemato	0.4			Aulerich and Ringer 1977 1254
			Bd Wt	0.4			
74	Mink (NS)	247 d (F)	Gastro	0.9		1.9 (gastric ulcers)	Bleavins et al. 1980 1242
			Bd Wt	0.9		1.9 (emaciation)	
75	Mink (NS)	28 d (F)	Gastro	1.8	3.9 (hemorrhage)		Hornshaw et al. 1986 1254
			Bd Wt	1.1	1.8 (10% body weight loss in treated, 7% weight gain in controls)		
	Immunological/Lymphoreticular						
76	Monkey (Rhesus)	11 mo (F)		0.1 F	0.2 F (decreased anti-SRBC hemolysin titers)		Thomas and Hinsdill 1978 1248
77	Monkey (Cynomolgus)	238 d (F)			0.1 F (decreased antibody response to SRBC antigen)		Truelove et al. 1982 1254

Table 3-2. Levels of Significant Exposure to PCB Mixtures - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
78	Rat (Fischer- 344)	5-15 wk 7 d/wk (GO)		1 M		10 M (decreased natural killer cells at 15 weeks, decreased thymus weight)	Smialowicz et al. 1989 1254
79	Mouse (BALB/c)	6 mo (F)		0.5 M	4.9 M (increased susceptibility to Moloney leukemia virus)		Koller 1977 1254
80	Mouse (BALB/c)	6 mo (F)		0.5 M	4.9 M (increased susceptibility to Moloney leukemia virus)		Koller 1977 1242
81	Mouse (BALB/c)	6 mo (F)		4.9 M			Koller 1977 1221
82	Mouse (BALB/c)	6 wk (F)				22 M (decreased resistance to bacterial endotoxin and protozoans leading to death)	Loose et al. 1978a 1242
83	Mouse (ARSF1)	5 wk (F)			13 F (increased sensitivity to bacterial endotoxin)	130 F (decreased resistance to bacterial infection resulting in death)	Thomas and Hinsdill 1978 1248
84	Gn pig (NS)	8 wk (F)			0.8 F (decreased gamma globulin- containing cells in lymph nodes)		Vos and de Roij 1972 1260
85	Gn Pig (albino)	6 wk (F)		0.8 F	4 F (decreased antibodies to tetanus toxoid and skin reactivity to tuberculin)		Vos and Van Driel-Grootenhuis 1972 Clophen A60

Table 3-2. Levels of Significant Exposure to PCB Mixtures - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
86	Rabbit (New Zealand)	8 wk (F)				0.18 M (marked thymic atrophy)	Street and Sharma 1975 1254
	Neurological						
87	Monkey (Cynomolgus)	20 wk Ld 1-140 1 x/d (G)			0.0075[b] M (decreased behavioral performance in nonspatial and spatial discrimination reversal tasks)		Rice 1997, 1998, 1999b; Rice and Hayward 1997, 1999a simulated human milk
88	Monkey (Macaque)	20 wk 7 d/wk (F)			0.8 (decreased dopamine content in caudate, putamen, substantia nigra, and hypothalamus)		Seegal et al. 1990 1016
89	Monkey (Macaque)	20 wk 7 d/wk (F)			0.8 M (decreased dopamine contents in brain areas)		Seegal et al. 1991b 1016
90	Monkey (Macaque)	20 wk 1 x/d (F)			3.2 M (significant reduction in dopamine in several brain areas)		Seegal et al. 1994 1016
91	Rat (Sprague-Dawley)	52 wk (F)		14.1 F			Freeman et al. 2000 1016
92	Rat (Sprague-Dawley)	52 wk (F)		7.5 F			Freeman et al. 2000 1242

Table 3-2. Levels of Significant Exposure to PCB Mixtures - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL				Reference Chemical Form
					Less Serious (mg/kg/day)		Serious (mg/kg/day)		
93	Rat (Sprague-Dawley)	52 wk (F)		6.9 F					Freeman et al. 2000 1254
94	Rat (Sprague-Dawley)	52 wk (F)		6.7 F					Freeman et al. 2000 1260
95	Rat (Long- Evans)	36 d Gd 6-21 Ld 1-21 (GO)			4	(elevated auditory threshold at 1 kHz)			Goldey et al. 1995 1254
96	Rat (Wistar)	80 d (F)					2.4	(impaired avoidance reaction and retention of a learned task)	Lilenthal and Winneke 1991 Clophen A-30
97	Rat (Wistar)	42 d (F)		0.13	1.3	(decreased motor coordination of pups, increased relative liver weight)	13.5	(50% neonatal death)	Overman et al. 1987 1254
98	Rat (Fischer- 344)	21 d ppd 1-21 1 x/d (GO)		1			2	(impaired learning, abnormal swimming behavior, decreased open field activity)	Pantaleoni et al. 1988 1242
	Reproductive								
99	Monkey (Rhesus)	2 mo (F)					0.8 F	(reduced conception rate, post-implant resorption and/or abortion)	Allen et al. 1974a 1248

Table 3-2. Levels of Significant Exposure to PCB Mixtures - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
100	Monkey (Rhesus)	38 wk 5 d/wk (F)				0.2 F (reduced conception rate, post-implant bleeding and abortion)	Arnold et al. 1990 1254
101	Monkey (Rhesus)	7 mo (F)			0.1 F (increased menstrual length)	0.2 F (reduced conception rate)	Barsotti et al. 1976 1248
102	Rat (Wistar)	1 mo 1 x/d (GO)				10 F (increased estrus, decreased receptivity, vaginal bleeding, delayed parturition)	Brezner et al. 1984 1254
103	Rat (Fischer- 344)	15 wk 7 d/wk (GO)		10 M	25 M (reduced seminal vesicle and epididymal weights and epididymal sperm counts following weanling exposure)		Gray et al. 1993 1254
104	Rat (Sherman)	67 d (F)		6.9		35.4 (decreased litter size)	Linder et al. 1974 1260
105	Mouse (ICR)	108 d (F)		1.25 F		12.5 F (55% decreased conception)	Welsch 1985 1254
106	Rabbit (New Zealand)	12-15 wk 3 x/wk (GO)		4 F			Seiler et al. 1994 1260
107	Mink (NS)	39 wk (F)				0.4 (decreased reproduction rates and litter size)	Aulerich and Ringer 1977 1254

Table 3-2. Levels of Significant Exposure to PCB Mixtures - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
108	Mink (NS)	21 wk (F)		0.2		0.9 (decreased reproduction rates and litter size)	Aulerich and Ringer 1977 1254
109	Mink (NS)	247 d (F)				0.9 (no reproduction)	Bleavins et al. 1980 1016
110	Mink (NS)	90 d (F)				1.3 F (48% reduced litter size with no live births)	Kihlstrom et al. 1992 1254
111	Mink (NS)	6 mo (F)		0.1 M			Wren et al. 1987b 1254
Developmental							
112	Monkey (Rhesus)	2 mo (F)				0.8 (2/3 resorption or abortion)	Allen et al. 1974a 1248
113	Monkey (rhesus, cynomolgus)	20 wk Ld 1 - 140 1x/d (G)			0.0075 (minimal reduction in IgM and IgG antibodies to SRBC, transient decrease in B lymphocytes)		Arnold et al. 1999 simulated human milk
114	Monkey (Cyno-molgus)	238 d (F)				0.1 (100% fetal death)	Truelove et al. 1982 1254
115	Rat (Wistar)	1 mo 1x/d (GO)				10 (35% decreased litter size, decreased pre- and post-weaning survival)	Brezner et al. 1984 1254

Table 3-2. Levels of Significant Exposure to PCB Mixtures - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference Chemical Form
116	Rat (Osborne-Mendel)	42 d Gd 1-Ppd 21 (F)					2.5	(decreased thyroid function of pups)	Collins and Capen 1980c 1254
117	Rat (Sprague-Dawley)	49 d Gd 1-Ppd 28 (F)			8	(decreased serum T_4 in 60-day pups after exposure through gestation and weaning)			Corey et al. 1996 1254
118	Rat (Long- Evans)	36 d Gd 6-21 Ld 1-21 (GO)			1	(decreased free and total T_4 serum levels in pups on Pnd 7, 14, and 21)	4	(15% pup mortality on postnatal day 21; 3% in controls)	Goldey et al. 1995 1254
119	Rat (Sprague-Dawley)	36 d Gd 1-21 Ld 1-15 (F)			3.1	(significant reduction in serum T_4 and in ChAT activity in brain from pups)			Juarez de Ku et al. 1994 1254
120	Rat (Sherman)	67 d (F)		6.9			35.4	(significantly reduced survival at weaning)	Linder et al. 1974 1260
121	Rat (Sherman)	187 d (F)		0.39	1.5	(enlarged liver cells and vacuolated cytoplasm in F2a)			Linder et al. 1974 1260
122	Rat (Sherman)	186 d (F)		7.2 F			37	(significant increase in preweaning mortality rate, lipid accumulation in hepatocytes from F1b)	Linder et al. 1974 1254

Table 3-2. Levels of Significant Exposure to PCB Mixtures - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
123	Rat (Sherman)	129 d (F)		0.32		1.5 (15-24% decreased litter size, lipid accumulation in hepatocytes)	Linder et al. 1974 1254
124	Rat (Wistar)	42 d (F)		0.13		13.5 (50% neonatal death)	Overman et al. 1987 1254
125	Rat (Sprague-Dawley)	51 d Gd 1-Ppd 30 (F)			0.1 (decreased thyroid serum T_3 and T_4 hormones in pups)		Provost et al. 1999 1254
126	Rat (Sprague-Dawley)	36 d Gd 1-Ppd 15 (F)			6.3 (reduced body serum temperature, T_4, oxygen consumption in offspring on day 15; body weight reduced 11%)	12.5 (27% reduction in pup body weight on day 15; reduced T_4; reduced body temperature)	Seo and Meserve 1995 1254
127	Rat (Sprague-Dawley)	35 d (gd 0-pnd 15) (F)			12.5 (reduced body temperature)		Seo and Meserve 1995 1254
128	Rat (Sprague-Dawley)	36 d Gd 6-ppd 21 (F)			1 (decreased thyroid serum T_4 hormone in pups)		Zoeller et al. 2000 1254
129	Mouse (ICR)	108 d (F)		12.5 F			Welsch 1985 1254
130	Gn pig (NS)	42 d Gd 18-60 1 x/d (GO)				2.5 F (34% increased fetal death)	Lundkvist 1990 Clophen A50

Table 3-2. Levels of Significant Exposure to PCB Mixtures - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL			Reference Chemical Form
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
131	Rabbit (New Zealand)	11 wk (F)				28 F	(focal liver necrosis in developing pups, severe vacuolization)	Thomas and Hinsdill 1980 1248
132	Rabbit (NS)	28 d Gd 1-28 1 x/d (GO)		10		12.5	(71% fetal death)	Villeneuve et al. 1971 1254
133	Mink (NS)	6 mo (F)				0.18	(neonatal death)	Wren et al. 1987b 1254

Table 3-2. Levels of Significant Exposure to PCB Mixtures - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
CHRONIC EXPOSURE							
Death							
134	Rat (Fischer- 344)	104-105 wk (F)				2.5 M (34% decreased survival)	NCI 1978 1254
Systemic							
135	Monkey (Rhesus)	17 mo (F)	Dermal		0.1 M (alopecia, acne periorbital edema)		Allen and Norback 1976 1248
			Bd Wt		0.1 M (body weight loss not quantitated)		
136	Monkey (Rhesus)	37 mo 1 x/d (C)	Hemato		0.02 F (decreased mean platelet volume)		Arnold et al. 1993a, 1993b 1254
			Hepatic		0.04 F (decreased serum cholesterol)		
			Endocr	0.08 F			
			Dermal		0.005 F (elevated and separated toenails)		
			Ocular		0.08 F (increased incidence of eye exudate; inflammation of Meibomian glands)		
			Bd Wt	0.08 F			

Table 3-2. Levels of Significant Exposure to PCB Mixtures - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
137	Monkey (Rhesus)	72 mo (F)	Resp	0.080 F			Arnold et al. 1997 1254
			Cardio	0.080 F			
			Gastro	0.080 F			
			Hemato	0.080 F			
			Hepatic	0.040	0.080 F (increased relative liver weights)		
			Renal	0.080 F			
			Endocr	0.080 F			
			Dermal	0.020	0.040 F (nail and nailbed changes)	0.080 F (severely altered finger and toenails)	
			Ocular	0.080 F			
			Bd Wt	0.080 F			
138	Monkey (Rhesus)	22 mo 1 x/d (C)	Endocr	0.08 F			Loo et al. 1989 1254
139	Monkey (Rhesus)	12 mo 5 d/wk (F)	Gastro		0.2 F (mucinous hypertrophy)		Tryphonas et al. 1986a 1254
			Hemato		0.2 F (hypoproliferative anemia)		
			Hepatic		0.2 F (hepatocyte necrosis, gall bladder and biliary duct hypertrophy)		
			Endocr	0.2 F			
			Dermal		0.2 F (nail loss, facial edema)		
			Ocular		0.2 F (conjunctivitis)		

Table 3-2. Levels of Significant Exposure to PCB Mixtures - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
140	Monkey (Rhesus)	28 mo 5 d/wk (F)	Gastro		0.2 F (hypertrophic gastropathy)		Tryphonas et al. 1986b 1254
			Hemato			0.2 F (severe normocytic anemia; 46-47% decreased hemoglobin and hematocrit)	
			Hepatic		0.2 F (liver hypertrophy and necrosis)		
			Endocr		0.2 F (thyroid desquammation)		
			Dermal		0.2 F (nail loss, gingival necrosis)		
			Bd Wt	0.2 F			
141	Rat (Sherman)	21 mo (F)	Bd Wt	5 F			Kimbrough et al. 1975 1260

Table 3-2. Levels of Significant Exposure to PCB Mixtures - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
142	Rat (Sprague-Dawley)	24 mo ad lib (F)	Resp	8.0 M 11.2 F			Mayes et al. 1998 1016
			Cardio	8.0 M 11.2 F			
			Gastro	8.0 M 11.2 F			
			Hemato	8.0 M	2.7 F (decreased RBC count and Hb concentration)		
			Musc/skel	8.0 M 11.2 F			
			Hepatic		2.0 M (hepatocellular 2.7 F hypertrophy and vacuolization)		
			Renal	8.0 M 11.2 F			
			Endocr	8.0 M 11.2 F			
			Ocular	8.0 M 11.2 F			
			Bd Wt	8.0 M 11.2 F			

Table 3-2. Levels of Significant Exposure to PCB Mixtures - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
143	Rat (Sprague-Dawley)	24 mo ad lib (F)	Resp	4.0 M 5.7 F			Mayes et al. 1998 1242
			Cardio	4.0 M 5.7 F			
			Gastro	4.0 M 5.7 F			
			Hemato	4.0 M 5.7 F			
			Musc/skel	4.0 M 5.7 F			
			Hepatic		2.0 M 2.8 F (hepatocellular hypertrophy and vacuolization, bile duct hyperplasia)		
			Renal	4.0 M 5.7 F			
			Endocr	 5.7 F	2.0 M (thyroid follicular cell hyperplasia)		
			Dermal	4.0 M 5.7 F			
			Ocular	4.0 M 5.7 F			
			Bd Wt	4.0 M 2.8 F	5.7 F (10% decreased final body weight)		

Table 3-2. Levels of Significant Exposure to PCB Mixtures - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
144	Rat (Sprague-Dawley)	24 mo ad lib (F)	Resp	4.3 M 6.1 F			Mayes et al. 1998 1254
			Cardio	4.3 M 6.1 F			
			Gastro	4.3 M 6.1 F			
			Hemato	4.3 M 6.1 F			
			Musc/skel	4.3 M 6.1 F			
			Hepatic		1.0 M (hepatocellular hypertrophy and vacuolization, bile duct hyperplasia, increased serum cholesterol) 1.4 F		
			Renal	4.3 M 6.1 F			
			Endocr	6.1 F	1.0 M (thyroid follicular cell hyperplasia)		
			Dermal	4.3 M 6.1 F			
			Ocular	4.3 M 6.1 F			
			Bd Wt	1.0 M	2.0 M (12% decreased final body weight) 1.4 F (15% decreased final body weight)	6.1 F (28% decreased final body weight)	

Table 3-2. Levels of Significant Exposure to PCB Mixtures - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
145	Rat (Sprague-Dawley)	24 mo ad lib (F)	Resp	4.1 M 5.8 F			Mayes et al. 1998 1260
			Cardio	4.1 M 5.8 F			
			Gastro	4.1 M 5.8 F			
			Hemato	4.1 M	1.4 F (decreased RBC count, Hb, and Hct)		
			Musc/skel	4.1 M 5.8 F			
			Hepatic		1.0 M (hepatocellular 1.4 F hypertrophy and vacuolization, bile duct hyperplasia)		
			Renal	4.1 M 5.8 F			
			Endocr	5.8 F	1.0 M (thyroid follicular cell hyperplasia)		
			Dermal	4.1 M 5.8 F			
			Ocular	4.1 M 5.8 F			
			Bd Wt	4.1 M 5.8 F			

Table 3-2. Levels of Significant Exposure to PCB Mixtures - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
146	Rat (Fischer- 344)	104-105 wk (F)	Dermal	1.25	2.5 (alopecia, facial edema)		NCI 1978 1254
			Ocular	1.25	2.5 (exophthalmia)		
			Bd Wt		1.25 F (10% decreased body weight gain)		
147	Rat (Wistar)	52 wk (F)	Hepatic	1	10 F (significant increase in absolute liver weight)		Phillips et al. 1972 1254
			Bd Wt	1 F		10 F (36% reduction in final body weight)	
Immunological/Lymphoreticular							
148	Monkey (Rhesus)	23 mo 7 d/wk (C)			0.005[c] F (reduced IgM and IgG antibody responses to sheep red blood cells)		Tryphonas et al. 1989 1254
Neurological							
149	Monkey (Rhesus)	16-21 mo Pmm 6- Ppm 3 (F)				0.1 (impaired learning and hyperactivity in offspring)	Bowman et al. 1978 1248
150	Monkey (Rhesus)	21.8 mo Pmm 7- Ppm 4 (F)		0.03			Levin et al. 1988 1016
151	Monkey (Rhesus)	18.2 mo Pmm 12- Ppm 4 (F)		0.007 F	0.03 F (decreased discrimination performance in offspring)		Schantz et al. 1989 1016

Table 3-2. Levels of Significant Exposure to PCB Mixtures - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
Reproductive							
152	Monkey (Rhesus)	17 mo (F)				0.1 M (decreased spermatogenesis, and libido)	Allen and Norback 1976 1248
153	Monkey (Rhesus)	37 mo 1 x/d (C)		0.005 F		0.02 F (42% reduced conception rate)	Arnold et al. 1995 1254
154	Rat (Sprague-Dawley)	24 mo ad lib (F)		8.0 M 11.2 F			Mayes et al. 1998 1016
155	Rat (Sprague-Dawley)	24 mo ad lib (F)		4.0 M 5.7 F			Mayes et al. 1998 1242
156	Rat (Sprague-Dawley)	24 mo ad lib (F)		4.3 M 6.1 F			Mayes et al. 1998 1254
157	Rat (Sprague-Dawley)	24 mo ad lib (F)		4.1 M 5.8 F			Mayes et al. 1998 1260
Developmental							
158	Monkey (Rhesus)	18.2 mo Pmm 3-Ppm 3 (F)				0.1 (50% mortality, dermal/ocular effects, and degenerative changes in thymus, spleen and lymph nodes)	Allen and Barsotti 1976 1248

Table 3-2. Levels of Significant Exposure to PCB Mixtures - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
159	Monkey (Rhesus)	18 mo (F)				0.1 F (72% infant deaths)	Allen et al. 1980 1248
160	Monkey (Rhesus)	~48 mo pmm 37-Ppw 22 1 x/d (C)			0.005 (inflammation of tarsal glands, nail lesions, gum recession, and reduced IgM antibody levels to SRBC in infant offspring)	0.02 (fetal and post-partum deaths in 4/4 impregnated monkeys)	Arnold et al. 1995 1254
161	Monkey (Rhesus)	72 mo (F)			0.005 F (inflammation of tarsal glands, nails and nail beds in infants)		Arnold et al. 1997 1254
162	Monkey (Rhesus)	12 mo (F)		0.007 F	0.03 (18% reduced birth weight)		Barsotti and Van Miller 1984 1016
163	Monkey (Rhesus)	21.8 mo Pmm 7-Ppm 4 (F)		0.007	0.03 (18% reduced birth weight)		Levin et al. 1988 1016
164	Monkey (Rhesus)	18.2 mo Pmm 12-Ppm 4 (F)		0.007 F	0.03 F (18% lower birth weight)		Schantz et al. 1989 1016
	Cancer						
165	Rat (Sherman)	21 mo (F)				5 F (CEL: liver hepatocellular carcinoma)	Kimbrough et al. 1975 1260

Table 3-2. Levels of Significant Exposure to PCB Mixtures - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
166	Rat (Sprague-Dawley)	24 mo ad lib (F)				5.4 F (CEL: liver hepatocellular adenoma)	Mayes et al. 1998 1016
167	Rat (Sprague-Dawley)	24 mo ad lib (F)				2.0 M (CEL: thyroid follicular cell adenoma) 2.8 F (CEL: liver hepatocellular adenoma)	Mayes et al. 1998 1242
168	Rat (Sprague-Dawley)	24 mo ad lib (F)				1.0 M (CEL: thyroid follicular cell adenoma) 1.4 F (CEL: liver hepatocellular adenoma)	Mayes et al. 1998 1254
169	Rat (Sprague-Dawley)	24 mo ad lib (F)				1.0 M (CEL: thyroid follicular cell adenoma) 1.4 F (CEL: liver hepatocellular adenoma)	Mayes et al. 1998 1260
170	Rat (Fischer-344)	104-105 wk (F)				1.25 (CEL: liver neoplastic nodules, adenoma and hepatocellular carcinoma)	NCI 1978 1254

Table 3-2. Levels of Significant Exposure to PCB Mixtures - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL				Reference Chemical Form
					Less Serious (mg/kg/day)		Serious (mg/kg/day)		
171	Rat (Sprague-Dawley)	24 mo (F)					4.2	(CEL: hepatocellular neoplasms)	Norback and Weltman 1985 1260

[a]The number corresponds to entries in Figure 3-2.
[b]Used to derive an intermediate oral minimal risk level (MRL) of 0.00003 mg/kg/day; dose divided by an uncertainty factor of 300 (10 for extrapolation from a LOAEL to a NOAEL, 3 for extrapolation from animals to humans, and 10 for human variability).
[c]Used to derive a chronic oral minimal risk level (MRL) of 0.00002 mg/kg/day; dose divided by an uncertainty factor of 300 (10 for extrapolation from a LOAEL to a NOAEL, 3 for extrapolation from animals to humans, and 10 for human variability).

Bd Wt = body weight; BUN = blood urea nitrogen; (C) = capsule; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); DHEA = dehydroepiandrosterone; DHS = dehydroepiandrosterone sulfate; Endocr = endocrine; F = female; (F) = feed; (G) = gavage; Gastro = gastrointestinal; Gd = gestational day; gest = gestation; Gn Pig = guinea pig; (GO) = gavage, oil; Hemato = hematological; Ld = lactation day; LDH = lactate dehydrogenase; LD_{50} = lethal dose, 50% kill; LOAEL = lowest-observable-adverse-effect level; M = male; mo = month(s); Musc/skel = musculoskeletal; NOAEL = no-observable-adverse-effect level; NS = not specified; Pmd = pre-mating day; Pmm = pre-mating month; PMN = polymorphonuclear; Ppd = post-parturition day; Ppw = post-parturition week; Ppm = post-parturition month; RBC = red blood cell; Resp = respiratory; SGOT= serum glutamic oxaloacetic transaminase; SRBC = sheep red blood cell; T3 = triiodothyronine; T4 = thyroxine; (W) drinking water; WBC = white blood cell; wk = week(s); x = time(s); 1016 = Aroclor 1016; 1221 = Aroclor 1221; 1242 = Aroclor 1242; 1248 = Aroclor 1248; 1254 = Aroclor 1254; 1260 = Aroclor 1260

Figure 3-2. Levels of Significant Exposure to PCB Mixtures - Oral

Acute (≤14 days)

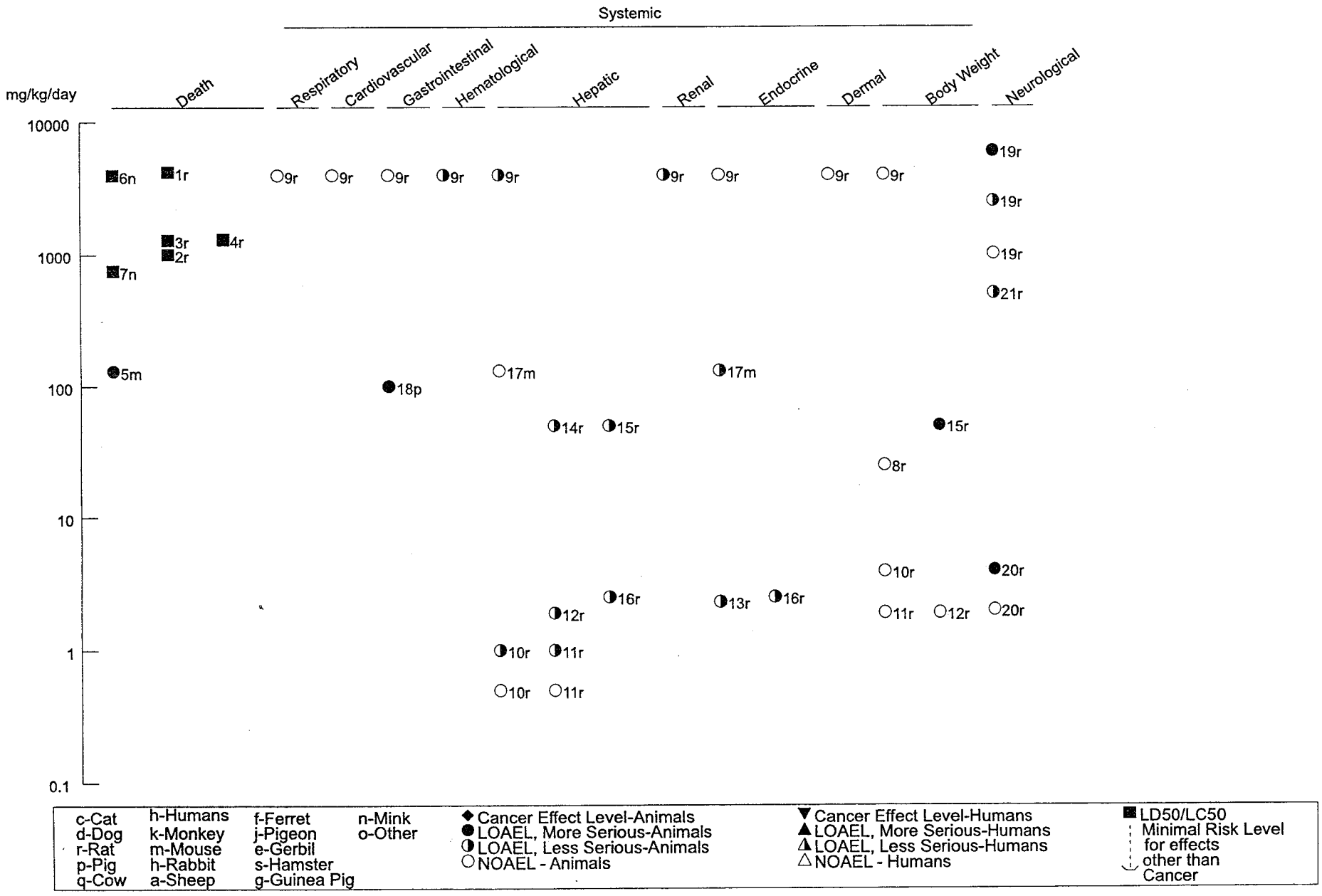


Figure 3-2. Levels of Significant Exposure to PCB Mixtures - Oral (*continued*)

Acute (≤14 days)

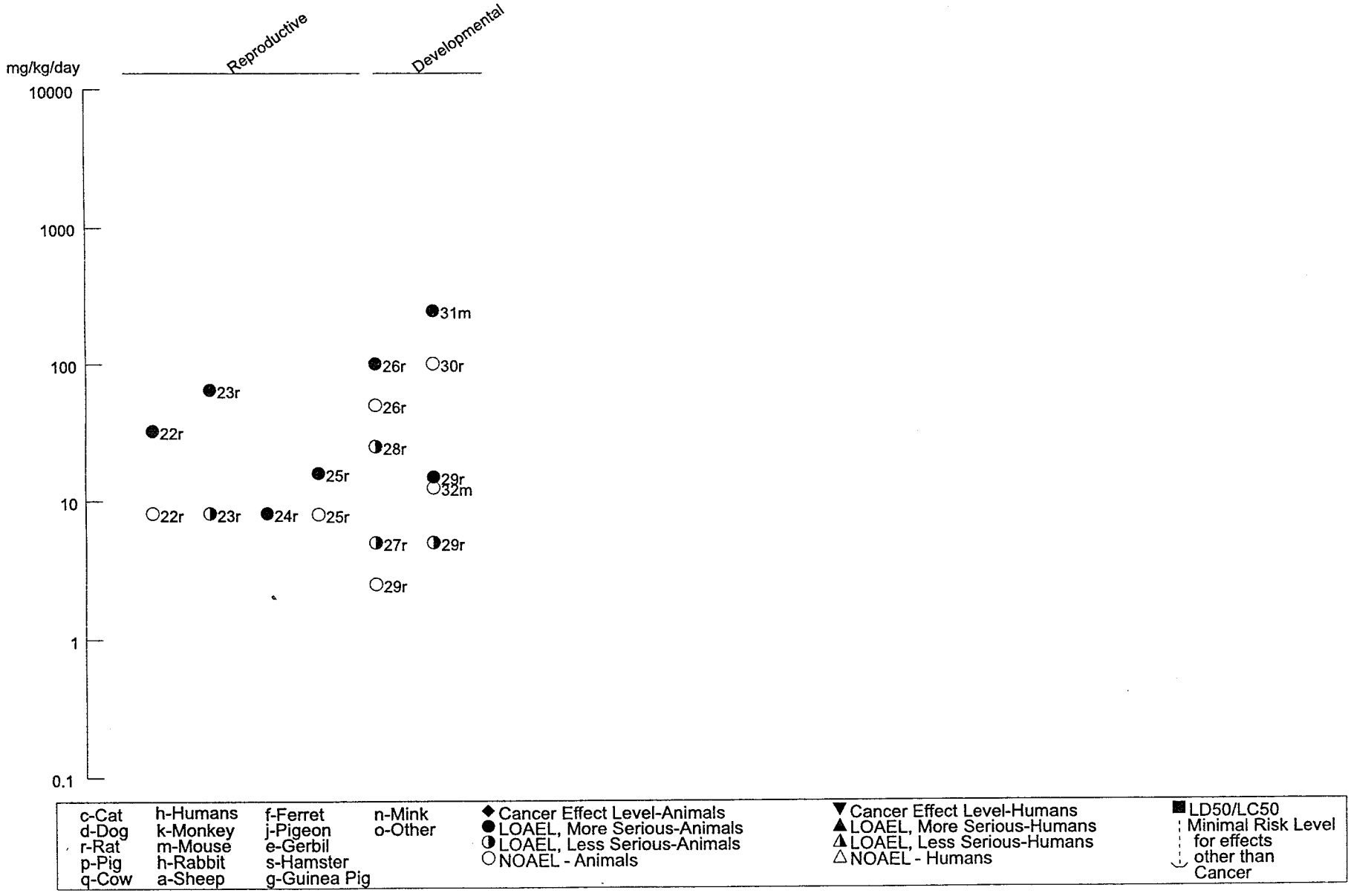


Figure 3-2. Levels of Significant Exposure to PCB Mixtures - Oral (*continued*)

Intermediate (15-364 days)

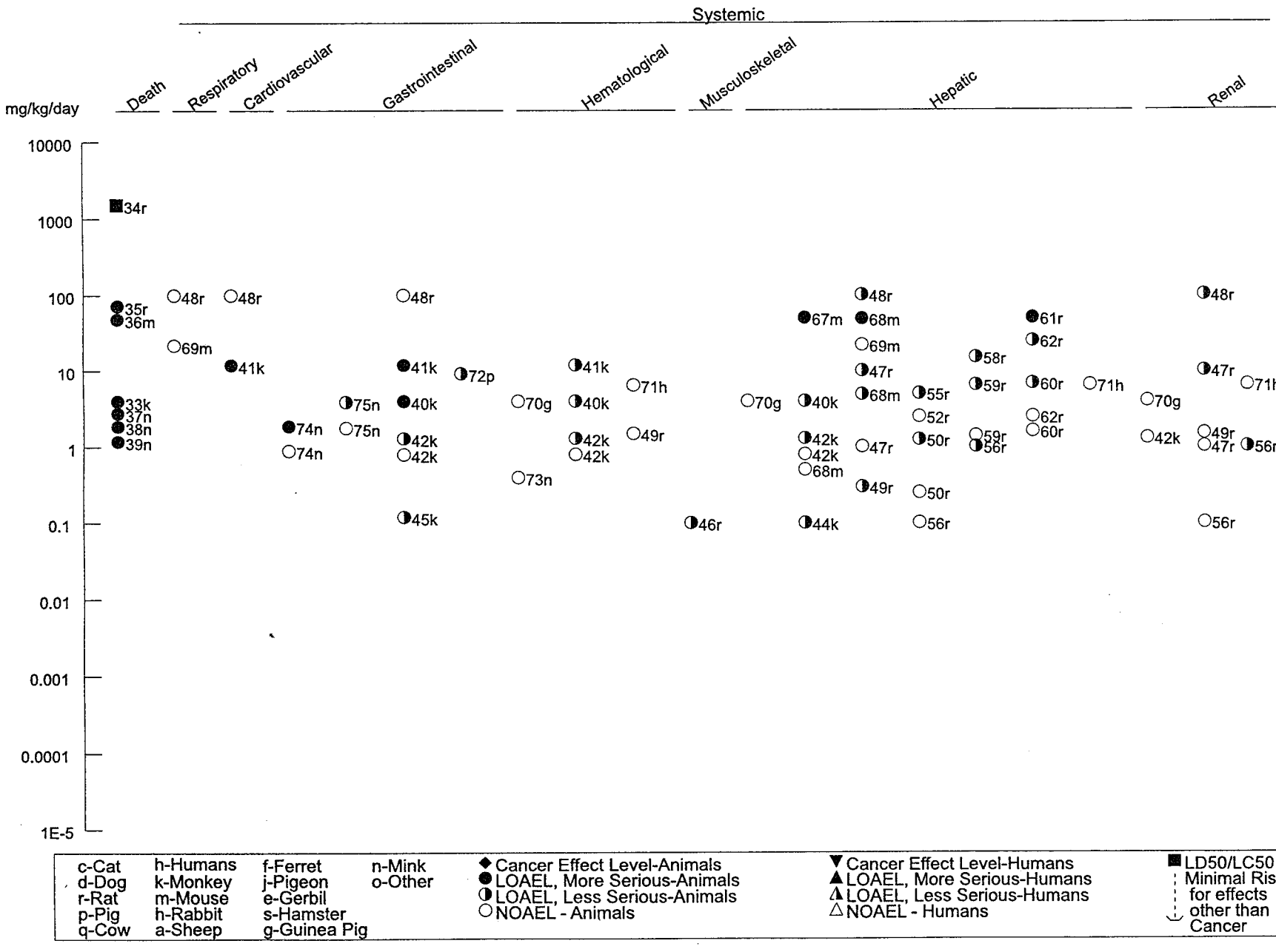


Figure 3-2. Levels of Significant Exposure to PCB Mixtures - Oral (*continued*)

Intermediate (15-364 days)

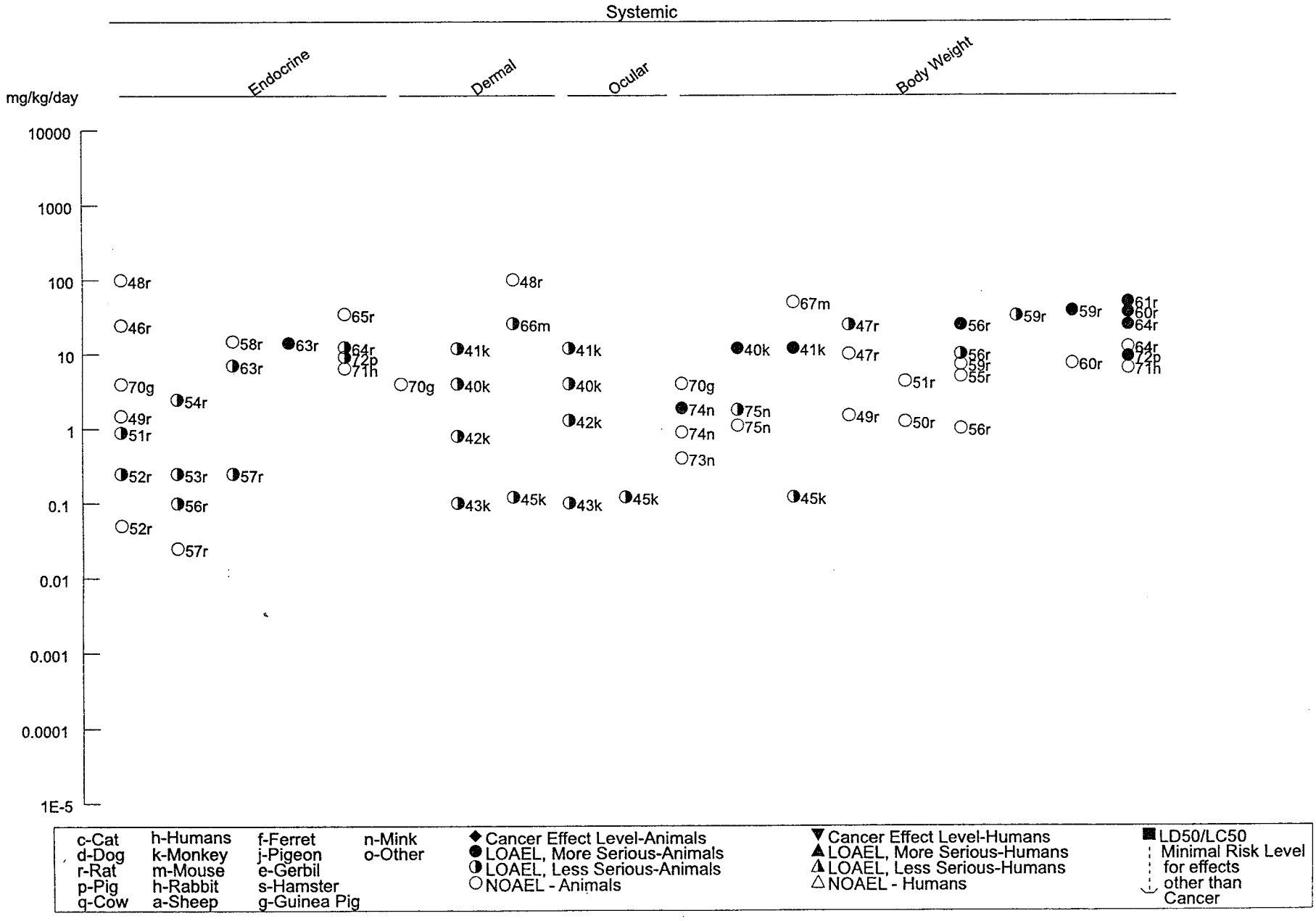


Figure 3-2. Levels of Significant Exposure to PCB Mixtures - Oral (*continued*)

Intermediate (15-364 days)

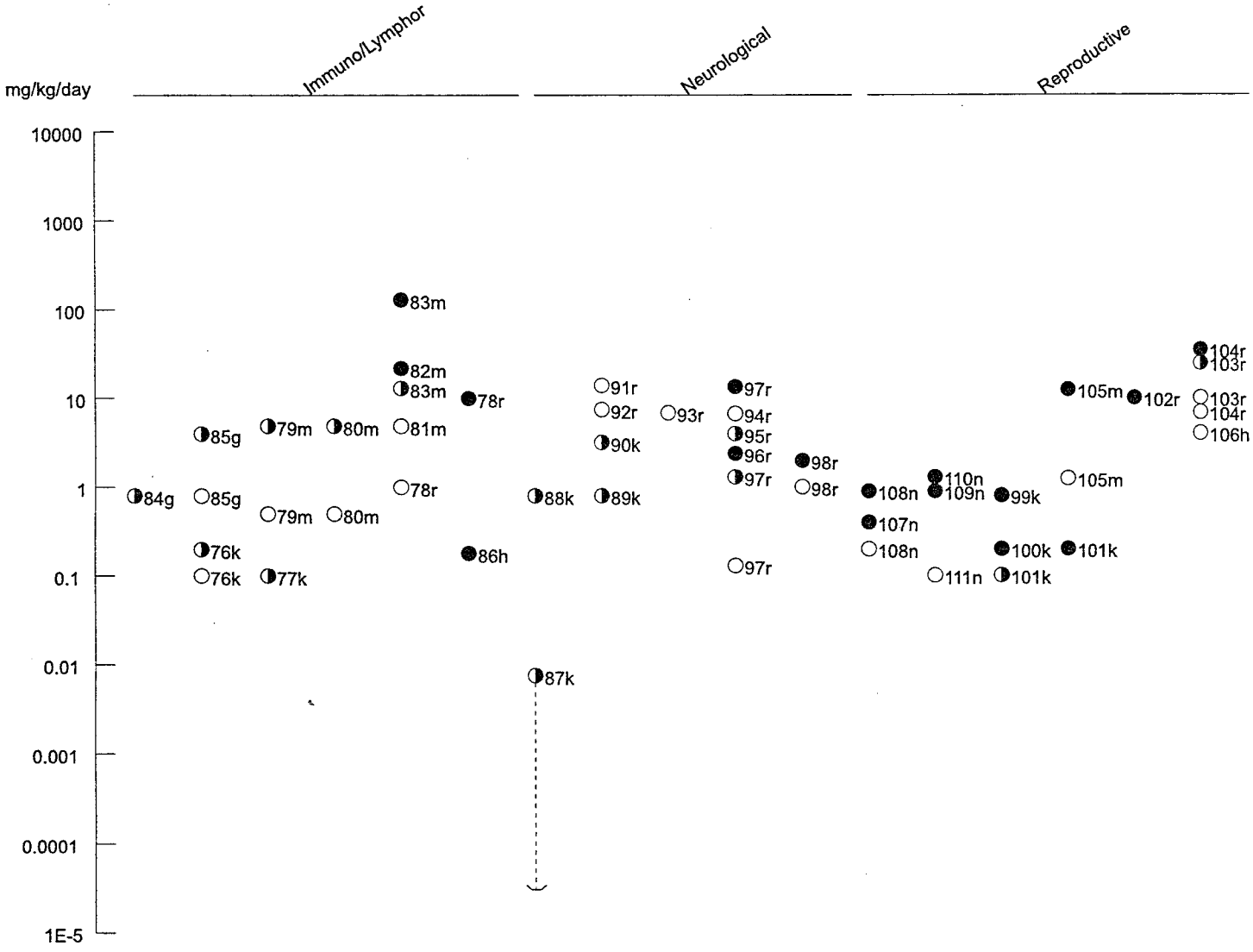


Figure 3-2. Levels of Significant Exposure to PCB Mixtures - Oral (*continued*)

Intermediate (15-364 days)

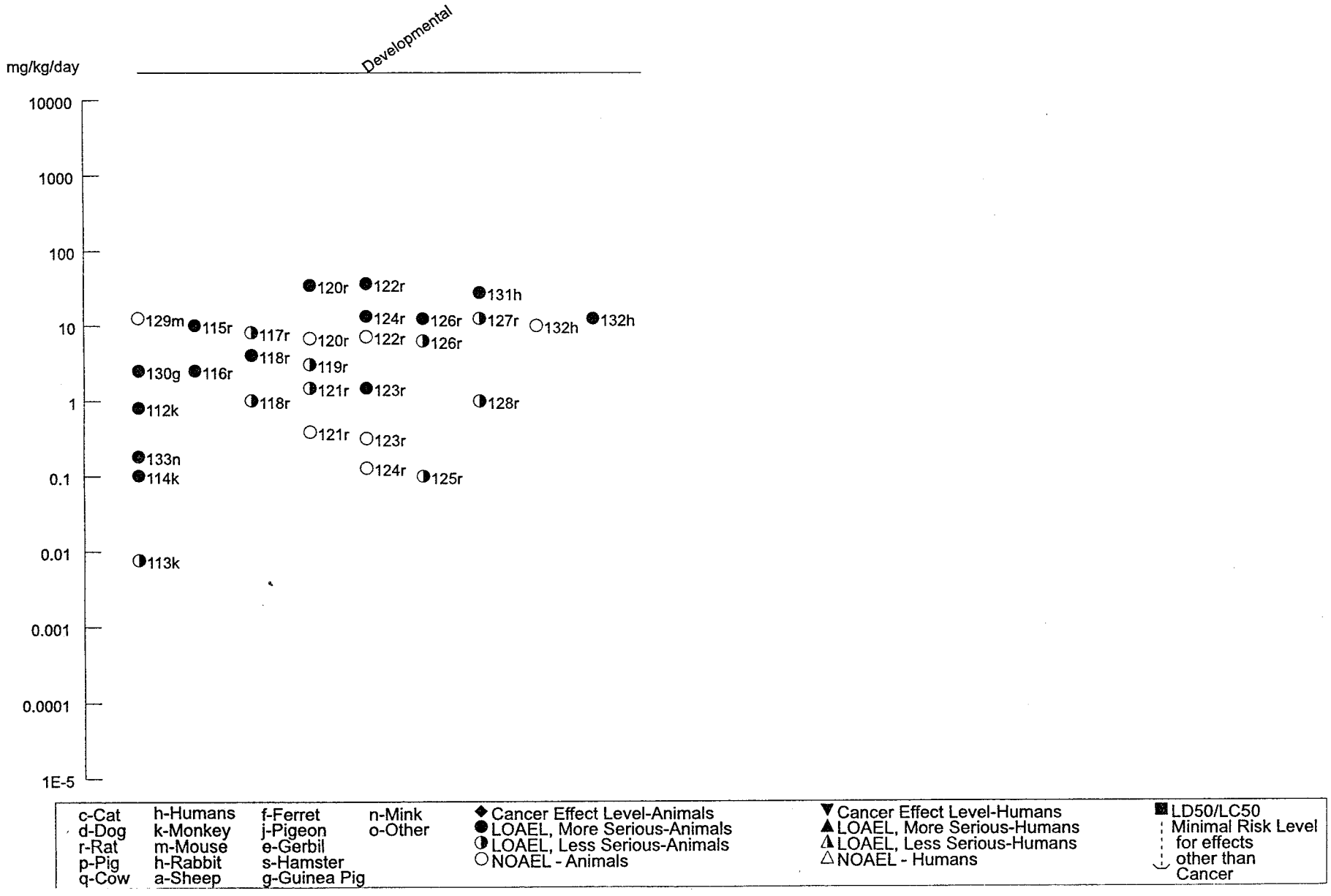


Figure 3-2. Levels of Significant Exposure to PCB Mixtures - Oral (*continued*)

Chronic (≥365 days)

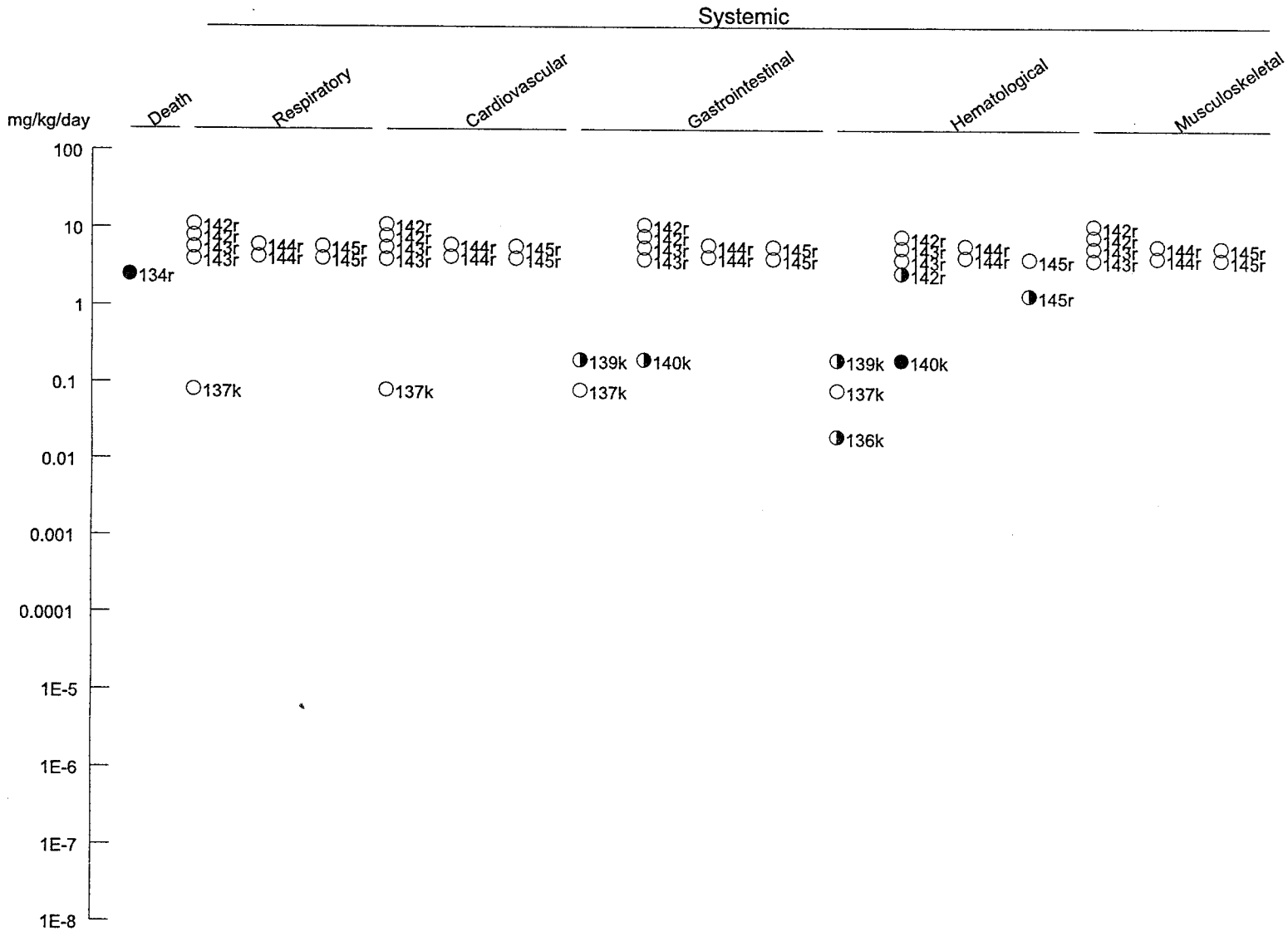


Figure 3-2. Levels of Significant Exposure to PCB Mixtures - Oral (*continued*)

Chronic (≥365 days)

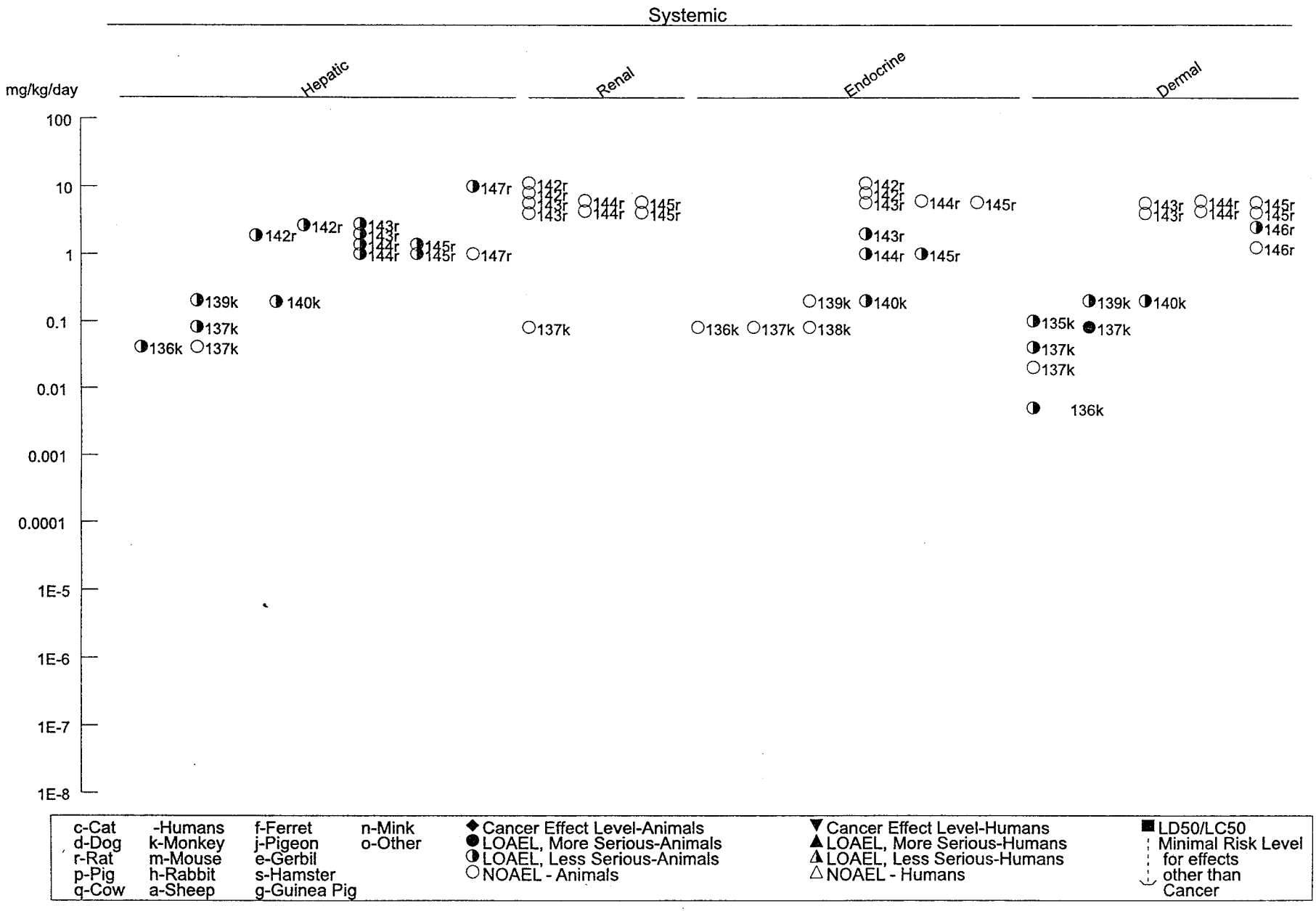


Figure 3-2. Levels of Significant Exposure to PCB Mixtures - Oral (*continued*)

Chronic (≥365 days)

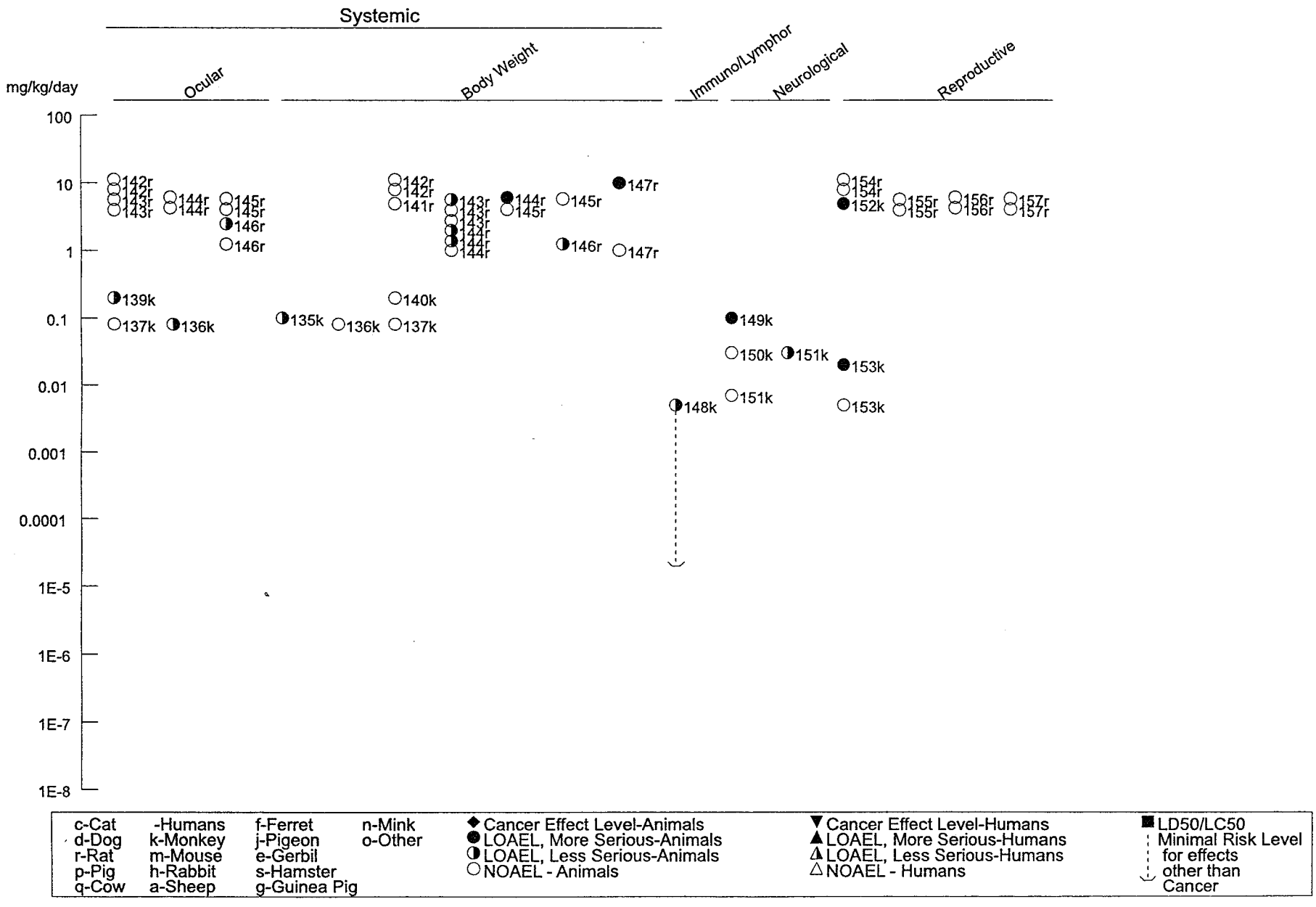


Figure 3-2. Levels of Significant Exposure to PCB Mixtures - Oral (*continued*)

Chronic (≥365 days)

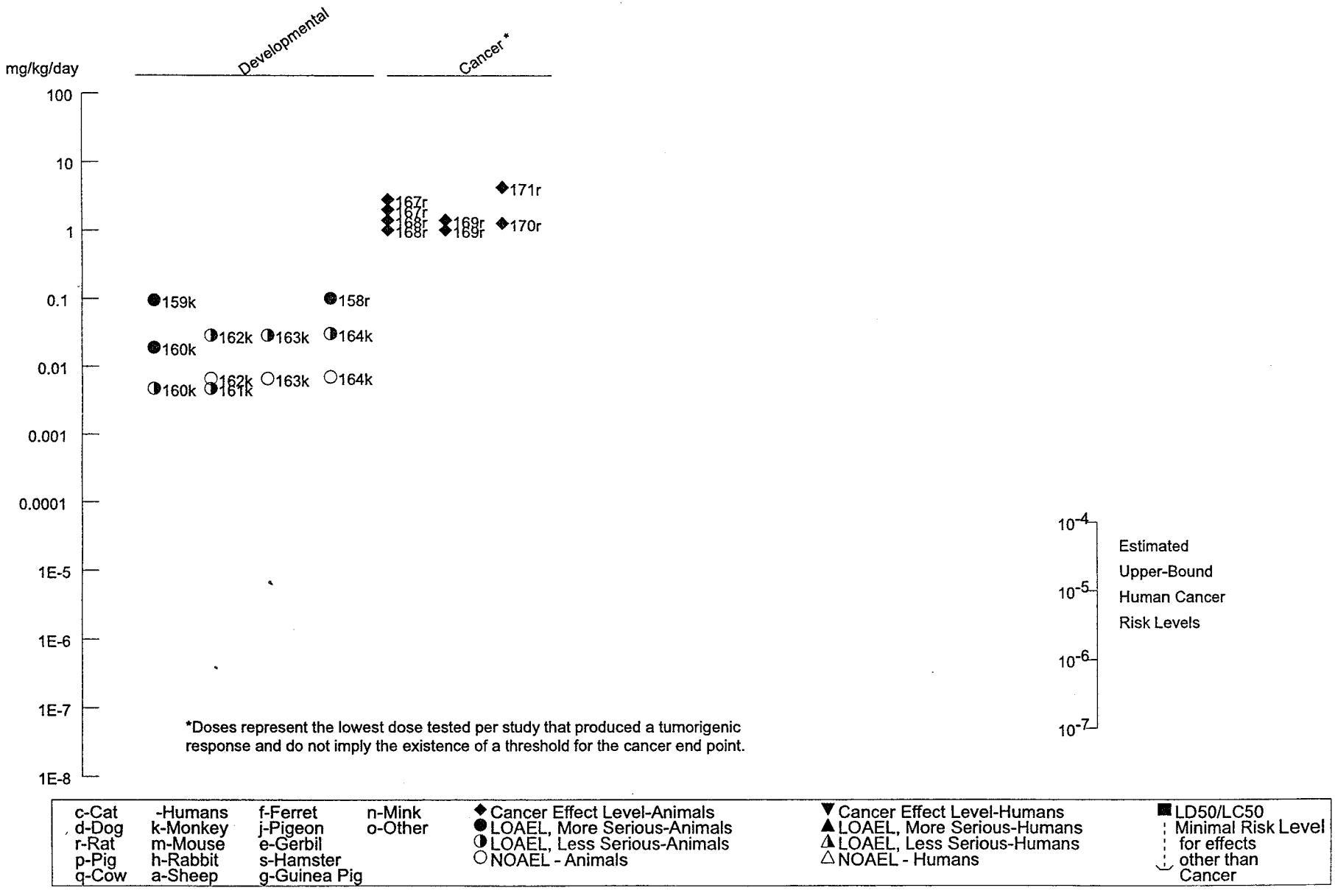


Table 3-3. Levels of Significant Exposure to PCB Mixtures - Dermal

Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
ACUTE EXPOSURE						
Death						
Mouse (Skh:HR-1)	once				2,273 F (unspecified number of deaths in a group of 3 animals)	Puhvel et al. 1982 1254
INTERMEDIATE EXPOSURE						
Systemic						
Mouse (Skh:HR-1)	6 wk 4 d/wk	Dermal	136 F			Puhvel et al. 1982 1254
Rabbit (New Zealand)	38 d 5 d/wk	Hemato	42.1 F			Vos and Beems 1971 1260
		Hepatic		42.1 F (centrilobular degeneration, focal necrosis, porphyria)		
		Renal		42.1 F (tubular degeneration)		
		Dermal		42.1 F (hyperkeratosis, acne)		
		Bd Wt		42.1 F (decreased body weight)		
Rabbit (New Zealand)	28 d 5 d/wk	Hepatic		44.4 F (hepatomegaly, centrilobular degeneration, focal necrosis, porphyria, increased SGPT and SGOT)		Vos and Notenboom-Ram 1972 1260
		Renal	44.4 F			
		Dermal		44.4 F (hyperkeratosis, acne)		
		Bd Wt		44.4 F (34% decrease in body weight gain)		

Table 3-3. Levels of Significant Exposure to PCB Miixtures - Dermal (continued)

Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
				Less Serious (mg/kg/day)	Serious (mg/kg/day)	
Immunological/Lymphoreticular						
Rabbit (New Zealand)	38 d 5 d/wk				42.1 F (thymic atrophy)	Vos and Beems 1971 1260
Rabbit (New Zealand)	28 d 5 d/wk				44.4 F (thymic atrophy)	Vos and Notenboom-Ram 1972 1260

Bd Wt = body weight; d = day(s); F = female; Hemato = hematological; LOAEL = lowest-observable-adverse-effect level; NOAEL = no-observable-adverse-effect level; SGOT = serum glutamic oxaloacetic transaminase; SGPT = serum glutamic pyruvic transaminase; wk = week(s); 1242 = Aroclor 1242; 1254 = Aroclor 1254; 1260 = Aroclor 1260

3.2 DISCUSSION OF HEALTH EFFECTS

3.2.1 Death

3.2.1.1 Human Studies

No studies were located regarding deaths in humans from acute exposure by any route. Some studies of longer-term occupational exposures found increased mortality from cardiovascular disease and cancer, as discussed in Sections 3.2.2.2.1 and 3.2.8.2, respectively.

3.2.1.2 Animal Studies

Inhalation Exposure. Intermittent exposure to near-saturation vapor concentrations of heated Aroclor 1242 (8.6 mg/m^3) over 24 days was not lethal in rats, mice, rabbits, or guinea pigs, and no signs of intoxication were reported (Treon et al. 1956). Pneumonia, apparently unrelated to PCB exposure, caused death in some of the test and control animals except those exposed to 8.6 mg/m^3 Aroclor 1242. The vapor concentrations are unknown, as the technique used to estimate them has since been shown to be invalid; possible CDF contamination was not reported because CDFs had not then been discovered. Similar exposures to lower concentrations of heated Aroclors 1242 and 1254 were also found not to produce lethality in these species. No data were located regarding lethality or decreased longevity of animals due to acute or chronic inhalation of PCBs.

Oral Exposure. There are no marked differences in acute LD_{50} values of Aroclor PCB mixtures for observation periods of <30 days. Single-dose LD_{50} values of 4,250 mg/kg for Aroclor 1242 (Bruckner et al. 1973), 1,010 to 1,295 mg/kg for Aroclor 1254 (Garthoff et al. 1981; Linder et al. 1974), and 1,315 mg/kg for Aroclor 1260 (Linder et al. 1974) have been reported in rats. In minks, single-dose LD_{50} values ranged between 750 and 1,000 mg/kg for Aroclor 1221, and were >3,000 mg/kg for Aroclor 1242 and 4,000 mg/kg for Aroclor 1254 (Aulerich and Ringer 1977). In addition to differences in PCB congener composition, the variation in LD_{50} values may be related to factors such as animal strain, age, sex, or formulation purity. There is evidence, for example, that immature rats (3–4 weeks old) are more susceptible than adults (Grant and Phillips 1974; Linder et al. 1974). Causes of death from acute exposure are unclear, but principal signs of toxicity in rats included diarrhea and respiratory depression, and dehydration may be a principal contributing factor (Bruckner et al. 1973). Single-dose oral lethality data for species other than rats and minks were not located.

Three of five mice fed Aroclor 1254 in the diet at an estimated dose of 130 mg/kg/day for 14 days died of unspecified causes by day 15 (Sanders et al. 1974). At the highest Aroclor 1254 dose of 520 mg/kg/day, 5 of 5 mice died within 7 days, but none of the 5 mice treated with 2.5 mg/kg/day died.

Estimated dietary doses of 4 mg/kg/day Aroclor 1248 for 2–3 months (Allen 1975; Allen and Norback 1976) and 0.12–4 mg/kg/day Aroclor 1242 for 92–245 days were lethal for monkeys (Becker et al. 1979). Survival effects were not clearly related to dose in the Becker et al. (1979) study, but this could be due to the small numbers tested (one per dose), which is not unusual in studies of nonhuman primates. Tryphonas et al. (1984) dosed Cynomolgus monkeys (*Macaca fasicicularis*) with Aroclors 1248 and 1254 at 2 and 5 mg/kg/day for 3 days/week for 4 weeks. Aroclor 1248 was more toxic than Aroclor 1254. Minks and monkeys appear to have similar susceptibility to lethal effects of intermediate-duration oral PCB exposure (Aulerich and Ringer 1977; Aulerich et al. 1986; Bleavins et al. 1980; Hornshaw et al. 1986; Ringer et al. 1981). LD_{50} values of 7.1–7.3 and 1 mg/kg/day were determined for minks fed Aroclor 1254 for 28 days (Aulerich et al. 1986; Hornshaw et al. 1986) and 9 months (Ringer et al. 1981), respectively. Death occurred in 33% of the minks fed 2.8 mg/kg/day Aroclor 1254 for 4 months (Aulerich and Ringer 1977). The average time to death in minks fed 1.9 mg/kg/day Aroclor 1242 ranged from 156 to 171 days, with .67% mortality occurring by 247 days (Bleavins et al. 1980). Death in minks was generally due to visceral hemorrhagic lesions. Female minks are more sensitive than males. Intermediate-duration gavage and feed studies in rats and mice reported that much higher doses of Aroclor 1254 or 1260 caused death (Garthoff et al. 1981; Kimbrough et al. 1972; Koller 1977). Although this may be due to species differences in susceptibility, the shorter and intermittent duration of exposure (2.5 weeks, 2 days/week) and mode of administration (gavage) in rats may account for some of the apparent differences.

Decreased survival occurred in male rats fed diets containing estimated doses $1.25 mg/kg/day Aroclor 1254 for 104–105 weeks (NCI 1978). A dose of 2.5 mg/kg/day induced a 34% decrease in survival. The cause of death was not specified. There was no effect on survival in similarly treated female rats, and a NOAEL for mortality was not identified. There was no attempt to identify or quantitate impurities in the Aroclor 1254 test compound. Decreased survival is not a universal finding in chronic PCB studies, as survival was unchanged or lifespan was extended in rats treated with estimated doses of 3.45–5 mg/kg/day 60% chlorine PCB mixtures (Aroclor 1260 and Clophen A60) via diet (Norback and Weltman 1985; Schaeffer et al. 1984).

Dermal Exposure. A single topical dose of 2,273 mg/kg Aroclor 1254 was fatal to hairless mice within 24 hours (Puhvel et al. 1982). It was not specified whether all three treated mice died or whether the Aroclor was administered in pure acetone or in acetone-mineral oil emulsion. Median lethal doses for single dermal applications of PCBs to rabbits were between 794 and 1,269 mg/kg for Aroclors 1242 and 1248, between 1,260 and 3,169 mg/kg for Aroclors 1221 and 1262, and between 1,260 and 2,000 mg/kg for Aroclors 1232 and 1260 (Fishbein 1974; Nelson et al. 1972). These PCBs were applied undiluted except for Aroclors 1260 and 1262, which were administered in corn oil. Other details regarding the exposure protocol were not provided. Cause of death was not reported, and there was no clear trend of toxicity with degree of chlorination. Lethality data for other species or durations of exposure were not located. The lethal dose from the Puhvel et al. (1982) study is recorded in Table 3-3.

3.2.2 Systemic Effects

3.2.2.1 Respiratory

3.2.2.1.1 Human Studies

There are limited data on potential respiratory effects of PCB exposure in humans. Cross-sectional studies provide suggestive evidence for an association. Upper respiratory tract or eye irritation (48%), cough (14%), and tightness of the chest (10%) were noted among 326 capacitor workers exposed to 0.007–11 mg/m^3 mean air concentrations of various Aroclors for >5 years (Fischbein et al. 1979; Warshaw et al. 1979). The significance of these effects is unknown due to lack of a control group; however, the prevalence of upper respiratory tract or eye irritation (48%) raises concern that they are exposure-related. Other limitations of this study include discrepancies between the reports of Fischbein et al. (1979) and Warshaw et al. (1979), poor definition of the cohort, and failure to distinguish between past and present symptoms. Additionally, capacitor manufacturing plants typically used large amounts of volatile degreasing agents that may have contributed to pulmonary symptom complaints. Chest pain while walking occurred more frequently (16%) in a group of 55 male transformer workers exposed to Aroclor/trichlorobenzene mixtures (Askarels) than in age-matched workers never occupationally exposed to PCBs (Emmett et al. 1988a). The workers were employed for a mean duration of 3.75 years, and the range of PCB personal exposures (primarily Aroclor 1260) measured in the breathing zone was 0.00001–0.012 mg/m^3. CDF contamination ranged from 13 to 116 ppb by weight. The chest pain symptom was not investigated further and was not attributed to a specific cause. A correlation between coughing on the job or soon after work and PCB blood levels in electrical capacitor manufacturing

workers has been reported (Smith et al. 1982). These workers were exposed to various Aroclors and Askarels, in PCB concentrations ranging from 0.003 to 0.08 mg/m^3 (duration of exposure was not reported).

In addition to these reported respiratory tract symptoms, changes in lung function were observed in the PCB workers discussed above. These include a significant decrease in 1-second forced expiratory volume (FEV_1) in the transformer workers (Emmett et al. 1988b); this is the same cohort evaluated by Emmett et al. (1988a). However, when adjusted for smoking habits, FEV was not statistically significant. Fourteen percent of 243 workers examined in the Warshaw et al. (1979) study showed reduced forced vital capacity (FVC) as compared to standard values. Decreased FVC was noted in 8% of the nonsmokers (12.5% males, 4.3% females) and in 17% of the current and former smokers (16% males, 18.7% females). Of all workers with reduced FVC, 80% demonstrated a restrictive pattern of impairment (increased FEV_1/FVC) without radiologic changes. Similar results were initially found in another spirometry study of 179 workers from the same plant population as that studied by Warshaw et al. (1979) (Lawton et al. 1986). The 1976 findings were not confirmed by followup evaluations performed in 1979 and 1983 after no further PCB exposure, and were considered to be artifactual due to deficient pulmonary function testing in 1976 and lack of radiologic changes to account for the restrictive impairment observed (Lawton et al. 1986). The workers had a history of clinically recognized respiratory illness and/or symptomatology, and obstructive impairment (increased FVC, decreased FEV_1/FVC) was found in about 15% of the workers in the initial and followup evaluations (1976 and 1979), but these effects could not be attributed solely to PCB exposure. The occurrence of self-reported respiratory effects was not elevated among residents who lived within 0.5 mile of three PCB-contaminated waste sites (Stehr-Green et al. 1986a).

Potential respiratory effects have also been reported in *Yusho* and *Yu-Cheng* patients. More frequent or severe respiratory infections (Kuratsune 1989; Rogan 1989) and chronic bronchitis accompanied by persistent cough and sputum production (Nakanishi et al. 1985; Shigematsu et al. 1971, 1977) have been reported.

3.2.2.1.2 Animal Studies

No studies were located regarding respiratory effects in animals after inhalation exposure to PCBs. There were no histological alterations in the lungs of rats administered a single 4,000 mg/kg dose of Aroclor 1242 by gavage and evaluated 24 hours posttreatment or in rats treated with 100 mg/kg/day Aroclor 1242 by gavage every other day for 3 weeks (Bruckner et al. 1973). Mice fed a diet that provided .22 mg Aroclor/kg/day for 6 weeks had no changes in lung weight or histology (Loose et al. 1978a, 1978b). Lung inflammation was observed in rats that died following dietary exposure to Phenoclor DP6 at .25 mg/kg/day for 8 days or .50 mg/kg/day for 6 days (Narbonne et al. 1978). Other respiratory end points were not examined in these studies. No histopathologic changes were observed in the trachea or lungs of male or female rats that were fed Aroclor 1016, 1242, 1254, or 1260 for 24 months at intake levels of 8.0–11.2, 4.0–5.7, 4.3–6.1, or 4.1–5.8 mg/kg/day, respectively (Mayes et al. 1998). Rhesus monkeys receiving daily doses of 0.005, 0.020, 0.040, or 0.080 mg/kg/day Aroclor 1254 for 72 months showed no effects on lung tissue (Arnold et al. 1997).

Intermediate-duration dietary exposure to single congeners did not result in histological damage in the lungs of rats fed diets providing #4.1 mg/kg/day of PCB 153 (Chu et al. 1996a), #4.2 mg/kg/day of PCB 128 (Lecavalier et al. 1997), #7.4 mg/kg/day of PCB 126 (Chu et al. 1994), #4.0 mg/kg/day of PCB 105 (Chu et al. 1998b), #3.7 mg/kg/day of PCB 28 (Chu et al. 1996b), #0.77 mg/kg/day of PCB 77 (Chu et al. 1995), or #0.17 mg/kg/day of PCB 118 (Chu et al. 1995).

The highest NOAEL values and all reliable LOAEL values for respiratory effects for each study are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.2 Cardiovascular

3.2.2.2.1 Human Studies

A number of occupational exposure studies have investigated the possible relationship between PCB exposure and increased risk of cardiovascular disease or altered blood pressure; the inconsistency of the results precludes drawing conclusions from these studies. Mortality from circulatory diseases was significantly increased in the high exposure subgroup of a cohort of 242 male capacitor manufacturing workers with >5 years exposure and >20 years latency (Gustavsson and Hogstedt 1997). The standardized mortality ratio (SMR) in the subgroup was 328 (5 observed/1.52 expected deaths, 95%

confidence interval [CI] 33–61, p value not reported). Kimbrough et al. (1999a) found no significant increases in mortality related to ischemic heart disease, hypertension with heart disease, other diseases of the heart, cerebrovascular disease, or circulatory system (arteries, veins, pulmonary circulation) in a study of 7,075 male and female capacitor workers. One of the subgroups (male salaried workers) in this study had a significantly decreased risk of mortality from ischemic heart as indicated by an SMR lower than 100 (44 observed/97.5 expected deaths, SMR=45, 95% CI 107–766, $p<0.01$). Neither of these studies reported adequate quantitative exposure data. The inconsistent results of these studies could be due to differences in exposure levels, durations, and latencies, as well as types of Aroclors and cohort sizes. Additional information on these studies is provided in Section 3.2.8.2.1.

Blood pressure measurements (systolic and diastolic) and electrocardiograms were normal in 194 capacitor plant workers (152 male, 43 female) who were exposed to Aroclors 1254, 1242, and 1016 for an average duration of 17 years (Lawton et al. 1985a). Limited exposure characterization, consisting of monitoring in one area of the plant several months prior to the cardiovascular evaluations, showed a geometric mean PCB concentration of 0.69 mg/m^3. No correlation was found between diastolic blood pressure in capacitor manufacturing workers, when adjusted for age and sex, and serum PCBs (Smith et al. 1982). Abnormal blood pressure measurements or other cardiovascular abnormalities were not reported in other studies of PCB-exposed workers that underwent general physical examinations and medical histories (Baker et al. 1980; Chase et al. 1982; Emmett et al. 1988a; Fischbein et al. 1979).

A 30% increase over the national average incidence of borderline and definite hypertension was observed in Triana, Alabama, residents (Kreiss et al. 1981). Increased systolic and diastolic blood pressure were significantly associated with serum PCB levels. However, the relationship between systolic blood pressure and serum PCB levels disappeared when serum cholesterol and triglyceride levels were factored in, but that between diastolic blood pressure and PCBs remained significant. Consumption of contaminated fish was the only known source of PCB exposure; the actual intake of PCBs was not reported. The population was also exposed to dichlorodiphenyltrichloroethane (DDT) via consumption of fish. Serum DDT and serum PCB levels were highly correlated. Multivariate analysis showed that the PCB-blood pressure association was independent of serum DDT levels, age, sex, and weight. The excess prevalence of hypertension cannot be attributed solely to PCBs (or DDT) with any degree of certainty due to the lack of a matched control group, co-linearity of DDT and PCB serum concentrations, and unknown effects of DDT residues on the metabolism or toxicity of PCBs (Kreiss 1985). Subsequent studies of environmentally exposed populations without exposure to DDT have failed to show an association between hypertension and PCBs. No excess of hypertension was found in 106 people who had lived near

PCB-containing hazardous waste sites for at least 5 years (Stehr-Green et al. 1986a). Mean PCB blood levels were <10 ppb. A significant association between increased diastolic blood pressure and serum PCB levels was observed, but the association failed to achieve statistical significance (p=0.08) when possible confounding effects of both age and smoking were controlled. There was no association between elevated systolic or diastolic blood pressure and serum levels of PCBs in 840 residents of New Bedford, Massachusetts, who were exposed via consumption of contaminated fish (Massachusetts Department of Public Health 1987). However, most subjects in this study had serum PCB levels that were within the typical range of the U.S. population.

3.2.2.2.2 Animal Studies

Data on the cardiovascular toxicity of PCBs in animals are limited to several oral exposure studies conducting histological examinations of the heart and blood vessels. Pericardial edema occurred in four of six monkeys given 12 mg/kg/day Aroclor 1248 in the diet for 3 months (Allen et al. 1973). However, Rhesus monkeys receiving daily doses of 0.005, 0.020, 0.040, or 0.080 mg/kg/day Aroclor 1254 for 25 months showed no effects on cardiac tissue (Arnold et al. 1997). Histological examination of the heart was normal in rats evaluated 24 hours following a single 4,000 mg/kg dose of Aroclor 1242 or 100 mg/kg/day Aroclor 1242 every other day for 3 weeks administered by gavage (Bruckner et al. 1973). No histopathologic changes were observed in the heart of male or female rats that were fed Aroclor 1016, 1242, 1254, or 1260 for 24 months at dose levels of 8.0–11.2, 4.0–5.7, 4.3–6.1, or 4.1–5.8 mg/kg/day, respectively (Mayes et al. 1998). Rhesus monkeys receiving daily doses of 0.005, 0.020, 0.040, or 0.080 mg/kg/day Aroclor 1254 for 25 months showed no effect on cardiac tissue (Arnold et al. 1997).

In a series of 13-week dietary exposure studies using single PCB congeners, no histological alterations in the heart or thoracic aorta were observed in rats fed diets providing #4.1 mg/kg/day of PCB 153 (Chu et al. 1996a), #4.2 mg/kg/day of PCB 128 (Lecavalier et al. 1997), #7.4 mg/kg/day of PCB 126 (thoracic aorta was not examined) (Chu et al. 1994), #4.0 mg/kg/day of PCB 105 (Chu et al. 1998b), #3.7 mg/kg/day of PCB 28 (Chu et al. 1996b), #0.77 mg/kg/day of PCB 77 (Chu et al. 1995), or #0.17 mg/kg/day of PCB 118 (Chu et al. 1995).

Hennig and associates (Hennig et al. 1999; Slim et al. 1999) demonstrated in *in vitro* studies that exposure to PCB 77 disrupts endothelial barrier function in the vascular endothelium; this was not seen for PCB 153.

The highest NOAEL values and all reliable LOAEL values for cardiovascular effects for each study are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.3 Gastrointestinal

3.2.2.3.1 Human Studies

Clinical observations suggestive of gastrointestinal damage have been reported in workers exposed to airborne PCBs and in the *Yusho* cohort. A statistically significant increase in loss of appetite was reported by PCB-exposed transformer workers (20%) as compared to the control group (4%) (Emmett et al. 1988a). PCB levels, primarily Aroclor 1260, ranged from 0.00001 to 0.012 mg/m^3. Gastrointestinal symptoms (anorexia, nausea, vomiting, and abdominal pain) and weight loss were also reported in 18% of capacitor workers exposed to various Aroclors at mean concentrations of 0.007–11 mg/m^3 (Fischbein et al. 1979). The statistical significance of the effects cannot be determined since a control group was not examined. A significant association was found between loss of appetite and increasing PCB blood levels in electrical equipment manufacturing workers who were exposed to various Aroclors and Askarels at PCB concentrations of 0.003–0.08 mg/m^3 (Smith et al. 1982).

Postprandial epigastric distress, epigastric pain with or without a burning sensation, postprandial headache, and intolerance to fatty foods were noted in 50% of workers exhibiting liver effects (Maroni et al. 1981a). The workers (40 males and 40 females) were exposed to concentrations of Pyralene 3010 or Apirolio (Italian PCB formulations) ranging from 0.048 to 0.275 mg/m^3 for an average duration of 12 years. Both of these products were PCB mixtures of unreported purity that had a 42% chlorine content. Some of these workers were also exposed to a PCB mixture containing 54% chlorine. There was no control group in this study, precluding a determination of the significance of the results. Gastrointestinal effects (vomiting and diarrhea) have been observed in *Yusho* patients (Kuratsune 1989). No signs of gastrointestinal effects were reported in community members exposed to PCB-contaminated sludge or in PCB exposed workers (Baker et al. 1980).

3.2.2.3.2 Animal Studies

No histopathologic effects were observed in the stomach or intestines of six rats 24 hours following a single near-lethal dose of 4,000 mg/kg of Aroclor 1242 by gavage (Bruckner et al. 1973). In contrast, hemorrhage into the stomach and foci of ulceration in the stomach and duodenum were observed in rats given a single lethal gavage dose (inadequately quantified) of Aroclor 1254 or 1260 (Kimbrough et al. 1972). Gastric ulcers were observed in two pigs that were treated with 100 mg/kg/day Aroclor 1254 for 11 days (Hansen et al. 1976). The lesions in the pigs were similar in gross and histological appearance to those observed in intermediate-duration studies with monkeys discussed below.

Intermediate-duration dietary administration of Aroclor 1248 (Allen 1975; Allen and Norback 1973, 1976; Allen et al. 1973, 1974a) and Aroclor 1242 (Becker et al. 1979) to monkeys produced gastritis with hypertrophy and hyperplasia of the gastric mucosa. The gastric changes progressed to include mucous-filled cysts in the mucosa penetrating into the submucosa, ulceration of the gastric mucosa resulting from ruptured cysts or erosion, and hemorrhage. Estimated doses of $1.3 mg/kg/day Aroclor 1248 or $0.12 mg/kg/day Aroclor 1242 for 2 months produced these gastric changes in monkeys (Allen 1975; Allen and Norback 1976; Allen et al. 1974a; Becker et al. 1979). Only a minimal number of Aroclor 1242-exposed animals were tested (mostly one monkey per dose group), although the severity of the histopathologic changes was dependent on both exposure length and dose. Gastric ulcers also occurred in minks at similar dietary doses of Aroclor 1016, 1242, or 1254 (Bleavins et al. 1980; Hornshaw et al. 1986), and there is evidence of gastric erosion and necrosis in pigs treated with 9.2 mg/kg/day Aroclor 1242 or 1254 for 91 days (Hansen et al. 1976). In seasoned sows, which are prone to gastric hyperemia, erosions were more severe in two of five sows receiving 9.2 mg/kg/day Aroclor 1242 (Hansen et al. 1975). Gastrointestinal lesions were also observed in Baltic seals, and found to be directly associated with body burdens of PCBs and/or metabolites (Bergman and Olsson 1985; Olsson et al. 1994). There were no histological changes in the stomach or intestines of rats treated with 100 mg/kg/day Aroclor 1242 by gavage 3 times/week for 3 weeks (Bruckner et al. 1973).

Re-examination of the National Cancer Institute (NCI 1978) cancer bioassay showed Aroclor 1254-induced intestinal metaplasia and some adenocarcinoma in the glandular stomach of Fischer 344 rats following chronic dietary treatment (Morgan et al. 1981; Ward 1985) (see Section 3.2.8.3.2). The intestinal metaplasia appeared to be dose-related. Nonproliferative gastric lesions were not observed. No histopathologic changes were observed in the gastrointestinal tract of male or female rats that were fed Aroclor 1016, 1242, 1254, or 1260 for 24 months at dose levels of 8.0–11.2, 4.0–5.7, 4.3–6.1, or

4.1–5.8 mg/kg/day, respectively (Mayes et al. 1998). Moderate mucinous hypertrophic gastropathy was evident in three of four Cynomolgus monkeys treated with 0.2 mg/kg/day Aroclor 1254 in the diet for 12–13 months (Tryphonas et al. 1984, 1986a) and in two of four Rhesus monkeys treated similarly for 28 months (Tryphonas et al. 1986b). No effects on stomach tissue were observed in Rhesus monkeys receiving daily doses of #0.080 mg/kg/day Aroclor 1254 for 72 months (Arnold et al. 1997).

No histological alterations were observed in the organs and tissues of the gastrointestinal tract of rats following a 13-week dietary exposure to #4.1 mg/kg/day of PCB 153 (Chu et al. 1996a), #4.2 mg/kg/day of PCB 128 (Lecavalier et al. 1997), #7.4 mg/kg/day of PCB 126 (Chu et al. 1994), #4.0 mg/kg/day of PCB 105 (Chu et al. 1998b), #3.7 mg/kg/day of PCB 28 (Chu et al. 1996b), #0.77 mg/kg/day of PCB 77 (Chu et al. 1995), or #0.17 mg/kg/day of PCB 118 (Chu et al. 1995).

The highest NOAEL values and all reliable LOAEL values for gastrointestinal effects for each study are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.4 Hematological

3.2.2.4.1 Human Studies

In general, hematological effects have not been observed in humans occupationally exposed to PCBs. Capacitor plant workers (152 males, 43 females) exposed to Aroclors 1254, 1242, and 1016 for an average duration of 17 years showed slightly decreased numbers of polymorphonuclear neutrophil (PMN) white cells and slightly increased lymphocyte, monocyte, and eosinophil counts when compared to normal values (Lawton et al. 1985a). Limited exposure characterization, consisting of monitoring in one area of the plant several months prior to hematological evaluation, showed a geometric mean PCB concentration of 0.69 mg/m^3. Values for other white cells, erythrocytes, hemoglobin, and hematocrit were within normal ranges. Other studies of PCB-exposed workers have reported essentially normal hematology including total and differential white blood cell counts (Chase et al. 1982; Emmett et al. 1988b; Fischbein et al. 1979; Maroni et al. 1981b; Ouw et al. 1976; Smith et al. 1982). Mild normocytic anemia and leukocytosis have been reported in *Yu-Cheng* patients (Rogan 1989).

3.2.2.4.2 Animal Studies

Erythrocyte count, leukocyte count, and hemoglobin level were evaluated in 3–6 rabbits and guinea pigs intermittently exposed to chamber concentrations of 5.4 mg/m^3 Aroclor 1254 or 6.8 mg/m^3 Aroclor 1242 over a period of 120 or 121 days, respectively (Treon et al. 1956). Alterations included increased erythrocytes in the rabbits (Aroclor 1254) and increased hemoglobin in the guinea pigs (both Aroclors); however, although statistically significant, neither change was regarded as physiologically significant.

Packed blood cell volume was increased in male rats given single lethal doses of 4,000 or 6,000 mg/kg Aroclor 1242 by gavage (Bruckner et al. 1973, 1974). Crenated erythrocytes and increased PMNs were observed at 4,000 mg/kg, but not at 6,000 mg/kg. The investigators indicated that the effect on cell volume reflected dehydration rather than a direct hematologic effect.

Anemia has been observed in monkeys treated with Aroclor 1248 or 1254 in intermediate-duration studies (Allen 1975; Allen and Norback 1973, 1976; Allen et al. 1973, 1974a) and chronic-duration studies (Allen 1975; Arnold et al. 1990; Tryphonas et al. 1984, 1986a, 1986b). The anemia was manifested by decreased hemoglobin content, decreased hematocrit, and hypocellularity of erythrocytic and other precursor cells in the bone marrow, occurred at doses of $4 mg/kg/day for 2 months (Allen 1975; Allen and Norback 1976) and $0.2 mg/kg/day for 12–28 months (Arnold et al. 1990; Tryphonas et al. 1986a, 1986b), and may be related to moribund condition of the monkeys. The anemia was not quantified in all studies, but the existing data indicate that it was moderate to severe after intermediate and chronic exposure. Numbers of circulating neutrophils were generally increased and lymphocytes were decreased in these studies. Hematological changes consistent with a picture of anemia have also been observed in monkeys treated with 0.08 mg/kg/day Aroclor 1254 for 37 months; a dose of 0.02 mg/kg/day produced a decrease in mean platelet volume (Arnold et al. 1993b). Rhesus monkeys receiving daily doses of #0.080 mg/kg/day Aroclor 1254 for 72 months, however, showed no effect on hematological parameters (Arnold et al. 1997).

Hematological changes do not appear to be a clear effect of PCB exposure in animals. Small numbers of rats (four per PCB) fed 50 mg/kg/day Aroclor 1248, 1254, or 1262 for 4–6 weeks showed marked neutrophilia and slightly increased hemoglobin and hematocrit (Allen and Abrahamson 1973). No consistent hematologic effects were observed in rats (6 per dose) fed #1.5 mg/kg/day Aroclor 1242 for 2–6 months (Bruckner et al. 1974), in guinea pigs (12 per dose) fed #4 mg/kg/day Aroclor 1260 for 8 weeks (Vos and de Roij 1972), or in rabbits (7 per dose) fed #6.5 mg/kg/day Aroclor 1254 for 8 weeks

(Street and Sharma 1975). There were no treatment-related changes in hemoglobin levels or hematocrit in minks (10 per PCB) fed 0.4 mg/kg/day Aroclor 1016, 1221, 1242, or 1254 for #39 weeks (Aulerich and Ringer 1977). Red blood cell count and hemoglobin concentration were reduced in female rats (50 per group) that were fed Aroclor 1016 or 1260 for 24 months at intake levels $2.7 or $1.4 mg/kg/day, respectively (Mayes et al. 1998). No hematologic effects were observed in female rats that were similarly exposed to #5.7 mg/kg/day Aroclor 1242 or #6.1 mg/kg/day Aroclor 1254, or in male rats exposed to Aroclor 1016, 1242, 1254, or 1260 at intake levels of #8.0, #5.7, #6.1, or #4.1 mg/kg/day, respectively.

Intermediate-duration exposure to single congeners has resulted in hematological effects in rats. Significant decreases in hemoglobin, hematocrit, mean corpuscular hemoglobin, mean corpuscular volume, and decreased eosinophils were observed in rats treated with 4.0 mg/kg/day of PCB 105 (Chu et al. 1998b). Decreases in hemoglobin, hematocrit erythrocyte count, mean corpuscular hemoglobin, mean corpuscular volume, and platelets were observed after exposure to 7.4 mg/kg/day of PCB 126 (Chu et al. 1994). In contrast, no hematological effects were observed similarly treated rats exposed to #0.77 mg/kg/day of PCB 77 (Chu et al. 1995), #0.17 mg/kg/day of PCB 118 (Chu et al. 1995), #3.7 mg/kg/day of PCB 28 (Chu et al. 1996b), or #4.2 mg/kg/day of PCB 128 (Lecavalier et al. 1997).

No effects on hemoglobin, hematocrit, or differential leukocyte count were observed in rabbits exposed to 60% chlorine PCBs in isopropanol (Aroclor 1260, Clophen A60, or Phenoclor DP6) applied to the shaved back skin 5 days/week for 38 days at estimated doses of 42 mg/kg/day (Vos and Beems 1971). Total leukocyte count was reduced, but insufficient information was provided to determine whether this effect was adverse, whether it was due to a direct effect on the reticuloendothelial system, or if it was secondary to other toxicity (hepatic and renal damage also occurred). CDFs were found only in the non-Aroclor PCBs (detection limit, 1 ppm).

The highest NOAEL values and all reliable LOAEL values for hematological effects for each study are recorded in Tables 3-1, 3-2, and 3-3, and plotted in Figures 3-1 and 3-2.

3.2.2.5 Musculoskeletal

3.2.2.5.1 Human Studies

There are limited data on the musculoskeletal toxicity of PCBs in humans. Only one report of musculoskeletal effects was located (Fischbein et al. 1979). Joint pain was reported by . 11% of the workers exposed to various Aroclors at mean area concentrations of 0.007–11 mg/m^3. A higher prevalence was noted in female workers (15.2%) than in males (7.7%). Muscle pain was reported by <4% of the males and females. Information on the severity or constancy of the joint and muscle pain was not reported, physiological testing was not performed, and there was failure to distinguish between past and present symptoms. The statistical significance of these symptoms cannot be determined because a control group was not examined. No studies were located regarding musculoskeletal effects in humans after oral exposure to PCBs, although a 10% prevalence of unspecified joint problems was reported among farm families who consumed dairy products and beef that were contaminated with PCBs (Humphrey 1983).

3.2.2.5.2 Animal Studies

Little information exists regarding musculoskeletal effects of PCBs in animals. Changes in femur bone morphology resulting in weaker bones occurred in growing (28-day-old) rats (10 per dose) that were treated with Aroclor 1254 by gavage for 10–15 weeks (Andrews 1989). Effects included increased femur density at $0.1 mg/kg/day; decreased cross-sectional and medullary areas at $10 mg/kg/day; and decreased femur weight, volume, length, and cortical area and strength at 25 mg/kg/day. No definite effects on bone flexibility were observed. Serum and urinary calcium levels were increased, but there were no treatment-related alterations in serum parathyroid hormone concentration

No histopathologic changes were observed in skeletal muscle of male or female rats that were fed Aroclor 1016, 1242, 1254, or 1260 for 24 months at dose levels of 8.0–11.2, 4.0–5.7, 4.3–6.1, or 4.1–5.8 mg/kg/day, respectively (Mayes et al. 1998). Similarly, there were no histological alterations in skeletal muscle of rats exposed to #4.1 mg/kg/day of PCB 153 (Chu et al. 1996a), #4.2 mg/kg/day of PCB 128 (Lecavalier et al. 1997), #4.0 mg/kg/day of PCB 105 (Chu et al. 1998b), #3.7 mg/kg/day of PCB 28 (Chu et al. 1996b), #0.77 mg/kg/day of PCB 77 (Chu et al. 1995), or #0.17 mg/kg/day of PCB 118 (Chu et al. 1995) in the diet for 13 weeks.

The highest NOAEL values and all reliable LOAEL values for musculoskeletal effects for each study are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.6 Hepatic Effects

3.2.2.6.1 Summary

In humans, clinical studies of PCB workers reported associations between increased serum levels of liver-related enzymes, lipids, and cholesterol and serum PCBs. Studies of people exposed to PCBs by ingestion of contaminated fish in Triana, Alabama or contaminated rice oil in the *Yusho* or *Yu-Cheng* incidents have reported increases in serum levels of some liver enzymes characteristic of microsomal enzyme induction or liver damage, but these effects cannot be attributed solely to PCBs due to the mixed chemical nature of the contaminated fish and heated rice oil exposures. Serum cholesterol, but not triglycerides, was increased in consumers of contaminated fish, whereas increased serum triglycerides, but not cholesterol, were associated with *Yusho* and *Yu-Cheng* exposure.

Hepatotoxicity of PCBs is well-documented in animals exposed to commercial mixtures or single congeners for acute, intermediate, or chronic durations by oral and other routes of exposure. PCB-induced liver effects in animals seem to be reversible when mild and include microsomal enzyme induction, liver enlargement, increased serum levels of liver-related enzymes and lipids, altered porphyrin and vitamin A metabolism, and histopathologic alterations that progress to non-neoplastic degenerative lesions (particularly fatty and necrotic changes) and/or tumors with higher doses or longer duration exposures. Intermediate- and chronic-duration oral studies indicate that monkeys are more sensitive than rats to PCB hepatotoxicity.

3.2.2.6.2 Human Studies

3.2.2.6.2.1 Liver Enzymes, Enlargement, and Pathology

Occupational Exposure. Hepatic effects have been investigated in a number of epidemiology studies and clinical surveys of PCB-exposed workers. Increased serum levels of liver-related enzymes, particularly gamma-glutamyl transpeptidase (GTP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), and/or lactate dehydrogenase (LDH), were reported in many of these studies (Chase et al. 1982; Emmett et al. 1988b; Fischbein 1985; Fischbein et al. 1979; Lawton et al.

1985a; Maroni et al. 1981a, 1981b; Ouw et al. 1976). Additionally, increases in levels of these serum enzymes have been correlated with serum PCB levels (Baker et al. 1980; Chase et al. 1982; Emmett et al. 1988b; Fischbein 1985; Fischbein et al. 1979; Lawton et al. 1985a; Smith et al. 1982).

Asymptomatic hepatomegaly and increased serum levels (elevated to slightly above normal range) of GTP, AST, and/or ALT were found in 14 of 80 capacitor manufacturing or repair workers who were exposed to non-Aroclor PCB mixtures with a 42% chlorine content (Italian formulations Pyralene 3010 or Apirolio) for an average of 12 years (Maroni et al. 1981a, 1981b). Two other workers had increased serum enzyme levels without liver enlargement. PCB levels ranged from 48 to 275 $\mu g/m^3$ in the workroom air, 2–28 $\mu g/cm^2$ on the skin surface (palms), and 41–1,319 µg/kg in the blood. The investigators considered the liver enlargement indicative of hepatic microsomal induction. Comparison of the 16 workers with abnormal liver findings and the 64 without abnormal findings showed that those with the abnormalities had statistically significant ($p<0.01$) higher mean concentrations of trichlorobiphenyls, pentachlorobiphenyls, and total PCBs in the blood. Additionally, significant positive correlations were found between the frequency of workers with the abnormal liver findings and increasing levels of blood trichlorobiphenyls ($p<0.001$), pentachlorobiphenyls ($p<0.05$), and total PCBs ($p<0.001$). No matched control group was included in the study, there was no apparent association between severity of hepatomegaly and blood PCB levels, and hepatomegaly was not reported in other studies that included physical examinations conducted even at similar or higher serum PCB levels (e.g., Fischbein et al. 1979; Smith et al. 1982).

Serum enzyme (AST, ALT, LDH, AP) and bilirubin levels were within normal limits in 16 workers exposed to PCBs (type not reported) primarily via dermal contact with used transformer oil containing . 600,000 ppm PCBs or secondary contact with contaminated clothes or shoes (Brandt-Rauf and Niman 1988). No correlation between serum triglyceride levels and serum PCB levels was found. Physical examinations showed no dermal or other abnormalities consistent with PCB exposure, but it is not specifically mentioned if the examinations looked for liver enlargement. Serum PCB concentrations in the study group were low (generally <10 ppb) in comparison to other occupational studies.

Clearance of antipyrine, a known substrate for microsomal hepatic enzymes, was used to test liver function in two studies of PCB-exposed workers (Alvares et al. 1977; Emmett et al. 1988b). A significantly lower mean half-life of antipyrine clearance from blood was found in five workers exposed for an average of 9 years to various Aroclors, including Aroclor 1260, compared to five control subjects matched for age, sex, and smoking/drinking habits (Alvares et al. 1977). The antipyrine clearance half-

lives were 10.8 and 15.6 hours in the exposed and control subjects ($p<0.005$), respectively, suggesting that exposure induced hepatic microsomal enzymes. The exposed workers were a subgroup of the population studied by Fischbein et al. (1979) who were exposed to mean PCB concentrations ranging from 0.007 to 11 mg/m^3. The second study (Emmett et al. 1988b) found no difference in antipyrine plasma half-life in transformer maintenance workers primarily exposed to lower concentrations of Aroclor 1260 (#0.012 mg/m^3) for an average of 3.75 years compared with controls matched for age, race, and marital status, but not for current smoking and drinking habits (Emmett et al. 1988b). The reason for the different antipyrine liver function test findings in these studies is not clear, but is most likely due to levels and durations of exposure since serum PCB levels were higher (up to 125 ppb) in the responding group (Fischbein et al. 1979) and (<15 ppb) in the Emmett et al. (1988b) group. The difference might also be related to smoking and/or drinking habits.

Contaminated Fish Consumption. Limited information is available on hepatic end points in populations who consumed fish contaminated with PCBs and other chemicals in Triana, Alabama (Kreiss et al. 1981) and the Baltic Sea area (Svensson et al. 1994). No data were located on liver effects in fisheaters from the Great Lakes/St. Lawrence River basin.

Serum γ-glutamyl transpeptidase (GGT) and cholesterol (Section 3.2.2.6.2.2), but not serum ALT or bilirubin, were positively correlated with serum PCB levels in 458 residents of Triana, Alabama (Kreiss et al. 1981). These associations were independent of factors such as age and alcohol and fish consumption, although the natural partitioning of PCBs into serum lipids could contribute to the correlation. Consumption of contaminated fish was the only known source of PCB exposure. The mean serum concentration of PCBs (analyzed as Aroclor 1260) was 17.2 ppb. Levels of DDT were also increased in residents and fish, and there was a strong positive correlation between serum concentrations of DDT and PCB. Serum DDT levels did contribute to the variance in serum GGT and other effects, but this does not preclude the possibility of an interaction between PCB and DDT.

A comparison of 23 Swedish males with a high consumption of Baltic Sea fish and 20 men with virtually no fish consumption showed no statistically significant differences in serum levels of AST, ALT, GGT, AP, or bilirubin (Svensson et al. 1994). The fisheaters had elevated blood levels of PCBs and other organochlorines, as well as increased erythrocyte levels of methylmercury.

Yusho and Yu-Cheng Exposures. Clinical alterations that have been observed in people exposed during the *Yusho* and *Yu-Cheng* PCB accidental ingestion incidents include increases in serum liver-related

enzymes and triglycerides and urinary uroporphyrins (Kuratsune 1989; Rogan 1989). Elevations in serum AST and ALT are generally consistent findings in *Yu-Cheng* patients (Rogan 1989), although few abnormalities in AST and ALT and other basic liver function indices have been associated with *Yusho* exposure (Kuratsune 1989; Masuda 1994). Results of non-routine serum tests (e.g., accelerated erythrocyte sedimentation rate, high titer in thymol turbidity, increased M fraction of lactate dehydrogenase, and increased alkaline phosphatase and ribonuclease levels) suggested liver damage in some *Yusho* patients, particularly severe cases (Masuda 1994).

The predominant morphological finding in the liver of *Yusho* patients appears to be ultrastructural changes suggestive of microsomal enzyme induction, particularly alterations in the endoplasmic reticulum and pleomorphic and enlarged mitochondria (Kuratsune 1989; Masuda 1994). Mortality from cirrhosis of the liver and from liver diseases excluding cirrhosis was increased in both sexes in a cohort of 1,940 *Yu-Cheng* victims (>95% of all registered cases) followed for 12 years after exposure (Hsieh et al. 1996). SMRs for cirrhosis and other liver diseases were 2.79 (95% CI 1.39–5.00) and 5.40 (CI 1.47–13.82), respectively, compared to the Taiwan national populations; rates were similarly increased compared to local populations. Mortality from all liver diseases during the first 3 years after exposure (SMR=10.76, 5.37–19.26) was more than 8 times higher than in the subsequent 9 years.

3.2.2.6.2.2 Serum Lipids, Triglycerides, and Cholesterol

Occupational Exposure. Levels of liver-regulated serum lipids, particularly triglycerides and cholesterol, have been studied in PCB-exposed workers. Serum triglycerides, total cholesterol, ALT, and albumin/globulin ratio were increased in capacitor plant workers with a mean length of employment of 17 years (Lawton et al. 1985a). These workers were exposed to various Aroclor mixtures at a mean concentration of 0.69 mg/m^3 (range, 0.2–2.0), based on monitoring performed in only one area of the plant several months prior to clinical evaluation. In other studies, no changes in serum cholesterol, triglycerides, high-density lipoproteins (HDL), low-density lipoproteins (LDL), very low-density lipoproteins (VLDL), and/or serum albumin levels were found in workers exposed primarily to Aroclor 1260 (#0.012 mg/m^3) for a mean of 3.75 years (Emmett et al. 1988b) or to an unspecified Aroclor mixture (PCB air concentration not reported) in transformer fluids for 4–17 years (Chase et al. 1982).

Significant positive correlations between serum triglyceride or cholesterol levels and serum PCBs in PCB-exposed workers have been reported (Baker et al. 1980; Chase et al. 1982; Emmett 1985; Emmett et al. 1988b; Lawton et al. 1985a; Smith et al. 1982), but not all studies were adjusted for all major

confounding variables. For example, when adjusted for all confounders, Emmett et al. (1988b) found no correlation between serum lipids and serum PCBs. Evidence from this and other studies indicates that correlations between serum lipids and PCBs may be due to the partitioning of PCBs between adipose tissue and lipids in the blood (Brown and Lawton 1984; Emmett 1985; Emmett et al. 1988b; Lawton et al. 1985a). Data from the *Yusho* and *Yu-Cheng* incidents (see subsection below) and animal studies (see Section 3.2.2.6.3.2), however, indicate that elevated serum lipids are an effect of oral exposure to high levels of PCBs.

Contaminated Fish Consumption. Serum cholesterol, serum GGT, and blood pressure, but not serum HDL cholesterol or triglycerides, were positively correlated with serum PCB levels in 458 residents of Triana, Alabama (Kreiss et al. 1981). These associations were independent of age, sex, fish consumption, body mass index, and alcohol consumption. Consumption of contaminated fish was the only known source of PCB exposure, but PCB intake was not estimated. DDT was also increased in the serum of the people and in the fish, and serum DDT and serum PCB levels were highly correlated. Serum DDT levels did not contribute to the variance in serum cholesterol, serum GGT, or blood pressure.

General Population Exposures. Serum cholesterol and triglycerides were increased in individuals with elevated serum PCB levels who had resided near waste sites for 5 years (Steer-Green et al. 1986a, 1986b). The increases were not substantially greater than normal, however, and neither levels of cholesterol nor triglycerides correlated with serum PCB concentrations. Other findings included a significant positive correlation of total bilirubin with serum PCB levels, and significant negative correlations of serum albumin with serum PCBs and of AST with serum lipid fraction-adjusted PCB levels. This study used pooled data from combined residential and occupational exposure. Similar results were reported by Steinberg et al. (1986) using uncorrected data. In addition, a positive correlation between the activities of ß-glucuronidase and 5ℕ-nucleotidase and total serum PCBs was observed in individuals who lived or worked near an electrical equipment manufacturing plant. Similar positive correlations were also found with serum dichlorodiphenyl dichloroethene (DDE) (a metabolite of DDT); no correlations were observed when potential confounding factors (e.g., age, cholesterol) were removed.

Yusho and Yu-Cheng Exposures. Markedly elevated serum triglyceride levels with unchanged total serum cholesterol was a laboratory finding characteristic of *Yusho* and *Yu-Cheng* exposures (Oxymora et al. 1979; Masuda et al. 1994; Uzawa et al. 1969). The elevated triglycerides generally persisted for several years following exposure and subsequently declined to normal levels.

3.2.2.6.2.3 Porphyria

Occupational Exposure. Sixty-seven PCB-exposed workers with a mean employment length of 12 years (range, 2–32 years) exhibited increased urinary excretion of total porphyrins and porphyrin homologues (coproporphyrin, pentaporphyrin, hexaporphyrin, heptaporphyrin, and uroporphyrin) compared to a control population of unexposed electrical workers (Colombi et al. 1982). No shift in the relative urinary levels of porphyrin homologues was observed between the exposed and control groups. The exposed workers were exposed to Aroclor 1254 (unquantitated) for up to 17 years and, subsequently, to 0.048–0.275 mg/m^3 Pyralene 3010 (42% chlorine content) for an unspecified duration; dermal exposure to both PCB mixtures could not be ruled out. In another study, urinary coproporphyrin, uroporphyrin, and porphobilinogen did not correlate with serum PCB levels in workers exposed to various Aroclors and Askarels in concentrations ranging from 0.003 to 0.08 mg/m^3 for >13 years (Smith et al. 1982).

Urinary porphyrin excretion and serum GGT activity were significantly increased in 51 workers who were exposed for a mean duration of 10 years, and 28 of 51 subjects had elevated concentrations of PCBs in the blood (Maroni et al. 1984). As discussed by James et al. (1993), average urinary excretion of porphyrins was almost twice as high as unexposed control group values, but no correlation was found between porphyrin excretion and blood PCB levels.

Yusho and Yu-Cheng Exposures. Type B hepatic porphyria (i.e., a uroporphyrin/coproporphyrin ratio greater than 1) is a consistent finding in *Yu-Cheng* patients, including children born to exposed mothers (Chang et al. 1980; Gladen et al. 1988; Hsu et al. 1994; Lu et al. 1980). Abnormal urinary porphyrin levels have rarely been associated with *Yusho* exposure (Masuda et al. 1994).

3.2.2.6.2.4 Evaluation of Human Studies

There is no clear indication that environmental exposure to PCBs has caused adverse liver effects in humans. Evidence for liver effects of PCBs in humans has been sought in numerous studies of exposed workers. Hepatic end points in these studies are essentially limited to serum enzymes (e.g., AST, ALT, and GGT) and other biochemical indices (e.g., bilirubin, serum lipids, and cholesterol) that are routinely-examined in clinical assays. Antipyrine elimination was evaluated in two studies of PCB workers (Alvares et al. 1977; Emmett et al. 1988b). Results suggest a threshold of 100 ppb in serum for phenobarbital-type induction in humans (Brown 1994). A positive correlation between the frequency of workers with hepatomegaly and elevated serum enzyme values and increasing levels of PCBs in the blood

was reported in one study (Maroni et al. 1981a, 1981b), but there was no apparent relationship between severity of the effect and PCB levels, and no matched control group was included in the study. Studies of people exposed to PCBs by ingestion of contaminated fish (Kreiss et al. 1981) or contaminated rice oil in the *Yusho* or *Yu-Cheng* incidents (Kuratsune 1989; Masuda 1994; Rogan 1989) have shown increases in serum levels of some liver enzymes and other hepatic indices that are indicative of microsomal enzyme induction or liver damage. Ultrastructural changes indicative of microsomal enzyme induction are predominant hepatic morphological findings in *Yusho* patients. Due to the mixed chemical nature of the fish and rice oil exposures, the results cannot be attributed solely to PCBs.

Increased levels of serum triglycerides and cholesterol have not been reported consistently in workers with long-term occupational exposure to PCBs. As discussed by James et al. (1993), the variable results can be explained, at least partially, by failure of the studies to control for variables known to affect serum lipid levels, particularly age, alcohol consumption, and medical history. Because tissue concentrations are generally considered to be a better measure of body burdens and dose received than serum lipid levels, this may explain the difficulty in showing a correlation between serum lipid levels and PCB dose. Additionally, both Emmett et al. (1988b) and Lawton et al. (1985a) showed that associations with serum lipid levels and serum PCB levels can be explained by the partitioning behavior of PCBs, suggesting that serum lipid levels may affect serum PCB levels rather than PCB exposure affecting serum lipid levels. However, as described in the following section, animal data indicate that exposure to PCBs can indeed increase serum lipid levels. A limited amount of information is available on serum lipid effects of PCBs in nonoccupational populations. Serum cholesterol, but not triglycerides, was increased in Triana, Alabama, consumers of contaminated fish (Kreiss et al. 1981), and increases in serum triglycerides, but not cholesterol, were associated with Yusho and Yu-Cheng exposure (Masuda et al. 1994; Oxymora et al. 1979; Uzawa et al. 1969).

Increased urinary excretion of porphyrins appears to be associated with occupational exposure to PCBs (Colombi et al. 1982; Maroni et al. 1984; Smith et al. 1982). Hepatic porphyria was commonly observed in people exposed during the *Yu-Cheng* PCB incident, although it was not a usual finding in *Yusho* cases (Chang et al. 1980; Gladen et al. 1988; Hsu et al. 1994; Lu et al. 1980; Masuda et al. 1994).

3.2.2.6.3 Animal Studies

The highest NOAEL values and all reliable LOAEL values for hepatic effects for each study are recorded in Tables 3-1, 3-2, and 3-3, and plotted in Figures 3-1 and 3-2.

3.2.2.6.3.1 Liver Enzymes, Enlargement, and Pathology

Inhalation Exposure

No histological changes occurred in the liver of adolescent male rats that were whole-body exposed to 0 or 900 ng/m^3 Aroclor 1242 vapor 23 hours/day for 30 days (Casey et al. 1999). The generation of the vapor-phase test atmosphere was based entirely on the evaporation of a liquid PCB mixture using a system that did not create aerosol droplets, and the concentration and congener composition of the test atmosphere was well characterized. Limitations of this study include only one exposure level and liver end point and a relatively small number of animals (8/group); however, uptake of PCBs in the liver was confirmed by tissue analysis, and the exposure was sufficient to induce effects in other tissues, including the thyroid, which is known to be particularly sensitive to PCBs.

Histopathologic lesions were found in the livers of rats, mice, rabbits, and guinea pigs that were intermittently exposed to chamber concentrations of 1.5 mg/m^3 Aroclor 1254 for 7 hours/day for 150 days over a total of 213 days (Treon et al. 1956). Alterations varied in severity depending upon species, ranging from cytoplasmic vacuolation in guinea pigs to fatty metamorphosis and other degenerative lesions in rats. Similar exposures of rats, mice, rabbits, or guinea pigs to Aroclor 1242 for 7 hours/day at 1.9 mg/m^3 for 150 of 214 days, or 8.6 mg/m^3 for 17 of 24 days, did not produce histopathology in the liver or other viscera. Relative liver weight, measured in rats, guinea pigs, and rabbits exposed for 7 hours/day to 6.8 mg/m^3 Aroclor 1242 for 82 of 120 days or 5.4 mg/m^3 Aroclor 1254 for 83 of 121 days was increased only in the rats exposed to Aroclor 1254; liver histology was not evaluated in these studies. None of the exposure scenarios produced treatment-related gross liver pathology in any of the species. It was necessary to vaporize the Aroclors by heating to 55–138 EC to attain the concentrations used in the study, although these temperatures are too low to cause formation of CDFs (Morita et al. 1978).

No information was located on hepatotoxicity in animals following acute- or chronic-duration inhalation exposure to PCBs.

Oral Exposure

Commercial PCB Mixtures. Relatively little information is available on hepatic effects of acute-duration oral exposure to PCBs. Liver microsomal enzyme activity (aminopyrine N-demethylation and acetanilide hydroxylation) was increased in rats exposed to 0.5 mg/kg/day (lowest tested level) Aroclor 1254 for durations as short as 1–3 days (Bruckner et al. 1977); no other hepatic end points were evaluated in this study. Relative liver weight and serum total cholesterol were increased in rats that were fed estimated doses of $1 mg/kg/day Aroclor 1254 for 4 days, but not 0.5 mg/kg/day (Carter 1984, 1985); histology was not evaluated. Acute-duration studies evaluating hepatic effects of PCBs other than microsomal enzyme induction at doses lower than those in the Carter (1984, 1985) studies were not located. Effects in rats exposed to higher doses of PCBs in acute-duration studies included increased liver weight, decreased liver glucose 6-phosphatase, and/or decreased serum cholesterol at $1.9 mg/kg/day Aroclor 1254 (Carter and Koo 1984; Price et al. 1988) and 50 mg/kg/day Aroclor 1248 (Kato and Yoshida 1980), as well as degenerative hepatic histopathological changes at PCB doses $50 mg/kg/day as discussed below. Additional information on PCB-induced hypercholesterolemia is included in Section 3.2.2.6.3.2.

The lowest reported hepatic effect levels in intermediate-duration oral studies are NOAELs for microsomal enzyme induction in rats (Bruckner et al. 1974, 1977; Litterst et al. 1972). Liver microsomal nitroreductase and demethylase were induced in rats that were fed $0.03 mg/kg/day (lowest tested dose) Aroclor 1242, 1248, 1254, or 1260 for 4 weeks (Litterst et al. 1972). All of these PCB mixtures also caused increased relative liver weight at $2.5 mg/kg/day and increased liver triglycerides at $25 mg/kg/day; however, histology was not evaluated. The effects were generally dose-related among the mixtures and the maximum increase in liver triglycerides was caused by Aroclor 1248. No histological changes were found in the liver of adolescent rats exposed to dietary doses of 0 or 0.033 mg/kg/day Aroclor 1242 for 30 days (Casey et al. 1999). Limitations of this study include a relatively small number of animals (8/group) and the lack of more than one dose level and hepatic end point, although tissue congener analyses confirmed uptake of PCBs in the liver. Hepatic microsomal enzymes, liver weight, and lipid deposition in the liver were increased in rats fed $0.25 mg/kg/day Aroclor 1242 for $2 months; no other hepatic histopathologic changes were observed, and serum levels of AST and ALT were not increased (Bruckner et al. 1974). Dietary ingestion of $0.25 mg/kg/day Aroclor 1254 for $35 days similarly induced hepatic microsomal enzymes in rats, but other liver effects (increased liver weight and triglyceride content; histology was not evaluated) only occurred at a higher dose of 1.25 mg/kg/day (Bruckner et al. 1977). Another study with Aroclor 1254 found no significant

change in liver weight in rats fed up to 2.5 mg/kg/day for 5 months (Byrne et al. 1988); no other hepatic end points were evaluated.

Increased relative liver weight and hepatocellular hypertrophy, but no additional histological changes in the liver, occurred in mice that were fed 22 mg/kg/day Aroclor 1242 for 6 weeks (Loose et al. 1978a, 1978b). Microsomal enzyme activity (as indicated by decreased pentobarbital-induced sleeping time) and liver weight were increased in mice fed 32.5 or 130 mg/kg/day Aroclor 1254 for 2 weeks (Sanders et al. 1974). No other liver end points (e.g., serum indices, histology) were evaluated, precluding the determination of whether these doses were hepatotoxic in mice. Liver weights were also increased in mice that were fed an estimated dose of 37.5 mg/kg/day Aroclor 1260 for 14 days, but not in mice administered a single 50 mg/kg dose by gavage (Whysner et al. 1998); no other liver toxicity end points were included in either study.

Fatty degeneration and necrotic changes are characteristic hepatic histopathological effects of PCBs that have been induced in rats and mice exposed to relatively high oral doses, including rats given a single 4,000 mg/kg dose of Aroclor 1242 by gavage (Bruckner et al. 1973); rats fed 100 mg/kg/day Aroclor 1242 for 3 weeks (Bruckner et al. 1973), 50 mg/kg/day Aroclor 1248 or 1254 for 2–4 weeks (Allen and Abrahamson 1973; Kling et al. 1978), or $6.5–7.5 mg/kg/day Aroclor 1254 or 1260 for 8 months (Kimbrough et al. 1972), and mice fed 4.88 mg/kg/day Aroclor 1254 for 6 months or 49.8 mg/kg/day for 11 months (Kimbrough and Linder 1974; Koller 1977). Additionally, lipid accumulation occurred in the liver of offspring of rats that were fed 1.5 mg/kg/day Aroclor 1254 or 1260 (Linder et al. 1974), and hepatocellular hypertrophy and vacuolar degeneration developed in weanling rats that ingested $1.0 mg/kg/day Aroclor 1254 for 10 weeks (Gray et al. 1993). Rabbits fed 2.1 or 6.5 mg/kg/day Aroclor 1254 for 8 weeks had increased relative liver weight, but no treatment-related histological alterations (Street and Sharma 1975); other hepatic end points were not evaluated. Similarly, there were no histological changes in the livers of guinea pigs with significantly increased relative liver weight fed #4 mg/kg/day Aroclor 1260 for 8 weeks (Vos and de Roij 1972).

The most comprehensive chronic toxicity study of PCBs in rodents provides comparative clinical and histology data on four Aroclor mixtures (Fish et al. 1997; General Electric Co. 1997a, 1997b; Mayes et al. 1998). Rats were fed Aroclor 1016, 1242, 1254, or 1260 for 24 months at two (Aroclor 1242) or three dose levels per sex at ranges of 2.0–11.2, 2.0–5.7, 1.0–6.1, or 1.0–5.8 mg/kg/day, respectively. Each lot of the basal feed contained <0.15 ppm of PCBs (estimated dose <0.01 mg/kg/day). As discussed in Section 3.2.8.3.2, the Aroclor 1254 test mixture had levels of congener 3,3',4,4',5-pentaCB (PCB 126)

that were about 2 times greater than that of "ordinary" Aroclor 1254. The liver was a target of all four PCB mixtures as indicated by increases in relative liver weight and hepatic mixed-function oxidases, serum enzyme and cholesterol levels, nonneoplastic lesions, and/or tumors. Hepatic enzyme induction varied with time and declined after reaching maxima, demonstrating the dynamic nature of the CYP end points (Fish et al. 1997). These effects were usually much more severe in females than in males and showed the following general pattern of Aroclor toxicity: 1254>1260 . 1242>1016. Carcinogenicity data from this study are summarized in Section 3.2.8.3.2. Nonneoplastic liver effects induced by Aroclor 1016 included increased hepatocellular hypertrophy and vacuolization at $2.0 mg/kg/day, and increased relative liver weight and bile duct hyperplasia at $2.7 mg/kg/day. Effects caused by Aroclor 1242 included increased hepatocellular hypertrophy and vacuolization, altered hepatocellular foci, and bile duct hyperplasia at $2.0 mg/kg/day, with increased liver weight, serum cholesterol, and bilirubin occurring at 5.7 mg/kg/day. Aroclor 1254 induced hepatocellular changes (hypertrophy, vacuolization, altered foci), bile duct hyperplasia, and increased serum cholesterol and liver weight at $1.0 mg/kg/day, with increases in serum AST, ALT, and GGT occurring at $2.9 mg/kg/day. Aroclor 1260 caused hepatocellular changes (hypertrophy, vacuolization, altered foci), bile duct hyperplasia, and increased liver weight at $1.4 mg/kg/day, and increased serum GGT and cholesterol at $2.8 mg/kg/day.

Histopathological changes in the liver also occurred in rats exposed to dietary Aroclor 1254 at 1.25–5 mg/kg/day for 2 years (Morgan et al. 1981; NCI 1978; Ward 1985), Aroclor 1260 at 5 mg/kg/day for 16 months followed by 2.5 mg/kg/day for 8 months and then no treatment for 5 months (Norback and Weltman 1985), or Aroclor 1260 at 5 mg/kg/day for 21 months (Kimbrough et al. 1975). Although preneoplastic and neoplastic liver lesions were induced in these as well as other rat studies (see Section 3.2.8.3.2), no nonproliferative changes, or nonproliferative lesions that did not progress to liver neoplasms after 1 year, were described.

Intermediate- and chronic-duration studies in monkeys indicate that this species is more sensitive than rodents to the hepatotoxic effects of PCBs. For example, lipid accumulation and focal necrosis were found in one female monkey that died after administration of 0.1 mg/kg/day Aroclor 1248 for 173 days and in one female monkey that died after being fed 0.2 mg/kg/day Aroclor 1248 for 310 days (Barsotti et al. 1976). Although only one animal per dose was examined, it is likely that these effects are treatment related due to the characteristic nature of the hepatic response and because similar effects on the liver occurred in monkeys at higher doses in other intermediate-duration studies (Allen 1975; Allen and Norback 1976; Allen et al. 1974a).

Cynomolgus monkeys that were fed relatively high doses of 2 mg/kg/day Aroclor 1248 or 5 mg/kg/day Aroclor 1254 for up to 20–23 weeks had serum biochemistry changes (increased ALT, AST, AP, LDH, cholesterol, triglycerides, and bilirubin) and histopathologic changes in the liver, including hyperplasia, fatty degeneration and degeneration of hepatocytes, and gall duct/gall bladder epithelial cell hypertrophy hyperplasia (Tryphonas et al. 1984). Hepatic effects observed in Rhesus monkeys after 12–28 months of dietary exposure to 0.2 mg/kg/day Aroclor 1254 included liver enlargement, fatty degeneration, hepatocellular necrosis, and hypertrophic and hyperplastic changes in the bile duct (Tryphonas et al. 1986a, 1986b). Rhesus monkeys that ingested capsules containing 0.005, 0.02, 0.04, or 0.08 mg/kg/day Aroclor 1254 for 72 months had increased liver weight attributed to hyperplasia (unspecified) at 0.08 mg/kg/day, as well as decreased serum levels of total bilirubin and cholesterol and increased serum triglycerides as summarized in Section 3.2.2.6.3.2 (Arnold et al. 1993b, 1997; Bell et al. 1994).

Defined Experimental Mixtures. Female Long-Evans rats were pre- and postnatally exposed to pelleted food containing Aroclor 1254 or a laboratory PCB mixture of 14 congeners resembling the congener pattern in human breast milk (Hany et al. 1999b). Exposure began 50 days prior to mating and was terminated at the day of birth (postnatal day [PND] 0), and the offspring were subsequently exposed via maternal milk until PND 21. The reported estimated average daily PCB intake by the dams was the same for both mixtures at 4 mg/kg/day. Relative liver weight was significantly higher than controls on PND 0 in both Aroclor 1254-exposed dams and their offspring, on PND 0 in offspring of the rats exposed to the simulated mixture, and on PND 21 in nonpregnant (unsuccessfully mated) females exposed to Aroclor 1254 or the simulated mixture. Additional information on the experimental design and results of this study, including the congener composition of the simulated mixture and nonhepatic data, are summarized in Section 3.2.6 (Developmental Effects).

Toxicity of a mixture of PCB congeners analogous to that in human breast milk (Canadian women) was studied in monkeys (Arnold et al. 1999). Groups of infant Cynomolgus monkeys (6 control males, 10 treated males) and Rhesus monkeys (2 control and 3 treated males, 1 control and 3 treated females) ingested the congener mixture in a total daily dose of 0 or 7.5 µg PCBs/kg/day from birth until 20 weeks old, and were observed until they were at least 66 weeks old. The dose represented the approximate daily intake of a nursing human infant whose mother's milk contained 50 ppb PCBs (the Health Canada guideline for maximum concentration in breast milk). Reported hepatotoxicity-related end points are limited to serum biochemical indices, including liver enzymes (ALT, AST, GGT, AP), bilirubin, triglycerides, and cholesterol; data for liver weight and histology are not yet published (as of July 2000). Although there were no statistically significant differences between the exposed and control groups for

any of the individual hepatic end points, significant increasing trends with time were found for serum cholesterol in both strains of monkeys and serum GGT in Rhesus monkeys.

Single Congeners. Multiple hepatic end points were evaluated in comparative studies of individual congeners in rats, mice, and monkeys. In the most comprehensive series of studies, rats were exposed to diets containing four dose levels of a congener for 13 weeks (Chu et al. 1994, 1995, 1996a, 1996b, 1998b; Gilroy et al. 1996, 1998; Lecavalier et al. 1997; MacLellan et al. 1994a, 1994b, 1994c; Peng et al. 1997; Singh et al. 1996, 1997). Eight congeners were tested based on frequent occurrence in environmental samples and human tissues or toxic potency. Hepatic effects included increased liver weight, biochemical changes (e.g., increased serum enzymes and cholesterol, increased liver porphyrins, and decreased liver vitamin A), and histopathology (e.g., cytoplasmic vacuolation and fatty alterations). The most toxic congener was PCB 126 with a LOAEL of 0.74 µg/kg/day, which was approximately 1/50 of the LOAEL of 39 µg/kg/day for PCB 105 (the next most toxic congener) and 1/500 of the LOAEL of 425 µg/kg/day for PCB 128 (the least toxic congener). Considering dose-response and severity of liver effects, the order of toxicity was PCB 126 > PCB 105 > PCB 118 . PCB 77 > PCB 153 . PCB 28 > PCB 128. In general, the non-*ortho* and mono-*ortho* substituted congeners were more potent than the di-*ortho* substituted congeners.

The comparative toxicity of four symmetrical hexachlorobiphenyl isomers was studied in mice (Biocca et al. 1981). Male mice were fed several dose levels of PCB 136, PCB 153, PCB 155, and PCB 169 daily for 28 days. The hepatic LOAEL (foamy cells and microabscesses) was 200 µg/kg/day for PCB 169 and much higher for the other congeners at 21.4 mg/kg/day. Liver effects induced at doses higher than the LOAEL included fatty metamorphosis (PCBs 155 and 169) and increased liver porphyrins (PCB 169).

Rhesus monkeys were exposed to PCB 52 or PCB 77 in estimated dietary doses of 0 or 60 µg/kg/day for 133 days (McNulty et al. 1980). Pathologic changes, including dilation of the extrahepatic biliary tree and hyperplastic intrahepatic biliary vessels, were induced by PCB 77 but not PCB 52. Additional liver data were not obtained for PCB 77 due to high systemic toxicity manifested as clinical signs, general emaciation, and marked effects in nonhepatic tissues.

Dermal Exposure. Limited information is available on liver toxicity of PCBs in dermally-exposed animals. Aroclor 1260, Clophen A60, or Phenoclor Dpb (all 60% chlorine PCB mixtures) was applied in isopropanol to the shaved back skin of female New Zealand rabbits (four/group) on 5 days/week for 28 or 38 days at estimated doses of 0 or 42–44 mg/kg/day (Vos and Beems 1971; Vos and Notenboom-Ram

1972). Hepatic effects included increased relative liver weights, histopathologic changes (e.g., centrilobular degeneration and hepatocyte atrophy, focal necrosis, and cytoplasmic hyalin degeneration), and increased fecal porphyrin levels. In general, the effects occurred in all treated animals and were least and most pronounced in the Aroclor 1260 and Clophen A60 groups, respectively. The CDF content of the Aroclor 1260 used in these experiments was below the detection limit (1 ppm); however, the analytical techniques available then were relatively insensitive.

3.2.2.6.3.2 Serum Lipids, Triglycerides, and Cholesterol

Oral Exposure

Commercial PCB Mixtures. Serum total cholesterol, HDL-cholesterol, and relative liver weight were increased in rats that were fed estimated doses of $1 mg/kg/day Aroclor 1254 for 4 days; no effects occurred at 0.5 mg/kg/day (Carter 1984, 1985). Serum LDL- and VLDL-cholesterol fractions were not increased in any dose group (#3.9 mg/kg/day). The lowest level causing increased HDL-cholesterol and liver weight was 1 mg/kg/day in the Carter (1984) study and 1.9 mg/kg/day in the Carter (1985) studies. Effects in rats exposed to PCBs in other acute-duration studies included increased serum cholesterol and liver weight at $1.9 mg/kg/day Aroclor 1254 (Carter and Koo 1984; Price et al. 1988) and 50 mg/kg/day Aroclor 1248 for 4 days (Kato and Yoshida 1980), as well as degenerative hepatic histopathological changes at 50 mg/kg/day Aroclor 1254 and 4,000 mg/kg/day Aroclor 1242 (Bruckner et al. 1973; Kling et al. 1978) as summarized above in Section 3.2.2.6.3.1.

Changes in serum lipid profiles commonly occurred in rats exposed to PCBs in intermediate-duration dietary studies (Andrews 1989; Bruckner et al. 1974, 1977; Gray et al. 1993; Kato et al. 1981a, 1981b, 1982b; Kling and Gamble 1982; Litterst et al. 1972). Effects included increased liver lipids at $0.3 mg/kg/day Aroclor 1242 for 2–6 months (Bruckner et al. 1974), increased liver triglycerides at 1.25 mg/kg/day Aroclor 1254 for 35 days (Bruckner et al. 1977), increased serum cholesterol at $10 mg/kg/day Aroclor 1254 for 5 weeks (Andrews et al. 1989), and increased liver lipids and liver and serum cholesterol at $15 mg/kg/day Aroclor 1248 for 20–24 days (Kato et al. 1981b, 1982b). Serum cholesterol, phospholipids, and triglycerides were similarly increased in rats fed 15 mg/kg/day Aroclor 1248 for 68 days (Oda and Yoshida 1994). Additional analyses performed by Oda and Yoshida (1994) showed that serum total lipoproteins were also elevated, with increases in protein, cholesterol, phospholipid, and triglycerides occurring among the lipoprotein fractions (VLDL, LDL, HDL1, HDL2).

Increased serum cholesterol was one of several manifestations of liver toxicity in rats found in the 24-month comparative study of several Aroclor mixtures (General Electric Co. 1997a, 1997b; Mayes et al. 1998) summarized in Section 3.2.2.6.3.1. Serum cholesterol was increased in females exposed to Aroclors 1242, 1254, and 1260 at 5.7, $1.4, and $2.8 mg/kg/day, respectively; no serum cholesterol changes were induced by Aroclor 1016 at doses as high as 11.2 mg/kg/day. Increased serum cholesterol levels observed in most PCB-exposed males appeared to be treatment-related only for Aroclor 1254. The effect in Aroclor 1254 males was minimal as the increase was slight and not clearly dose-related (statistically significant at 1.0 and 4.3 mg/kg/day, but not at 2.0 mg/kg/day). Increases in serum cholesterol in males exposed to Aroclor 1016, 1242, and 1260 were not consistently dose- or time-related and were considered to be equivocal. Considering the effect levels and sizes of increases in females, the order of toxicity was Aroclor 1254 followed by 1260, 1242, and 1016.

Effects in monkeys that ingested Aroclor 1254 in capsules daily for 37 months included normal plasma lipid profiles at doses #0.02 mg/kg/day, decreased total and VLDL + LDL cholesterol at $0.04 mg/kg/day, and decreased HDL cholesterol and total carnitine (which is involved in fatty acid metabolism) at 0.08 mg/kg/day (Arnold et al. 1993b; Bell et al. 1994). Plasma triglycerides were significantly elevated an apparent maximum of 30–40% at all tested doses (0.005–0.08 mg/kg/day) except 0.04 mg/kg/day. Bell et al. (1994) found statistically significant correlations supporting a causal relationship between PCB intake and the plasma lipid/lipoprotein changes, including an indication that the elevation in plasma triglycerides was not due to the partitioning of PCBs between adipose tissues and blood lipids. No correlation was found between the increases in triglycerides and HDL cholesterol.

Single Congeners. A comprehensive series of toxicity studies was performed in rats that were fed various individual congeners for 13 weeks, as detailed in Section 3.2.2.6.3.1 (Chu et al. 1994, 1995, 1996a, 1996b, 1998b; Gilroy et al. 1996, 1998; Lecavalier et al. 1997; MacLellan et al. 1994a, 1994b, 1994c; Peng et al. 1997; Singh et al. 1996, 1997). Effects included increased serum cholesterol levels that were caused by exposure to PCB 126 at $7.4 µg/kg/day and PCB 105 at $3,960 µg/kg/day. No changes in serum cholesterol were induced by PCB 28 at #3,956 µg/kg/day, PCB 77 at #892 µg/kg/day, PCB 118 at #683 µg/kg/day, PCB 128 at #4,397 µg/kg/day, or PCB 153 at #4,125 µg/kg/day.

3.2.2.6.3.3 Porphyria

Oral Exposure

Commercial PCB Mixtures. Urinary coproporphyrin levels were increased in rats that ingested 0.3 or 1.5 mg/kg/day Aroclor 1242 in the diet for 2–6 months (Bruckner et al. 1974). Rats treated with 5 mg/kg/day Aroclor 1254 in the diet had maximum increases in liver microsomal P-450 concentration and liver weight after 1 week, but onset of porphyria and induction of δ-aminolevulinic acid (ALA) synthetase was delayed until 2–7 months of treatment (Goldstein et al. 1974). A marked accumulation of uroporphyrins occurred in the liver, and urinary excretion of coproporphyrin and other porphyrins was increased; the largest increase was in uroporphyrins. The uroporphyrins in the liver and urine of the treated rats consisted primarily of 8- and 7-carboxyporphyrins.

Single Congeners. Increased hepatic uroporphyin is one of the effects observed in rats that were fed various single PCB congeners for 13 weeks (Chu et al. 1994, 1995, 1996a, 1996b, 1998b; Gilroy et al. 1996, 1998; Lecavalier et al. 1997; MacLellan et al. 1994a, 1994b, 1994c; Peng et al. 1997; Singh et al. 1996, 1997). Liver uroporphyrin was increased by exposure to PCB 126 at $0.74 µg/kg/day, PCB 105 at $3,960 µg/kg/day, or PCB 128 at $4,210 µg/kg/day, but not by PCB 28 at #3,956 µg/kg/day, PCB 77 at #892 µg/kg/day, PCB 118 at #683 µg/kg/day, or PCB 153 at #4,125 µg/kg/day. Additional information on the design and results of these studies is summarized in Section 3.2.2.6.3.1.

Dermal Exposure. Groups of four New Zealand rabbits were dermally treated with 0 or 42–44 mg/kg/day estimated doses of Aroclor 1260, Clophen A60, or Phenoclor Dpb (all 60% chlorine PCB mixtures), on 5 days/week for 28 or 38 days (Vos and Beems 1971; Vos and Notenboom-Ram 1972). The PCBs were dissolved in isopropanol and applied to shaved back skin. All three PCB mixtures caused significantly increased fecal levels of coproporphyrin and protoporphyrin, and ultraviolet fluorescence, indicative of porphyrin accumulation, was increased in the liver and other tissues. Similar dermal exposure to the congener PCB 153 caused higher fecal levels of coproporphyrin and protoporphyrin than those in rabbits exposed to the same dose of Aroclor 1260 (Vos and Notenboom-Ram 1972).

3.2.2.6.3.4 Other Hepatic Effects

Vitamin A homeostasis was altered in rats that were exposed to 100 mg/kg/day (only tested dose) of PCB 169 in the diet for 77 days (Bank et al. 1989). Effects included significantly decreased hepatic vitamin A, increased renal vitamin A, increased serum retinol, decreased plasma clearance and half-time of injected retinol (i.e., intravenously administered [^{3}H]retinol-labeled retinol binding protein-transthyretin complex), decreased hepatic and increased renal uptake uptake of injected retinol, and increased urinary and fecal excretion of injected retinol.

Vitamin A levels in the liver were also reduced in rats following oral exposure to various other congeners for 13 weeks (Chu et al. 1994, 1995, 1996a, 1996b, 1998b; Gilroy et al. 1996, 1998; Lecavalier et al. 1997; MacLellan et al. 1994a, 1994b, 1994c; Peng et al. 1997; Singh et al. 1996, 1997). This effect occurred following ingestion of PCB 126 at $0.74 µg/kg/day, PCB 77 at $768 µg/kg/day, and PCB 153 at $4,125 µg/kg/day), but not by exposure to PCB 28 at #3,956 µg/kg/day, PCB 105 at #4,327 µg/kg/day, PCB 118 at #683 µg/kg/day, or PCB 128 at #4,397 µg/kg/day.

3.2.2.6.3.5 Evaluation of Animal Studies

The hepatotoxicity of commercial PCBs is well-documented in numerous intermediate- and chronic-duration studies in animals, particularly in rats and monkeys, which are the most extensively tested species. These studies also indicate that monkeys are more sensitive to PCBs than rats and other laboratory species. Liver effects are similar in nature among species, appear to be reversible when mild, and characteristically include hepatic microsomal enzyme induction, increased serum levels of liver-related enzymes indicative of possible hepatocellular damage, liver enlargement, fat deposition, fibrosis, and necrosis. Ultrastructural changes include hepatocyte alterations associated with microsomal enzyme induction (e.g., proliferation of endoplasmic reticulum, enlarged and pleomorphic mitochondria), lipid droplets, and enlarged parenchymal cells. There is relatively little information on hepatic effects of commercial PCB mixtures in animals exposed by acute-duration oral exposure or the inhalation or dermal routes, although available data are consistent with the findings of the intermediate- and chronic-duration oral studies. The results of a comprehensive comparative 24-month oral toxicity study in rats indicate that the general pattern of hepatotoxicity was Aroclor 1254 > Aroclor 1260 . Aroclor 1242 > Aroclor 1016 (Mayes et al. 1998).

Other liver-related effects of PCBs include altered lipid and porphyrin metabolism. Increased serum levels of total lipids, triglycerides, and/or cholesterol are characteristic effects of short- and long-term oral exposures to PCBs that are well-documented in rats and monkeys (Carter 1984, 1985; Kato and Yoshida 1980; Kato et al. 1982a, 1982b; Oda and Yoshida 1994; Quazi et al. 1984). The results of comparative studies in rats exposed to various Aroclor mixtures for 24 months (Mayes et al. 1998) or single congeners for 13 weeks (Chu et al. 1994, 1995, 1996a, 1996b, 1998b; Gilroy et al. 1996, 1998; Lecavalier et al. 1997; MacLellan et al. 1994; Peng et al. 1997; Singh et al. 1996, 1997) indicate that Aroclor 1254 and 3,3',4,4',5-pentaCB (PCB 126) are particularly effective in increasing serum cholesterol. Hepatic porphyria is a well-documented effect that has been induced in rats, rabbits, and other species following oral or dermal exposure to PCBs (Bruckner et al. 1974; Chu et al. 1994, 1995, 1996a, 1996b, 1998b; Gilroy et al. 1996, 1998; Goldstein et al. 1974; Lecavalier et al. 1997; MacLellan et al. 1994a, 1994b, 1994c; Peng et al. 1997; Singh et al. 1996, 1997; Vos and Beems 1971; Vos and Notenboom-Ram 1972).

3.2.2.7 Renal Effects

3.2.2.7.1 Human Studies

Urinalysis of PCB-exposed capacitor plant workers showed no abnormalities in blood urea nitrogen (BUN) or other routinely-examined kidney function indices (Fischbein et al. 1979; Lawton et al. 1985a). Most of the workers studied by Fischbein et al. (1979) were exposed to mean concentrations of Aroclors 1254 and 1242 and/or other PCBs ranging from 0.007 to 11 mg/m^3 for $5 years; 40% of the workers were employed for $20 years. The workers in the Lawton et al. (1985a) study were exposed to various Aroclor mixtures for a mean duration of 17 years; the mean PCB concentration was 0.69 mg/m^3 (range, 0.2–2.0), based on monitoring performed in only one area of the plant several months prior to clinical evaluation.

3.2.2.7.2 Animal Studies

Information on the renal toxicity of PCBs comes from an inhalation study, a number of oral exposure studies, and several dermal exposure studies involving PCB mixtures or single congeners. Slight degeneration of the renal tubules was observed in rats exposed to chamber concentrations of 1.5 mg/m^3 Aroclor 1254 over 213 days (Treon et al. 1956). No information was reported on renal histological effects in other species (mice, guinea pigs, and rabbits) exposed to Aroclor 1254 under the same conditions or in rats, mice, guinea pigs, or rabbits similarly exposed to 1.9 mg/m^3 Aroclor 1242.

Interpretation of gross pathology data in this study is complicated by imprecise reporting and/or small numbers of animals, but it appears that there were no gross renal changes. The concentrations of PCBs are uncertain due to an invalid analytical technique and differential enrichment of the more volatile PCB congeners in the vapor phase.

A single near-lethal gavage dose of 4,000 mg/kg of Aroclor 1242 produced renal tubular damage in an unreported percentage of rats evaluated 24 hours following treatment (Bruckner et al. 1973). Effects included vacuolated tubular epithelial cells with fatty deposits and epithelial cells and proteinaceous casts in the tubular lumens and urine. Neither serum sodium or potassium ion concentrations or blood pH values were altered significantly by treatment, but lack of changes in these indices does not necessarily indicate that there was no functional damage in the kidney. No effect on kidney weight was observed in pregnant C57BL/6J mice given #21 mg/kg PCB by gavage on 5 consecutive days beginning on day 1, 6, or 11 of pregnancy (Rodriguez et al. 1997).

Cortical tubular protein casts were observed in the kidneys of rats treated with $1.0 mg/kg/day Aroclor 1254 for 15 weeks (Gray et al. 1993). The same group of investigators had previously observed increased kidney weight and biochemical alterations suggestive of functional renal damage, including increased urinary lactate dehydrogenase and urinary protein in rats treated with $10 mg/kg/day Aroclor 1254 by gavage for 5–15 weeks (Andrews 1989). Histology was not evaluated in the Andrews (1989) study. Renal histopathologic changes (lipid vacuolization and sloughing of the tubular epithelium) occurred in rats with no increase in kidney weight when treated with 100 mg/kg/day Aroclor 1242, 3 days/week for 3 weeks (Bruckner et al. 1973); these degenerative effects are similar to those observed in the acute study described above. No histological effects were observed in the kidneys of rats treated with 1.5 mg/kg/day Aroclor 1242 in the diet for 2–6 months (Bruckner et al. 1974). Similarly, no renal histopathologic changes were observed in male or female rats that were fed Aroclor 1016, 1242, 1254, or 1260 for 24 months at dose levels of 8.0–11.2, 4.0–5.7, 4.3–6.1, or 4.1–5.8 mg/kg/day, respectively (Mayes et al. 1998). There were no treatment-related renal organ weight changes or histological effects in rabbits fed #6.5 mg/kg/day Aroclor 1254 for 8 weeks (Street and Sharma 1975) or guinea pigs fed #4 mg/kg/day Aroclor 1260 for 8 weeks (Vos and de Roij 1972). No renal histological effects were observed in one monkey that died after 128 days of dietary treatment with 1.3 mg/kg/day Aroclor 1248 (Allen et al. 1974a). Rhesus monkeys receiving daily doses of 0.005, 0.020, 0.040, or 0.080 mg/kg/day Aroclor 1254 for 72 months also showed no effects on renal tissue (Arnold et al. 1997).

In a series of 13-week dietary exposure studies using single PCB congeners, no histological alterations in the kidneys were observed in rats fed diets providing #4.1 mg/kg/day of PCB 153 (Chu et al. 1996a), #4.2 mg/kg/day of PCB 128 (Lecavalier et al. 1997), #7.4 mg/kg/day of PCB 126 (Chu et al. 1994), #4.0 mg/kg/day of PCB 105 (Chu et al. 1998b), #3.7 mg/kg/day of PCB 28 (Chu et al. 1996b), or #0.77 mg/kg/day of PCB 77 (Chu et al. 1995). In similarly treated rats exposed to PCB 118, minimal histological damage (cytoplasmic shedding and inclusions in the renal tubules) was observed at 0.17 mg/kg/day (Chu et al. 1995).

Hydropic degeneration of the convoluted tubules, destruction of tubular epithelial cells, tubular dilation, and proteinaceous casts were observed in half of the rabbits treated with Aroclor 1260 in an isopropanol vehicle applied 5 days/week for 38 days at an estimated dose of 42 mg/kg/day (Vos and Beems 1971). No kidney effects were observed in a similar study in which 44 mg/kg/day Aroclor 1260 was applied in the same manner to adult female New Zealand rabbits 5 days/week for 28 days (Vos and Notenboom-Ram 1972). The reason for the discrepancy in the results is unclear since the doses are essentially the same, but it may be related to the small numbers treated (four per study) and to the longer duration of the 1971 study. The Aroclor 1260 used in both studies had undetectable (<1 ppm) levels of CDFs.

The highest NOAEL values and all reliable LOAEL values for renal effects for each study are recorded in Tables 3-1, 3-2, and 3-3, and plotted in Figures 3-1 and 3-2.

3.2.2.8 Endocrine Effects

This section describes effects of exposure to PCBs on the thyroid and other non-reproductive endocrine systems. Estrogenic, anti-estrogenic, and anti-androgenic effects of PCBs are discussed in Sections 3.2.5 (Reproductive Effects), 3.5.2 (Mechanisms of Toxicity), and 3.6 (Endocrine Disruption).

3.2.2.8.1 Summary

A number of studies have examined the relationships between PCB exposure and thyroid hormone status in both children and adults. The results suggest that PCBs can induce thyroid toxicity as well as a variety of changes in thyroid hormone levels. Differing results have been reported for differing Aroclor mixtures and PCB congeners, as well as for differing exposure scenarios and differing ages at the time of exposure. Increased thyroid gland volume has been found among workers at a PCB production facility as well as among nearby residents. An elevated odds ratio for goiter has been found among the *Yu-Cheng* cohort.

In addition, numerous statistically significant positive and/or negative correlations (for a number of different age groups) have been reported between circulating levels of TSH, T_4, and T_3, and varying measures of PCB exposure.

Evidence for a thyroid hormone involvement in PCB toxicity in animals is much stronger and includes findings in rodents and nonhuman primates. Depending on dose and duration, PCBs can disrupt the production and disposition of thyroid hormones at a variety of levels. The major findings include (1) histological changes in the thyroid gland indicative of both stimulation of the gland (e.g., similar to that induced by TSH or a hypothyroid state) and a disruption of the processing of follicular colloid needed for normal production and secretion of thyroid hormone; (2) depression of serum T_4 and T_3 levels, which may effectively create a hypothyroid state (in some studies, low doses resulted in elevated serum T_4 levels while depressed levels occurred at higher PCB doses); (3) increased rates of elimination of T_4 and T_3 from serum; (4) increased activities of T_4-uridine diphosphate-glucuronyl transferase (UDP-GT) in liver, which is an important metabolic elimination pathway for T_4 and T_3; (5) decreased activity of iodothyronine sulfotransferases in liver which are also important in the metabolic elimination of iodothyronines; (6) decreased activity of iodothyronine deiodinases including brain Type-2 deiodinase, which provide the major pathways for the production of the active thyroid hormone, T_3; and (7) decreased binding of T_4 to transthyretin, an important transport protein for both T_4 and T_3. Other effects of PCBs on endocrine function that have been observed in experimental animals include effects on the adrenal glands and serum adrenal steroid levels.

3.2.2.8.2 Human Studies

Occupational Exposures. Total thyroxine (T_4) and free T_4 (T_4 index) were significantly lower (approximately 10%) in a group of 55 transformer maintenance workers compared to a comparison control group of workers (Emmett et al. 1988b), even though thyroid hormone levels were in the normal range for adults in both groups. The transformer workers were primarily exposed to Aroclor 1260 at levels ranging from 0.00001 to 0.012 mg/m^3; the mean length of exposure was approximately 4 years. Although there was a statistically significant increase in thyroxine levels in the PCB-exposed cohort, there was no correlation between PCB levels in serum or adipose tissue and serum T_4 concentrations (adjusted for age, smoking, and alcohol consumption).

Langer et al. (1998) measured thyroid volumes in 238 employees of a factory that produced PCBs, and in 572 adults from “less polluted areas” of Slovakia, which formed a sex- and age-matched control group.

Various serum indices of thyroid status were measured in subsamples of these groups, including total serum T_4, serum TSH, thyroglobulin (TGB); and antibodies for thyroid peroxidase (TPO Ab), thyroglobulin (Tg Ab), and TSH receptor (TSHR Ab). Mean thyroid volume was significantly greater in the workers compared to the control group (18.85±0.69 mL vs. 13.47±0.48 mL, $p<0.001$). Workers also had a significantly elevated prevalence of TPO Ab, Tg Ab, and TSHR Ab. There were no differences between the worker and control groups with regard to serum T_4, TSH, or TGB concentrations. Although larger thyroid volume could reflect a difference in the iodine intakes between the two groups, the investigators indicated that this was not likely because iodine intakes were considered sufficient in Slovakia and urinary iodine concentrations were similar in the worker and control groups (data not reported).

Yusho and Yu-Cheng Exposures. In a case-control study of the Taiwan *Yu-Cheng* cohort, 795 exposed subjects and 693 sex- and age-matched controls were interviewed for information about health and medical history (Guo et al. 1999). The odds ratio (OR) for goiter (men and women combined) was 2.8 (CI, 1.2–7.1) and 4.0 (CI, 1.5–13.9) for goiter that was treated with medication or surgery. The ORs for hypothyroidism or hyperthyroidism were not significant (males, 0.95; females, 1.7).

General Population Exposures. Several studies have examined relationships between indices of PCB exposure and thyroid hormone status, as indicated from measurements of serum thyroid hormones. The results of these studies have been mixed, with negative, positive, or no correlations observed. Osius et al. (1999) examined the relationship between whole blood concentrations of various PCB congeners and serum TSH, free T_4, and free T_3 in children who lived near a hazardous waste incinerator. Although the median and 5th–95th percentile ranges of the hormone concentrations in the study population (671 children, ages 7–10 years) were within expected ranges for children, a significant positive ($\beta=7.129$, $p=0.039$) association was found between concentrations of TSH in serum and PCB 118 in blood. Significant negative associations were found between serum T_3 and PCBs 138, 153, 180, 183, and 187.

Several studies have examined relationships between thyroid hormone levels in infants and maternal or neonatal PCB concentrations, or mixed PCB and CDD concentrations (Koopman-Esseboom et al. 1994a; Longnecker et al. 2000; Nagayama et al. 1998a; Winneke et al. 1998a). Hormone levels were within normal ranges in these studies. In describing hormone levels in the serum or plasma, the designations TT_4 or TT_3 have been used to denote total hormone concentrations, whereas free concentrations are denoted as FT_4 or FT_3. If not specified in the report, the notations T_4 or T_3 have been used. Longnecker et al. (2000) compared PCB concentrations in breast milk of 880 mothers to serum TSH, TT_4, and FT_4 concentrations

in cord blood at delivery. The subjects in this study are from the North Carolina Breast Milk and Formula Project cohort summarized in Section 3.2.4.2.1.2. Concentrations of T_4 and TSH were not shown to be related to breast milk PCB concentrations. However, a significant positive correlation (r=0.15, p=0.029)was found between TSH concentrations in cord blood and total serum PCBs in 170 infants from the German cohort in the European Background PCB Study summarized in Section 3.2.4.2.1.2 (Winneke et al. 1998b). Nagayama et al. (1998) examined the relationship between serum TSH, TT_4, and TT_3 in infants and estimated intake of 2,3,7,8-TCDD toxic equivalent (TEQ) in breast milk during the first year of postnatal life. Significant negative correlations were found for serum TT_4 and TT_3; no relationship was apparent between infant serum TSH or thyroxine binding globulin (TBG) and TEQ intake. The mean total TEQ intake was 34 ng/kg; however, the co-planar PCB contribution to the estimated TEQ intake, and intakes of other PCBs were not reported. As part of the Dutch Mother-Child Study cohort summarized in Section 3.2.4.2.1.2, Koopman-Esseboom et al. (1994a) compared TEQ levels of PCBs and dioxins in maternal milk with TT_3 and TT_4 concentrations in maternal plasma, TSH concentrations in cord plasma at delivery, and TSH concentrations in venous plasma of the infants at ages 2 weeks and 3 months. Higher levels of total PCB-dioxin TEQ, dioxin TEQ, and both planar and non-planar-PCB TEQ in milk were significantly correlated with lower maternal plasma TT_3 concentrations in the last month of pregnancy, lower maternal plasma TT_3 and TT_4 concentrations in the 2^{nd} week after delivery, and higher plasma TSH concentrations in the infants at 2 weeks and 3 months of age.

Langer et al. (1998) measured thyroid volumes in 454 adolescents in Slovakia who lived near a factory that produced PCBs, and in 956 adolescents who lived in “less polluted areas” of Slovakia, which formed a sex- and age-matched control group. Various serum indices of thyroid status were measured in subsamples of these groups, including TSH and TPO Ab. Mean thyroid volume was significantly greater in the group who lived near the factory compared to the control group (9.37±0.17 mL vs. 8.07±0.10 mL, p<0.001). There were no differences between the groups with regard to serum TSH or TPO Ab concentrations. As with the worker cohort (discussed with the occupational studies), the investigators indicated that a difference in the iodine intakes between the two groups was not likely because iodine intakes were considered to be sufficient and urinary iodine concentrations were similar in the two groups (data were not reported).

An ongoing epidemiologic study is investigating the potential for health effects in Native Americans from exposure to persistent toxic substances (Dellinger et al. 1997; Tarvis et al. 1997). Fish consumption, species consumed, and medical histories were obtained from 541 Native Americans on eight reservations in Minnesota, Wisconsin, and Michigan. Preliminary results indicated elevated serum PCB levels (mean

was 3.7 ppb and the maximum was 9.6 ppb) were correlated with self-reported diabetes and liver disease in two of the cohorts (Ojibwa and Red Cliff). The average annual fish consumption rate was 23 g/day. No additional information was available regarding the potential link between PCBs and diabetes, although there is growing evidence of an association between dioxin exposure and diabetes (ATSDR 1998).

Evaluation of Human Studies. The epidemiological literature suggests a link between PCBs exposure and thyroid hormone anomalies in humans. Studies that have examined relationships between PCB exposure and thyroid hormone status, in children or adults, have reported a variety of different results, with findings of both negative and positive significant correlations between PCB exposure and circulating levels of TSH, T_4 or T_3 depending on the specific type of analysis for PCB exposure, the age of the cohort, and the specific exposure scenario (Emmett et al. 1988b; Koopman-Esseboom et al. 1994a; Langer et al. 1998; Longnecker et al. 2000; Nagayama et al. 1998; Osius et al. 1999; Winneke et al. 1998a). A comparison of PCB levels in blood and breast milk in some of these studies is included in Appendix A. Although many of the populations examined had thyroid hormone levels within normal ranges, many of these studies also showed statistically significant differences in circulatory thyroid hormone levels in exposed cohorts compared to unexposed controls. In addition, a significantly elevated OR for goiter was found among the *Yu-Cheng* cohort (Guo et al. 1999), suggesting the possibility of excess thyroid disease in a population that experienced relatively high exposures to mixtures of PCBs and CDDs. Other observations include reports of increased thyroid gland volume among workers at a PCB production facility, as well as among nearby residents (Langer et al. 1998). Considering the epidemiologic data as well as the much stronger findings in thyroid studies in animals discussed in the following section, there is mounting evidence of thyroid hormone involvement in PCB toxicity in humans.

3.2.2.8.3 Animal Studies

The highest NOAEL values and all reliable LOAEL values for endocrine effects for each study are recorded in Tables 3-1 and 3-2, and plotted in Figures 3-1 and 3-2.

Effects on Thyroid Gland and Hormones

Commercial PCB Mixtures. Various effects on the thyroid gland and thyroid hormone system have been observed in rats exposed to Aroclor 1254 by the oral route. Descriptions of the histological changes in

the rat are reasonably consistent across studies. Typical findings, depending on the dose, include hyperplasia, hypertrophy, and increased vacuolization of follicular cells, depletion of follicular colloid and reduced follicular size, and thyroid enlargement (Collins and Capen 1980a; Collins et al. 1977). These changes are similar to the histological appearance of the gland during prolonged TSH stimulation (Capen 2000). Additional abnormalities have also been noted when the gland has been examined at the ultrastructural level. Collins and Capen (1980a) observed in PCB-treated rats the accumulation of colloid droplets and large, abnormally shaped lysosomes in the follicular cells that were indicative of a disruption of the normal lysosomal processing of colloid. They also noted distinct abnormalities in follicular microvilli (shortening and abnormal branching) that were uncharacteristic of the TSH-stimulated or iodide-stimulated gland. Thus, the effects of Aroclor 1254 on the thyroid gland are not completely explained solely by a direct or indirect stimulation of the gland through a TSH mechanism. A complex mechanism is further indicated from observations of the forementioned structural changes with or without concurrent changes in circulating thyroid hormone (T_4 or T_3) or TSH levels, or changes in hormone levels without changes in thyroid gland, or changes in hormone levels that vary in magnitude and direction over time (Hood et al. 1999; Saeed and Hansen 1997). Thus, while a general consensus has emerged that Aroclor 1254 produces a stimulation of the thyroid gland and thyroid hormone production (Byrne et al. 1987), it is not clear to what extent this results from a direct effect on the thyroid gland or as an indirect effect resulting from changes in circulating thyroid hormone and induction of TSH. It is likely that both contribute to varying degrees depending on the dosage and duration of exposure (Saeed and Hansen 1997). It is important to emphasize that characteristic structural changes that have been attributed to Aroclor 1254 may not be apparent when the gland is viewed only at the light microscopic level, which has been the approach used in most studies. Furthermore, histopathology of the gland should not be inferred from observed changes in circulating thyroid hormone or TSH levels, alone. Experimental studies that provide evidence for Aroclor-mediated effects on the thyroid gland and/or thyroid hormone status are noted below. In describing hormone levels in the serum or plasma, the designations TT_4 or TT_3 have been used to denote total hormone concentrations, whereas free concentrations are denoted as FT_4 or FT_3. If not specified in the report, the notations T_4 or T_3 have been used.

In an acute-duration study, Hood et al. (1999) observed significant depression of serum TT_4 and FT_4 in rats fed $25 ppm Aroclor 1254 in food (2.3 mg/kg/day) for 7 days and depression of TT_3, but not FT_3 in rats fed $50 ppm (4.6 mg/kg/day). PCBs at exposure levels up to 200 ppm (18 mg/kg/day) had no effect of serum TSH levels or on thyroid structure. TT_4 levels were reduced in rats fed $2.5 mg/kg/day for 7 days, but there were no treatment-related changes in serum TT_3 (Price et al. 1988). This study reports histological changes in the thyroid gland that are typical of Aroclor 1254-related changes that have been

observed in intermediate- and chronic-duration studies; however, it is not clear from the report whether the changes occurred at the 2.5 mg/kg/day dosage or at higher dosages.

Collins et al. (1977) conducted one of the more comprehensive evaluations of the histopathology of intermediate-duration exposures to Aroclor 1254 in rats. Rats were fed 5, 50, or 500 ppm Aroclor 1254 in food for 4 weeks (approximately 0.44, 4.4, or 44 mg/kg/day). Ultrastructural changes in the thyroid were evident at the lowest exposure level and became more pronounced and evident with light microscopy at the 50 ppm exposure level (4.4 mg/kg/day). Serum concentrations of TT_4 were significantly depressed (42%) at the 50 ppm level and both TT_4 and TT_3 were depressed (79 and 13%, respectively) at the 500 ppm level. Thyroid lesions in rats that were exposed to 500 ppm for 6 weeks followed by 250 ppm for 6 weeks were largely absent after a subsequent 12 weeks on a control diet, suggesting substantial recovery, and were not evident at all after a period of 35 weeks on the control diet (Collins and Capen 1980a). A similar time course of recovery of serum TT_4 concentrations was observed. Thus, the observed lesions in this study and at these doses appeared to be reversible.

In other intermediate-duration studies, oral exposures of rats for 1–5 months decreased serum levels of T_4 and T_3 and/or produced histological changes in the thyroid (Byrne et al. 1987; Gray et al. 1993; Kasza et al. 1978). Thyroid effects in rats occurred at oral doses as low as 0.09 mg/kg/day for 35 days (Byrne et al. 1987). In Sprague-Dawley rats, serum levels of T_4 decreased when rats received daily gavage dosages of $0.1 mg/kg/day Aroclor 1254 for 15 weeks; however, no histopathologic alterations were observed in the thyroid after gavage dosages of up to 25 mg/kg/day Aroclor 1254 (Gray et al. 1993). The lack of effect of this dose of Aroclor 1254 on the histological status of the thyroid in the Sprague-Dawley rat, in comparison to histological changes observed in Osborne-Mendel rats at similar dosages and durations (Collins and Capen 1980a; Collins et al. 1977), suggests a possible strain-related difference, or some other unaccounted variable in either study. Byrne et al. (1987) attempted to discern the relative contributions of production and metabolism in the depression of serum T_4 concentrations that occur during Aroclor 1254 exposure. In rats exposed to Aroclor 1254 in diet at concentrations $1 ppm (0.09 mg/kg/day) for $35 days, serum TT_4 and TT_3 levels were depressed; however, the rate of clearance of injected radiolabeled T_4 was not changed by the PCB exposures, relative to a control group, suggesting that the decline in T_4, and possibly T_3 concentrations, was primarily the result of a decline in T_4 production in the thyroid.

In chronic-duration studies, enlarged thyroid glands and follicles with desquamated cells were observed in Rhesus monkeys exposed to 0.2 mg/kg/day Aroclor 1254 for 28 months (Tryphonas et al. 1986b);

serum levels of thyroid hormones were not evaluated. In Cynomolgus monkeys, treatment for 12 months with 0.2 mg/kg/day Aroclor 1254 did not induce histological alterations in the thyroid (Tryphonas et al. 1986a). Rhesus monkeys receiving daily doses of 0.005, 0.020, 0.040, or 0.080 mg/kg/day Aroclor 1254 for 72 months showed no effect on thyroid tissue (Arnold et al. 1997). After 37-months of exposure to 0.08 mg/kg/day Aroclor 1254, serum TT_4 and FT_4 (T_4 index) were not different from controls (Arnold et al. 1993b). The incidence of follicular cell hyperplasia (generally minimal or mild) was increased in a non-dose-related pattern in male rats that were fed Aroclor 1242, 1254, or 1260 for 24 months at dose levels of $2.0, $1.0, and $1.0 mg/kg/day, respectively (Mayes et al. 1998). This thyroid lesion was not observed following exposure to similar doses of Aroclor 1016 in male rats or Aroclor 1016, 1242, 1254, or 1260 in female rats. Thyroid follicular cell adenomas were also increased in the male rats as discussed in the animal cancer section (3.2.8.3.2).

The effects of gestational exposures to Aroclor 1254 on the thyroid gland and thyroid hormone status of neonates have been examined in numerous studies. Lesions in the thyroid were observed in pups born to dams that were exposed to 50 or 500 ppm Aroclor 1254 (2.5 or 25 mg/kg/day) from gestation day 1 through postnatal day 21 (Collins and Capen 1980c). The pups also had depressed serum levels of TT_4 and TT_3. Aroclor 1254 (3.1, 6.2, or 12.5 mg/kg/day, oral) administered to rats during gestation and lactation depressed serum TT_4, but not TT_3, in the neonatal rats (Juarez de Ku et al. 1994). Aroclor 1254 administered to rats on days 10–16 of gestation (5 or 25 mg/kg/day, oral) depressed plasma TT_3 in the dams and both plasma TT_4 and FT_4 in fetuses and 5-day neonates (Morse et al. 1996c). Fetal brain levels of T_4, but not T_3, were also depressed. No changes were detected in fetal or neonatal plasma TSH concentration. Other effects observed that are relevant to the thyroid hormone system included an increase in Type II thyroxine 5'-deiodinase activity in fetal brain and depression of activity in 21-day-old pups, and an increase in T_4-UDP-GT activity in fetal and pup liver. Provost et al. (1999) observed a depression in both serum total T_4 and T_3 concentrations in rats that had been exposed to 1.25 or 12.5 ppm Aroclor 1254 (approximately 0.1 or 1 mg/kg/day) during gestation and through postnatal day 30. Zoeller et al. (2000) fed pregnant rats 0, 1, 4, or 8 mg/kg/day Aroclor 1254 beginning on day 6 of gestation through weaning of pups. Dosages $1 mg/kg/day depressed serum TT_4 levels in the pups. Serum concentrations of TT_4 and FT_4 were depressed in dams and fetuses after gavage doses of 25 mg/kg/day Aroclor 1254 on gestation days 10–20 (Schuur et al. 1998a). Although serum concentrations of the sulfate ester of T_4 were not affected by Aroclor 1254 treatment, the activity of 3,3'-T_2-sulfotransferase in liver cytosol preparations was lower in the treatment group relative to the control group. Activities of iodothyronine deiodinase in liver was also decreased in the dams and fetuses in the treatment group. Type II deiodinase activity was significantly increased in fetal, but not maternal brain in the treatment

group. T_4-UDP-GT activity in maternal liver was significantly increased in the treatment group. These observations suggest that Aroclor 1254 can potentially affect thyroid hormone status by modifying several different metabolic pathways for T_4, including glucuronide and sulfate conjugation, and deiodination of iodothyronines.

Aroclor 1254 (1, 4, or 10 mg/kg/day, oral) administered on gestation day 6 through postnatal day 21 depressed postnatal (day 7–21) serum TT_4 concentration ($1 mg/kg/day) and T_3 concentration ($4 mg/kg/day), without a change in serum TSH concentration (Goldey et al. 1995). Thyroid hormone levels recovered from depressed levels with time and were nearly at control levels by postnatal day 45. Neurobehavioral deficits were observed in the pups, including decreased motor activity and changes in acoustic startle response (see Section 3.2.4.3.3); these changes were significantly attenuated in pups that received subcutaneous injections of T_4 that increased serum T_4 and T_3 concentrations (Goldey and Crofton 1998). Rates of elimination of both hormones from serum were accelerated in the pups that had been exposed to Aroclor 1254, relative to controls. These observations suggest that the observed neurobehavioral deficits may have been attributable to deficits in thyroid hormones. The increased elimination of T_4 and T_3 from serum is consistent with an induction of UDP-GT or other elimination pathways for thyroid hormones (e.g., deiodination of T_4 to T_3).

In a longer feeding study, pregnant rats were exposed to 125 or 250 ppm Aroclor 1254 in food from gestation day 1 through weaning of pups (Corey et al. 1996). The weaned pups either continued the exposure until postnatal day 60 or were fed the control diet. Reported dosages during gestation were 8 or 18 mg/kg/day, and during lactation were 37 or 62 mg/kg/day. Serum TT_4 concentrations, but not TT_3, were depressed (>90% decrease) at postnatal day 60 in all of the exposure groups. In pups that were removed from the PCB exposure after weaning, serum TT_4 concentrations partially recovered, but, unlike the Collins and Capen (1980a) study previously discussed, remained significantly lower than control levels.

Seo and Meserve (1995) reported the effects of maternal ingestion of Aroclor 1254 in pregnant and lactating rats on the development of thermoregulation in neonates. Female pregnant Sprague-Dawley rats (n=6–8) were fed *ad libitum* 125 or 250 ppm (6.3 or 12.5 mg/kg/day) Aroclor 1254 and continued on the diet from conception to completion of the experiment when pups were 15 days old. Serum TT_4 levels were depressed in female rats treated with 6.3 and 12.5 mg/kg/day during pregnancy and lactation. Relative thyroid weight increased (19 mg/100 g ±1.3) significantly in animals given 6.5 mg/kg/day compared to controls, but not at the 12.5 mg/kg/day dose.

In contrast to the depression of circulating levels of thyroid hormone levels that has been observed in rats exposed to Aroclor 1254, rats exposed to Aroclor 1242 (32 µg/kg/day in food or 900 ng/m^3 vapor, whole body exposure) for 30 days had higher serum concentrations of TT_3 and TT_4 than rats in a control group (Casey et al. 1999). Histological changes in the thyroid observed in the rats exposed to the aerosol included increased vacuolization of thyroid follicle cells with reduced follicular colloid, changes that are typical of TSH stimulation of the gland. The elevation in thyroid hormone levels observed in this study supports earlier observations of increased serum T_4 levels following low doses of PCB 153 (Li et al. 1994), PCB 110 (Li et al. 1998), a mixture of PCBs collected in the air over a landfill (Li and Hansen 1996b), and Aroclor 1242, but not Aroclor 1254, in chick embryos (Gould et al. 1997). The rats exposed to the Aroclor 1242 vapor may have received a substantial ingestion dose, because of deposition of PCBs on the fur and because the animals were exposed and housed two animals per cage.

Cooke et al. (1996) demonstrated a possible thyroid hormone-mediated response of the testes to Aroclor 1254. This study found increased testes weight and sperm production in 135-day-old rats that were administered subcutaneous doses of Aroclor 1254 (. 40 mg/kg/day) or Aroclor 1242 (. 80 mg/kg/day) on postnatal days 1–25. Serum TT_4 concentrations were also depressed in these rats, and the effects on the testes were attenuated by injections of T_4 on postnatal days 1–25. In contrast to the results of this study, Gray et al. (1993) found no effect of oral exposure to Aroclor 1254 (up to 25 mg/kg/day) on testes weights or sperm numbers in rats that had substantially depressed levels of serum TT_4 and TT_3; however, the study initiated the dosing of the rats on postnatal day 31, after the development of Sertoli cells is complete in the rat, and, thus, may have missed a vulnerable period in the postnatal development of the testes.

Defined Experimental Mixtures. PCBs were extracted from an NPL site and doses ranging from 3 to 96 mg/kg were administered to 20-day-old female rats for 2 days (Hansen et al. 1995). The animals were sacrificed 24 hours after the last dose. Serum total T_4 declined significantly at doses of $36 mg/kg/day; however, at doses >12 mg/kg/day, thyroid follicular cells increased in size, while the colloid area decreased to <60% of control values, indicative of thyroid gland stimulation. Depression of serum T_4 was also observed in 21-day-old rats that received the same soil mixture and a charcoal filtered mixture, which had considerably lower TCDD equivalents (Li and Hansen 1996a). When compared to extracts of superficial dust and debris and airborne PCBs, the soil extract was somewhat less potent than the air extract (Li and Hansen 1996b).

Single Congeners. Histopathologic lesions of the thyroid gland developed in rats that were exposed to single PCB congeners in food for 13 weeks (Chu et al. 1994, 1995, 1996a, 1996b, 1998b; Lecavalier et al. 1997). The lesions consisted of a reduction in size and collapse of the thyroid follicles, reduced follicle colloid density, and cellular changes, including cytoplasmic vacuolization and nuclear vesiculation of follicle cells. These changes were evident to varying degrees of severity at the following dosages: PCB 28 at $36 mg/kg/day; PCB 77 at $0.070 mg/kg/day; PCB 105 at $0.039 mg/kg/day; PCB 118 at $0.17 mg/kg/day; PCB 126 at $0.00074 mg/kg/day; PCB 128 at $0.43 mg/kg/day; and PCB 153 at $0.35 mg/kg/day.

Depressed concentrations of T_4 have been observed in rats exposed to single PCB congeners in food for 13 weeks (Desaulniers et al. 1997; Van Birgelen et al. 1992, 1994a, 1994b, 1995). Effective dosages were as follows: 75 µg/kg/day PCB 77 decreased serum TT_4, but not serum TSH (Desaulniers et al. 1997); 50 µg/kg/day PCB 126 decreased $FT_4$and TT_4 plasma concentrations; 1.2 mg/kg/day; PCB 156 decreased plasma FT_4 concentration; and 6 mg/kg/day of PCB 156 depressed both free and TT_4 concentrations (Van Birgelen et al. 1995). These same dosages increased activity of UDP-GT in liver homogenate, including activity when either T_4, *p*-nitrophenol, or 1-naphthol were the substrates (Desaulniers et al. 1997; Van Birgelen et al. 1995). This is consistent with the induction of UDP-GT1A1, which utilizes T_4 and simple phenols as a substrate, and with the induction of cytochrome P-450 1A1 at these same dosages of PCB congeners (Chu et al. 1995; Van Birgelen et al. 1995). These observations suggest a possible involvement of the Ah receptor in modifying the metabolism and, thereby circulating levels of T_4.

Rice (1999a) administered oral doses (0.25 or 1.0 µg/kg/day) of 3,3',4,4',5-pentaCB (PCB 126) to female rats, 5 days/week beginning 5 weeks prior to and through pregnancy, gestation, and lactation. Serum T_4 levels were depressed in 21-day-old pups, but not in 60-day-old pups or in the dams. Darnerud et al. (1996a) found no changes in plasma FT_4 or TT_4 concentrations in maternal mice given a single oral dose of up to 10 mg/kg PCB 77 in corn oil on day 13 of gestation; however, FT_4 and TT_4 concentrations in plasma of the 13-day-old fetus were depressed (36 and 45%, respectively). This study also found substantial binding of 4-hydroxylated metabolites of PCB 77 in fetal serum to serum transthyretin and that binding of T_4 to transthyretin was substantially decreased in serum of the exposed pups, relative to the control group. This observation suggests that hydroxylated metabolites of certain PCBs may compete for binding of T_4 to transthyretin, and is consistent with the results of *in vitro* binding studies that have estimated transthryretin binding affinities of 4-OH metabolites of PCB congeners to be similar to that of T_4 (Cheek et al. 1999; Lars et al. 1994).

An intraperitoneal dose of 8 mg/kg/day of 2,3,3',4',6-pentaCB (PCB 110), administered to female rat pups on postnatal days 21 and 22, increased serum TT_4 levels, while higher doses resulted in a dose-dependent decrease in serum TT_4 levels (Li et al. 1998). A PCB 110 preparation contaminated with 0.4% 3,3',4,4',5-pentaCD (PCB 126) produced a dose-dependent decrease in serum T_4 levels (4 mg/kg/day or higher) without an increase in serum T_4 levels.

Effects on the Adrenal Gland and other Endocrine Systems

Commercial PCB Mixtures. PCB-related effects on the adrenal gland have been reported after repeated oral exposure to PCBs; however, a single dose of 4,000 mg Aroclor 1242/kg did not induce histological alterations in the adrenals or pancreas in rats (Bruckner et al. 1973). Significantly increased serum corticosterone levels were reported in mice following $8.1 mg/kg/day Aroclor 1254 for 2 weeks (Sanders et al. 1974); adrenal weight was increased at 130 mg/kg, but histology was not evaluated. Intermediate-duration studies with rats found that serum corticosterone levels were increased by dietary exposure to 15 mg/kg/day Aroclor 1248 for 20 days (Kato et al. 1982a), 35 mg/kg/day Aroclor 1221 for 10 weeks (Wassermann et al. 1973), and 0.1 mg/kg/day Aroclor 1254 for 15 weeks (Miller et al. 1993b). Bergman and Olsson (1984) have attributed much of the pathology in PCB-contaminated Baltic seals to adrenal cortical hyperplasia. Rats fed 0.05–2.5 mg/kg/day Aroclor 1242 or 1221 for 5 months had decreased serum levels of the adrenal cortex hormones, dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHS). The decreases in DHEA, DHS, and corticosterone occurred at $0.25 mg/kg/day Aroclor 1254, but not at 0.05 mg/kg/day; no corticosterone data were reported for Aroclor 1242 or 1221 (Byrne et al. 1988). The degree of hormone inhibition was dose related and generally increased with increasing degree of mixture chlorination. These reductions in circulating hormones were accompanied by decreased adrenal weight with Aroclor 1254 (not evaluated with Aroclor 1242 or 1221). There were no changes in plasma corticosteroids in rats fed #1.5 mg/kg/day Aroclor 1242 for 2–6 months or in adrenal weight in rats fed 100 mg/kg/day Aroclor 1242 for 3 weeks (Bruckner et al. 1973, 1974). In another study (Miller et al. 1993b), no histopathological changes were observed in the adrenals from Fischer 344 rats treated with up to 25 mg/kg/day Aroclor 1254 by gavage for 15 weeks. Rao and Banerji (1993), however, reported degenerative changes in the adrenals of Wistar rats treated with $7.1 mg/kg/day Aroclor 1260 in the diet for 120 days. The differing results from these two studies may reflect differences in the congeneric composition of the Aroclors, in strains of animals, and/or in the methods of administration.

Adrenal weight was unchanged in rabbits fed #6.5 mg/kg/day Aroclor 1254 for 8 weeks (Street and Sharma 1975), and adrenal cortex histology was normal in monkeys fed 0.2 mg/kg/day Aroclor 1254 for 12 months (Tryphonas et al. 1986a), but hormone evaluations were not performed. Pigs treated with 9.2 mg/kg/day Aroclor 1242 for 91 days exhibited increased relative weight of the adrenals (Hansen et al. 1976). No histological alterations were observed in adrenals from guinea pigs treated with #4.0 mg/kg/day Aroclor 1260 in the diet for 8 weeks (Vos and de Roij 1972). Monkeys treated with dietary doses of #0.08 mg/kg/day Aroclor 1254 for up to 22 months showed no treatment-related changes in serum hydrocortisone levels (Loo et al. 1989). Histological examinations and higher doses were not tested, and levels of other adrenal cortex hormones were not evaluated. However, Arnold et al. (1997) reported that Rhesus monkeys that received daily doses of 0.005, 0.020, 0.040, or 0.080 mg/kg/day Aroclor 1254 for 72 months showed no effect on adrenal tissue.

No histopathologic changes were observed in the adrenal, pancreas, pituitary, or parathyroid glands of male or female rats that were fed Aroclor 1016, 1242, 1254, or 1260 for 24 months at dose levels of 8.0–11.2, 4.0–5.7, 4.3–6.1, or 4.1–5.8 mg/kg/day, respectively (Mayes et al. 1998). Serum parathyroid hormone levels were not affected in rats treated with up to 25 mg/kg/day Aroclor 1254 for up to 15 weeks (Andrews 1989).

Single Congeners. Histopathologic evaluations of the adrenal glands, ovary, parathyroid, pancreas, and pituitary revealed no treatment-related changes in rats that were exposed to single PCB congeners in food for 13 weeks (Chu et al. 1994, 1995, 1996a, 1996b, 1998b; Lecavalier et al. 1997). The highest dosages in the studies were as follows: PCB 28, 3.8 (males) and 4.0 (females) mg/kg/day; PCB 77, 0.77 (males) and 0.89 (females) mg/kg/day; PCB 105, 4.0 (females) and 4.3 (males) mg/kg/day; PCB 118, 0.17 (females) and 0.68 (males) mg/kg/day; PCB 126, 7.4 (males) and 8.7 (females) µg/kg/day; PCB 128, 4.2 (males) and 4.4 (females) mg/kg/day; and PCB 153, 3.5 (males) and 4.1 (females) mg/kg/day.

Serum concentrations of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone concentrations were measured in rats exposed for 13 weeks to PCB 28 or PCB 77 in food (Desaulniers et al. 1997). No changes, relative to the control group, were observed at doses of 500 µg/kg/day PCB 28 or 75 µg/kg/day PCB 77. Acute intraperitoneal administration of PCB 126 in adult rats caused decreased serum concentrations of T_4 at $6.25 µg/kg/day, T_3 at $25 µg/kg/day, LH at $100 µg/kg/day, and FSH at 400 µg/kg/day (Desaulniers et al. 1999). Similar administration of PCB 153 caused an increase in serum T_4 and decrease in serum LH at a dose level of 25 mg/kg/day.

Evaluation of Animal Studies. Studies in animals, including rodents and nonhuman primates, provide strong evidence of thyroid hormone involvement in PCB toxicity. Although the studies differ in design and, the emerging picture is that, depending of dose and duration, PCBs can disrupt the production and levels of thyroid hormones, both in the thyroid and in peripheral tissues, can interfere with their transport to peripheral tissues, and can accelerate the metabolic clearance of thyroid hormones. Exposure to PCBs *in utero* and/or during early development (e.g., through breast milk) can deplete levels of circulating thyroid hormones in the fetus or neonate, which may give rise to effectively a hypothyroid state during development. The most convincing evidence that PCBs can exert toxicity by disrupting thyroid hormone system derives from two studies in rats. In one study, neurobehavioral deficits in pups that experienced exposures to Aroclor 1254 *in utero* and during nursing were significantly attenuated by subcutaneous injections of T_4 that increased serum T_4 and T_3 concentrations that were otherwise depressed in the PCB-exposed animals (Goldey and Crofton 1998). While this study examined relatively high doses of Aroclor 1254 ($1 mg/kg/day), it nevertheless demonstrated neurodevelopmental effects that are directly relevant to observations made in epidemiological studies and to neurological sequelae of fetal hypothyroidism, including disturbances of motor function and hearing. In the second study, increased testes weight and sperm production in rats that were administered Aroclor 1254 on postnatal days 1–25 were attenuated by injections of T_4 on postnatal days 1–25, which also prevented the depression in serum T_4 concentrations (Cooke et al. 1996). Here again, although produced by relatively large doses of Aroclor 1254 (.40 mg/kg/day, subcutaneous), similar effects can be produced by other hypothyroid-inducing agents, including 6-propyl-2-thiouracil (PTU). Furthermore, the effects observed may reflect a disruption of the normal sexual maturation process, which is known to be associated with neonatal hypothyroidism in humans (Longcope 2000).

Certain PCBs or certain exposures to PCBs may increase serum T_4 levels at low doses and decrease serum T_4 in a dose-dependent manner at higher doses (Gould et al. 1997; Li and Hansen, 1996b; Li et al. 1994, 1998). This effect may reflect stimulation of the thyroid gland as suggested by concurrent morphological changes in the thyroid follicles.

Other effects of PCBs on endocrine function that have been observed in experimental animals include effects on the adrenal glands and serum adrenal steroid levels (Byrne et al. 1988; Kato et al. 1982a; Miller et al. 1993b; Rao ad Banerji 1993; Sanders et al. 1974; Wasserman et al. 1973). Studies that have shown depressed levels of adrenal cortical steroids in PCB-exposed animals are also relevant because depressed levels of adrenal steroids have been associated with hypothyroidism in humans (Dluhy 2000). In

hypothyroidism, this effect is thought to result from decreases in both secretion and metabolism of adrenal steroids.

3.2.2.9 Dermal Effects

3.2.2.9.1 Summary

Chloracne and other dermal alterations are well known markers of exposure to PCBs and structurally-related halogenated aromatic hydrocarbons. Chloracne and other dermal alterations have been reported in subjects occupationally exposed to PCBs and in individuals exposed by accidental ingestion of rice oil contaminated with high concentrations of PCBs, CDFs, and related chemicals (*Yusho* and *Yu-Cheng*). In general, chloracne appears in individuals with serum PCB levels 10–20 times higher than those of the general population, but there is great variability among individuals. Therefore, chloracne is not a sensitive marker of PCB exposure. Long-term oral administration of relatively low doses of PCBs to monkeys resulted in dermal alterations similar to those observed in humans exposed to high concentrations of PCBs. The dermal effects were observed in the monkeys at serum PCB levels not much higher than serum PCB levels in humans with no known point source exposure to PCBs. Offspring from monkeys exposed during gestation and nursed by exposed mothers also developed dermal alterations after a few weeks of suckling. There are reports of rodents also developing skin alterations, but only after high exposures to PCB.

3.2.2.9.2 Human Studies

3.2.2.9.2.1 Occupational Exposure

Chloracne is the most easily recognized effect of exposure to PCBs and structurally-related chlorinated organic chemicals (Rice and Cohen 1996). Chloracne is a high-dose response in animals and humans; and its presence in humans indicates exposure to PCBs and/or other chlorinated organic compounds, but its absence does not preclude such exposure. Furthermore, the variability of the response in more highly exposed individuals suggests that susceptibility varies greatly among individuals. Chloracne can first occur on the face, particularly under the eyes and behind the ears. With increasing exposure, the rest of the face and neck, upper arms, chest, back, abdomen, outer thighs, and genitalia may be affected. When severe, chloracne can cover the entire body. Clinically, changes vary from an eruption of comedones to

the occurrence of papules and pustules. Histologically, the lesions consist of keratinous cysts caused by squamous metaplasia of sebaceous glands. The acute stage is followed by vermiculite skin atrophy.

Mild to moderate chloracne was observed in 7 of 14 workers exposed to 0.1 mg/m^3 Aroclors (formulation not specified) for an average duration of 14.3 months (Meigs et al. 1954). Because PCBs were used as a heat exchange material, it is possible that the workers were exposed to such pyrolysis products as CDFs. In these workers, the chloracne was found primarily on the face, especially the cheeks, forehead, and ears. Three cases of chloracne occurred among an unspecified number of autoclave operators exposed to 5.2–6.8 mg/m^3 Aroclor 1254 for 4–7 months in 1954 (Bertazzi et al. 1987), but pyrolytic formation of CDFs is a confounding factor. In 1977, four more cases of chloracne were diagnosed among 67 workers from the same plant who were engaged in impregnating capacitors with Pyralene 3010 (0.048–0.275 mg/m^3) and had skin contact confirmed as a major exposure route. An increased incidence of nonadolescent acneform eruptions was reported in workers exposed to 0.007–11 mg/m^3 mean concentrations of various Aroclors for >5 years; 40% of the workers were exposed for >20 years (Fischbein et al. 1979, 1982). Maroni et al. (1981a, 1981b) reported 10 cases of acne and/or folliculitis and 5 cases of dermatitis among 80 capacitor manufacturing workers examined in Italy. All of the workers with chloracne were employed in high exposure jobs. Their blood PCB concentrations ranged from 300 to 500 ppb.

Other dermal effects reported in workers include skin rashes, pigmentation disturbances of skin and nails, erythema and thickening of the skin, and burning sensations (Fischbein et al. 1979, 1982; Ouw et al. 1976, 1982; Smith et al. 1982). In these studies, the workers were exposed to various Aroclors at levels as low as 0.003 mg/m^3 for >5 years. Statistically significant associations between dermatologic effects and plasma levels of higher chlorinated PCB congeners have been reported (Fischbein et al. 1979, 1982; Smith et al. 1982). No relationships between the incidence of skin rash or dermatitis and plasma levels of lower chlorinated PCBs were found (Smith et al. 1982).

3.2.2.9.2.2 Accidental Exposure

Effects in the skin were widely reported among victims of the *Yusho* and *Yu-Cheng* poisoning episodes exposure (Guo et al. 1999; Kuratsune 1989; Lu and Wu 1985; Rogan 1989). It is important to mention, however, that the findings from the studies of these groups cannot be attributed solely to exposure to PCBs since the victims also were exposed to CDFs and other chlorinated chemicals (ATSDR 1994). Characteristic skin changes included marked enlargement, elevation and keratotic plugging of follicular

orifices, comedo formation, acneform eruptions, hyperpigmentation, hyperkeratosis, and deformed nails. The acne most commonly developed on the face and other parts of the head, axillae, trunk, and external genitalia, with follicular plugging occurring in the axillae, groin, glenoid regions such as elbow and knee flexures, trunk, thigh, and outer aspect of the forearm. Dark-colored pigmentation frequently occurred in the gingival and buccal mucosa, lips, and nails, and improved only gradually in most patients (Fu 1984; Kuratsune 1989; Lu and Wu 1985; Rogan 1989). Improvement of the dermal changes was gradual. Evaluation of *Yu-Cheng* subjects 14 years after the poisoning incident showed that men and women exposed to PCBs/PCDFs had a higher lifetime prevalence of chloracne, abnormal nails, hyperkeratosis, and gum pigmentation (Guo et al. 1999). Skin lesions were commonly observed in children born to mothers with *Yusho* or *Yu-Cheng* exposure. The dermal changes are consistent with those observed in exposed adults and included hyperpigmentation of the skin, nails and gingivae, deformed nails, and acne (Funatsu et al. 1971; Gladen et al. 1990; Hsu et al. 1985; Rogan et al. 1988; Taki et al. 1969; Yamaguchi et al. 1971; Yoshimura 1974). These effects generally diminished as the babies grew older.

3.2.2.9.2.3 Evaluation of Human Studies

There is conclusive evidence that exposure to high concentrations of PCBs (and other chlorinated hydrocarbons) induce adverse dermal effects in humans. A typical dermal sign of exposure is chloracne and is generally present in individuals with blood PCB levels several times higher than background levels as observed among capacitor workers in the past and in *Yusho* and *Yu-Cheng* victims (Fischbein et al. 1979, 1982; Guo et al. 1999; Hsu et al. 1994; Maroni et al. 1981a, 1981b; Masuda 1994). It is generally accepted that chloracne is induced by exposure to dioxin-like substances (ATSDR 1998); therefore, the contribution of PCBs to this effect in *Yusho* and *Yu-Cheng* was probably minor compared to that of CDFs, which were the main contributors to the total dioxin TEQs of the rice oil. High incidence of chloracne was seen among *Yu-Cheng* victims 14 years after exposure, at a time when the body burden of PCBs and CDFs was still considerably higher than local controls (Guo et al. 1997). No adverse dermal effects have been reported in subjects with high consumption of Great Lakes fish contaminated with PCBs and other environmentally persistent chemicals or in other cohorts from the general population, although it is unknown if this outcome was systematically studied in these cohorts.

3.2.2.9.3 Animal Studies

The highest NOAEL values and all reliable LOAEL values for dermal effects for each study are recorded in Tables 3-2 and 3-3, and plotted in Figure 3-2.

Oral Exposure

Commercial Mixtures. Very limited information is available regarding dermal effects of commercial PCB mixtures following acute-duration oral exposure. Skin histology was normal in rats that were treated with a single gavage dose of 4,000 mg Aroclor 1242/kg and evaluated after 24 hours, no follow-up observations were conducted (Bruckner et al. 1973). Treatment of rats with 100 mg Aroclor 1242/kg/day by gavage every other day for 3 weeks did not result in histological alterations in the skin (Bruckner et al. 1973). Rats exposed in the diet to 2.5 mg/kg/day Aroclor 1254 for 104 weeks or to 5 mg/kg/day for 72 weeks developed alopecia and facial edema (NCI 1978); these effects did not occur after 104 weeks at 1.25 mg/kg/day. No histopathologic changes were observed in the skin of rats that were fed Aroclor 1016, 1242, 1254, or 1260 for 24 months at dose levels of 8.0–11.2, 4.0–5.7, 4.3–6.1, or 4.1–5.8 mg/kg/day, respectively (Mayes et al. 1998). Guinea pigs fed a diet that provided up to approximately 4 mg Aroclor 1260/kg/day for 8 weeks showed no treatment-related gross or microscopical alterations of the skin (Vos and de Roij 1972). Mice treated with 26 mg Aroclor 1254/kg/day in the diet for 23 weeks developed erythema, altered sebaceous gland differentiation, and thickening with occasional hyperkeratosis and cysts in the pinna; other skin areas were not examined (Bell 1983).

Dermal effects were reported in Rhesus monkeys fed diets containing Aroclors for intermediate durations (Allen and Norback 1973, 1976; Allen et al. 1973, 1974a; Barsotti et al. 1976; Becker et al. 1979; Ohnishi and Kohno 1979; Thomas and Hinsdill 1978). These include facial edema (particularly in the periorbital area), acne, folliculitis, and alopecia. The effects appear to be reversible and have been produced by estimated doses as low as 0.1 mg/kg/day Aroclor 1248 for 2 months (Barsotti et al. 1976) and 0.12 mg/kg/day Aroclor 1242 for 2 months (Becker et al. 1979). NOAELs for these effects in monkeys cannot be identified from the available studies. Chronic dietary treatment with 0.1 mg Aroclor 1248/kg/day for 12 months (Allen and Norback 1976) or 0.2 mg/kg/day Aroclor 1254 for 12–28 months (Arnold et al. 1990; Tryphonas et al. 1986a, 1986b) produced progressive dermal effects in monkeys, including alopecia, periorbital edema, acne, fingernail loss, and gingival hyperplasia and necrosis of varying severity (Tryphonas et al. 1986b). The same group of investigators also reported fingernail and toenail changes in monkeys during treatment with as little as 0.005 mg/kg/day

Aroclor 1254 over a 37-month period or 0.04 mg/kg/day over a 72-month period (Arnold et al. 1993a, 1997). Offspring from Rhesus monkeys treated before mating and during gestation with 0.03 mg Aroclor 1016/kg/day showed hyperpigmentation (Barsotti and Van Miller 1984). Doses even smaller of Aroclor 1254 (0.005 mg/kg/day) produced clear signs of PCB intoxication manifested as inflammation and/or enlargement of the tarsal glands, and nail and gum lesions (but not acne or hyperpigmentation) in monkeys exposed during gestation and via breast milk for 22 weeks (Arnold et al. 1995, 1997). Most of these alterations were seen after the infants had been weaned. The concentration of PCBs in breast milk from dams treated with 0.005 mg/kg/day ranged from 5.6 to 15.6 ppm. The geometric mean concentration of PCBs in the blood of these infants after 22 weeks of nursing was 47 ppb (Arnold et al. 1995).

Single Congeners. Treatment of female and male weanling Sprague-Dawley rats for 90 days with several PCB congeners in the diet, both dioxin-like and nondioxin-like (PCBs 28, 77, 105, 118, 126, 128, 153), did not result in any treatment-related histological alterations in the skin (Chu et al. 1994, 1995, 1996a, 1996b, 1998a, 1998b; Lecavalier et al. 1997). Doses ranged from 0.009 mg/kg/day for the dioxin-like PCB 126 to approximately 4 mg/kg/day for some mono- and di-*ortho*-substituted congeners.

Dermal Exposure

Commercial Mixtures. Skin appearance and histology was normal in three hairless mice dermally treated with Aroclor 1254 in estimated doses of up to 136 mg/kg/day on 4 days/week for 6 weeks (Puhvel et al. 1982). Aroclor 1254 was applied in either pure acetone or in acetone-mineral oil emulsion; few experimental details were provided in this study. Dermal effects were produced by application of Aroclor 1260 in an isopropanol vehicle to the shaved back skin of female New Zealand rabbits 5 days/week for 28 or 38 days at estimated doses of 42–44 mg/kg/day (Vos and Beems 1971; Vos and Notenboom-Ram 1972). Effects included thickening of the skin and acneform lesions resulting from hyperplasia and hyperkeratosis of the epidermal and follicular epithelium.

3.2.2.9.4 Evaluation of Animal Studies

PCB-related cutaneous effects are well characterized in monkeys after long-term oral exposure to commercial PCB mixtures and are generally similar to those observed in humans. Infant monkeys exposed *in utero* and via breast milk also developed similar dermal lesions. Chronic-duration oral exposure studies in monkeys showed that adverse dermal effects can occur at dose levels lower than had

been previously observed (Arnold et al. 1993a, 1993b, 1995, 1997). It should be pointed out that dermal effects in monkeys appeared with doses that resulted in tissue (5 ppm) and blood levels (10 ppb) of PCBs near background concentrations found in the general human population. In general, adverse cutaneous effects in rodents followed exposure to relatively high oral doses of PCBs. The series of studies with single congeners by Chu and coworkers found no significant dermal effects at the dose levels tested, and no conclusions regarding a potential ranking for dermatotoxicity of congeners can be drawn based on these studies (Chu et al. 1994, 1995, 1996a, 1996b, 1998a, 1998b; Lecavalier et al. 1997).

3.2.2.10 Ocular Effects

3.2.2.10.1 Summary

Along with dermal alterations, adverse ocular effects are markers of exposure to PCBs and structurally-related halogenated aromatic hydrocarbons. Ocular effects consisting primarily of hypersecretion of the Meibomian glands and abnormal pigmentation of the conjunctiva have been reported in subjects occupationally exposed to PCBs and in individuals exposed by accidental ingestion of rice oil contaminated with high concentrations of PCBs, CDFs, and related chemicals (*Yusho* and *Yu-Cheng*). In general, these effects appear in individuals with serum PCB levels 10–20 times higher than those of the general population, but there is great variability among individuals. Long-term oral administration of relatively low doses of PCBs to monkeys resulted in ocular alterations similar to those observed in humans exposed to high concentrations of PCBs. The ocular effects were observed in the monkeys at serum PCB levels not much higher than serum PCB levels in humans with no known high exposure to PCBs. Offspring from monkeys exposed during gestation and nursed by exposed mothers developed similar ocular alterations after a few weeks of suckling.

3.2.2.10.2 Human Studies

3.2.2.10.2.1 Occupational Exposure

The primary ocular effects reported by workers exposed to airborne PCBs were eye irritation, tearing, and burning (Emmett et al. 1988a; Ouw et al. 1976; Smith et al. 1982). The workers had been exposed to a variety of Aroclors at concentrations between 0 and 2.2 mg/m^3 for >3 years. A significant relationship between the incidence of irritated, burning eyes and plasma levels of higher and lower chlorinated PCB congeners has been found (Smith et al. 1982). Emmett et al. (1988a) suggested that because PCBs have

low volatility and are relatively nonirritating, 1,1,1-trichloroethane used to clean up spills or trichlorobenzene in Askarel may have been responsible for the complaints.

An ocular examination of 181 workers at a capacitor manufacturing plant revealed a 13% prevalence of edema of the upper eyelid, congestion or hyperemia of conjunctiva, eye discharge, and enlargement of Meibomian glands following exposure to 0.007–11 mg/m^3 mean concentrations of various Aroclors for >5 years (Fischbein et al. 1985). The median blood value of lower homologues of PCBs was approximately 60 ppb and of the higher homologues, 18 ppb. There was no significant association between ocular abnormalities and blood concentrations of PCBs (Fischbein et al. 1985).

3.2.2.10.2.2 Accidental Exposure

In addition to dermal effects, ocular effects were the most obvious manifestations of *Yusho* and *Yu-Cheng* exposure (Fu 1984; Kuratsune 1989; Lu and Wu 1985; Rogan 1989). As previously mentioned, victims of these poisoning episodes also were exposed to CDFs and other chlorinated chemicals (ATSDR 1994). Hypersecretion of the Meibomian glands and abnormal pigmentation of the conjunctiva were commonly observed (Masuda 1994). Typical cases showed swollen Meibomian glands filled with yellow infarct-like contents. Abnormal changes in the Meibomian glands as well as eye discharge were still seen 10 years after the poisoning incident (Kono and Yamana 1979). The incidence of ocular signs was closely related to PCB concentrations and patterns in blood. Babies born to *Yusho* mothers also had increased eye discharge. Similar findings were seen in children born to *Yu-Cheng* mothers who also showed high incidence of conjunctivitis, swelling of the eyelid, and eye discharge (Rogan et al. 1988).

3.2.2.10.2.3 Evaluation of Human Studies

There is sufficient evidence that exposure to high concentrations of PCBs (and other chlorinated hydrocarbons) induce adverse ocular effects in humans. Typical responses include hypersecretion of the Meibomian glands and abnormal pigmentation of the conjunctiva. This has been observed among capacitor workers (Fischbein et al. 1985) and in *Yusho* and *Yu-Cheng* victims (Hsu et al. 1994; Kono and Yamana 1979; Kuratsune 1989; Masuda 1994; Rogan et al. 1988). The contribution of PCBs to this effect in *Yusho* and *Yu-Cheng* is unknown since the victims also were exposed to CDFs and other structurally-related chemicals. In the occupationally-exposed subjects described by Fischbein et al. (1985), PCBs seemed to have been responsible for the high incidence of ocular effects since there was no apparent exposure to CDFs or similar chemicals, although such possibility could not be completely ruled

out. No adverse ocular effects have been reported in subjects with high consumption of Great Lakes fish contaminated with PCBs and other environmentally persistent chemicals or in other cohorts from the general population, although it is unknown if this outcome was systematically studied in these cohorts.

3.2.2.10.3 Animal Studies

The highest NOAEL values and all reliable LOAEL values for ocular effects for each study are recorded in Table 3-2 and plotted in Figure 3-2.

Oral Exposure

Commercial Mixtures. Ocular effects were commonly observed in Rhesus monkeys fed diets containing Aroclors for intermediate durations (Allen and Norback 1973, 1976; Allen et al. 1973, 1974a; Barsotti et al. 1976; Becker et al. 1979; Ohnishi and Kohno 1979; Thomas and Hinsdill 1978). The effects consisted of swelling and reddening of the eyelid and eyelid discharge. Females appear to be more sensitive than males. The effects appear to be reversible and have been produced by estimated doses as low as 0.1 mg/kg/day Aroclor 1248 for 2 months (Barsotti et al. 1976) and 0.12 mg/kg/day Aroclor 1242 for 2 months (Becker et al. 1979). NOAELs for these effects in monkeys were not identified in the available studies. Monkeys exposed to 0.005–0.08 mg/kg/day Aroclor 1254 for 37 months showed characteristic dose-related ocular and dermal effects, including eye exudate, inflammation and/or prominence of the tarsal (Meibomian) glands, and various finger and toe nail changes (Arnold et al. 1993a). Eye inflammation is a result of metaplastic changes in the Meibomian glands, which cause the glands to be come keratinaceous. Conjunctivitis was observed in Rhesus monkeys treated in the diet with 0.2 mg/kg/day Aroclor 1254 for 12 months (Tryphonas et al. 1986a). Exophthalmia was observed in rats treated in the diet with 2.5 mg/kg/day Aroclor 1254 for 104–105 weeks (NCI 1978); a dietary level of 1.25 mg/kg/day Aroclor 1254 was a NOAEL. No histopathologic changes were observed in the eye of male or female rats that were fed Aroclor 1016, 1242, 1254, or 1260 for 24 months at dose levels of 8.0–11.2, 4.0–5.7, 4.3–6.1, or 4.1–5.8 mg/kg/day, respectively (Mayes et al. 1998).

Single Congeners. Treatment of female and male weanling Sprague-Dawley rats for 90 days with several PCB congeners in the diet, both dioxin-like and nondioxin-like (PCBs 28, 77, 105, 118, 126, 128, 153), did not result in any treatment-related histological alterations in the eye or optic nerve (Chu et al. 1994, 1995, 1996a, 1996b, 1998b; Lecavalier et al. 1997). Doses ranged from 0.009 mg/kg/day for the dioxin-like PCB 126 to approximately 4 mg/kg/day for some mono- and di-*ortho*-substituted congeners.

3.2.2.10.4 Evaluation of Animal Studies

PCB-induced ocular effects are well characterized in monkeys after long-term oral exposure to commercial PCB mixtures and are generally similar to those observed in humans. Infant monkeys exposed *in utero* and via breast milk also developed similar ocular lesions (Arnold et al. 1995). Chronic-duration oral exposure studies in monkeys showed that adverse ocular and dermal effects occurred at doses of 0.005 mg/kg/day (Arnold et al. 1993a, 1993b, 1995, 1997). Because these effects occurred at the lowest tested dose of any PCB mixture in any species, they are used as part of the basis for the chronic-duration MRL for oral exposure as discussed in Chapter 2 and Appendix A. It is worth mentioning that ocular effects appeared in monkeys given PCB doses that resulted in tissue (5 ppm) and blood levels (10 ppb) of PCBs near background concentrations found in the general human population. The series of studies with single congeners by Chu and coworkers found no significant effects in the eye and optic nerve at the dose levels tested and no conclusions regarding a potential ranking for oculotoxicity of congeners can be drawn based on these studies (Chu et al. 1994, 1995, 1996a, 1996b, 1998; Lecavalier et al. 1997).

3.2.2.11 Body Weight Effects

3.2.2.11.1 Human Studies

No studies were located regarding body weight effects in humans after exposure to PCBs.

3.2.2.11.2 Animal Studies

A number of animal studies have shown that inhalation, oral, or dermal exposure to PCBs results in decreases in body weight gain. Body weight gain was decreased in guinea pigs and mice that were intermittently exposed to 5.4 mg/m^3 Aroclor 1254 over 121 days or in guinea pigs exposed to 1.5 mg/m^3 Aroclor 1254 over 213 days (Treon et al. 1956). Exposure-related changes in body weight were not observed in rats or rabbits that were similarly exposed to 1.5 or 5.4 mg/m^3 Aroclor 1254 or to 8.6 mg/m^3 Aroclor 1242 over 24 days. The concentrations of PCBs are uncertain due to an invalid analytical technique and differential evaporation of the most volatile PCB congeners. A decrease in body weight gain was also observed in rats exposed to 0.009 mg/m^3 Aroclor 1242 for 30 days (Casey et al. 1999); the rate of body weight gain was 33% as compared to 39% in controls.

Reduced body weight (or reduced weight gain) is a characteristic effect of oral exposure to PCBs in animals. Acute-duration studies have shown moderate to severe weight decreases in rats following a single gavage dose of $4,000 mg/kg Aroclor 1242 or dietary administration of 50 mg/kg/day Aroclor 1254 for 14 days (Bruckner et al. 1973; Kling et al. 1978). No significant effect on weight gain was reported in rats administered up to 25 mg/kg/day Aroclor 1254 in several acute-duration studies (Brown and Lamartiniere 1995; Carter 1984, 1985; Carter and Koo 1984) or in rats administered four daily doses of 25 mg/kg of Aroclor 1221 (Brown and Lamartiniere 1995). The weight loss following single high doses appears to be due to dehydration (Bruckner et al. 1973). Effects on animal body weight are often pronounced following intermediate- and chronic-duration dietary administration, constituting a wasting syndrome. Decreases in body weight or body weight gain relative of different toxic doses have been observed with various species and Aroclor mixtures, including rats and minks fed Aroclor 1254 (Andrews 1989; Bleavins et al. 1980; Gray et al. 1993; Hornshaw et al. 1986; Kimbrough et al. 1972; Kling et al. 1978; Mayes et al. 1998; NCI 1978; Phillips et al. 1972), rats fed Aroclor 1260 (Kimbrough et al. 1972), pigs fed Aroclor 1254 or 1242 (Hansen et al. 1976), and monkeys fed Aroclor 1242 or 1248 (Allen 1975; Allen and Norback 1976; Allen et al. 1973; Becker et al. 1979), but not Aroclor 1254 (Arnold et al. 1993a, 1993b, 1997; Tryphonas et al. 1986b). The body weight from guinea pigs treated with 4 mg/kg/day Aroclor 1260 for 187 days was not altered by treatment (Vos and de Roij 1972). In general, Aroclors administered to rats in doses of #5 mg/kg/day for intermediate durations did not significantly affect body weight (Bruckner et al. 1974, 1977; Byrne et al. 1987; Goldstein et al. 1974; Huang et al. 1998a, 1998b). Rats that were fed Aroclor 1254 for 24 months at dose levels of 1.4–6.1 mg/kg/day had final body weights that were 12–28% lower than unexposed animals (Mayes et al. 1998). Decreased body weight was also observed following similar exposure to Aroclor 1242 (10% reduction at 5.7 mg/kg/day), but not to Aroclor 1016 (2.0–11.2 mg/kg/day) or Aroclor 1260 (1.0–5.8 mg/kg/day). The existing data indicate that monkeys and minks may be particularly susceptible species, as effect levels were higher in rats and adverse effects on body weight were not observed in rabbits and mice fed Aroclor 1254 (Kimbrough and Linder 1974; Street and Sharma 1975). Food and water consumption were not measured in most of these studies, but in general decreases in food and water intake were not sufficient to account for the decreases in body weight. In swine and sheep fed Aroclors 1242 and 1254 at 20 ppm in the diet, feed efficiency (unit gain/unit feed) were decreased about the same degree as by diet variations (Hansen et al. 1976).

Body weight gain was not adversely affected in rats fed diets containing #4.1 mg/kg/day of PCB 153 (Chu et al. 1996a), #4.2 mg/kg/day of PCB 128 (Lecavalier et al. 1997), #4.0 mg/kg/day of PCB 105 (Chu et al. 1998b), #3.7 mg/kg/day of PCB 28 (Chu et al. 1996b), #0.77 mg/kg/day of

PCB 77 (Chu et al. 1995), or #0.17 mg/kg/day of PCB 118 (Chu et al. 1995). A significant decrease in body weight gain was observed in rats fed diets containing 7.4 mg/kg/day of PCB 126 (Chu et al. 1994) for 13 weeks.

A significant reduction in body weight gain was observed in rabbits that received estimated doses of 42–44 mg/kg of Aroclor 1260 in isopropanol 5 days/week for 28 or 38 days applied to the shaved back skin (Vos and Beems 1971; Vos and Notenboom-Ram 1972). These studies tested small numbers of rabbits (four) and used Aroclor 1260 that had undetectable levels (<1 ppm) of CDFs.

The highest NOAEL values and all reliable LOAEL values for body weight effects for each study are recorded in Tables 3-1, 3-2, and 3-3, and plotted in Figures 3-1 and 3-2.

3.2.2.12 Other Systemic Effects

Inhalation and oral exposure to Aroclor 1242 resulted in epithelial hyperplasia in the urinary bladders of rats near continuously (23 hours/day) exposed to 0.009 mg/m^3 or 0.033 mg/kg/day in the diet for 30 days (Casey et al. 1999). In contrast, no effects on the urinary bladder were reported in a study by Mayes et al. (1998) involving chronic oral exposure to approximately #6 mg/kg/day Aroclor 1242, 1254, and 1260 or #11 mg/kg/day Aroclor 1016. Additionally, no urinary bladder effects were noted in a series of dietary exposure studies on single PCB congeners in which rats were exposed to #4.1 mg/kg/day of PCB 153 (Chu et al. 1996a), #4.2 mg/kg/day of PCB 128 (Lecavalier et al. 1997), #4.0 mg/kg/day of PCB 105 (Chu et al. 1998b), #3.7 mg/kg/day of PCB 28 (Chu et al. 1996b), #0.77 mg/kg/day of PCB 77 (Chu et al. 1995), #0.17 mg/kg/day of PCB 118 (Chu et al. 1995) or #7.4 mg/kg/day of PCB 126 (Chu et al. 1994) for 13 weeks.

3.2.3 Immunological and Lymphoreticular Effects

3.2.3.1 Summary

Immunologic changes have been observed in human populations exposed to mixtures of PCBs and other persistent toxic substances. Alterations have been associated with consumption of contaminated fish and other marine foods, consumption of contaminated rice oil in the *Yusho* and *Yu-Cheng* poisoning incidents, and general environmental exposures. Findings include increased susceptibility to respiratory tract infections in adults and their children, increased prevalence of ear infections in infants, decreased total

serum IgA and IgM antibody levels, and/or changes in T lymphocyte subsets. Overall, there is a consistency of effects among the human studies suggesting sensitivity of the immune system to PCBs, particularly in infants exposed *in utero* and/or via breast feeding. However, due to the mixed chemical nature of the exposures and generally insufficient information on exposure-response relationships, the human studies provide only limited evidence of PCB immunotoxicity. In contrast to the human data, immunotoxicity of PCBs has been documented in animals that were exposed via commercial mixtures, mixtures of congeners analogous to human breast milk, Great Lakes fish, or single congeners. Effects of commercial PCBs in rats, mice, guinea pigs, and rabbits included morphological and functional changes, such as thymic and splenic atrophy, reduced antibody production against foreign antigens, such as tetanus toxoid and sheep red blood cells (SRBC), and increased susceptibility to microbial infection. Oral studies of commercial mixtures in monkeys confirm the observations in other species and further indicate that the immune system of nonhuman primates is particularly sensitive to PCBs. Suppressed antibody responses to SRBCs is the parameter most consistently affected by PCBs in monkeys and have been observed in adult animals, infants exposed during gestation and lactation, and infants exposed postnatally to a PCB congener mixture simulating the congener content of human milk. Immunological assessments of rodents that were fed Great Lakes fish containing PCBs and other chemicals were generally limited although some alterations were observed that are similar to those in animals exposed to commercial PCB mixtures.

3.2.3.2 Human Studies

Occupational Exposures. A limited amount of information is available on immunological end points in PCB-exposed workers because assessments in most occupational studies were limited to routine clinical measurements of white blood cell (WBC) counts and serum proteins and did not include assessment of immunocompetence. Total and differential WBC counts were determined in 194 capacitor plant workers (152 males, 42 females) who were exposed to Aroclors 1254, 1242, and/or 1016 for an average duration of 17 years (Lawton et al. 1985a). Mean area air concentrations of PCBs were 0.69 mg/m^3 in 1975 and 0.16 mg/m^3 in 1983, and average personal time-weighted average (TWA) levels in 1977 were 0.17 mg/m^3; all PCB use was discontinued in 1977. Clinical examinations in 1976 showed some elevations in total WBCs associated with decreased PMN cells and increased lymphocytes, monocytes, and eosinophils. In 1979, the WBC and lymphocyte counts were near normal and the increases in monocytes and eosinophils were marginal, although there was a strong association between serum PCB levels and monocyte counts.

Other studies of PCB-exposed workers did not report any effects on total and differential WBC counts or changes in serum albumin, globulin, and/or total proteins (Chase et al. 1982; Maroni et al. 1981b; Smith et al. 1982). These included studies of 86 men exposed to unreported levels of unspecified PCBs via transformer fluids for an average of 17 years (Chase et al. 1982), 40 men and 40 women exposed mainly to Pyralene 3010 or Apirolio (Italian PCB formulations containing 42% chlorine) at concentrations ranging from 0.048 to 0.275 mg/m^3 for an average duration of 12 years (Maroni et al. 1981b), 228 electrical equipment manufacturing workers exposed to Aroclor 1242 and 1016 (sex and exposure duration not reported) at a median personal TWA air concentration of 0.081 mg/m^3 (Smith et al. 1982), and 14 and 25 electrical utility workers exposed to Askarel (Aroclor 1254 or 1260 either alone or in combination with tri- or tetrachlorobenzenes) at personal TWA levels of 0.037–0.215 mg/m^3 and 0.0031–0.0823 mg/m^3, respectively (Smith et al. 1982). Exposure durations and worker gender were not reported in the Smith et al. (1982) study.

Delayed-type hypersensitivity was not affected in 55 transformer repairmen compared to 56 unexposed workers who were matched for age, race, and marital status (Emmett et al. 1988a, 1988b). The mean length of employment of the exposed workers was 3.75 years, most (38) of the workers were currently exposed to PCBs, and the predominant exposure was from Aroclor 1260. Measurements of air PCB levels at four work areas showed 8-hour TWA concentrations of 0.0167–0.024, 0.0032–0.007, 0.00001–0.0004, and 0.0007–0.0124 mg/m^3. The percentages of exposed and control workers with positive skin responses to mumps antigen (92 vs. 89%) and trichophyton antigen (17 vs. 8%) were not significantly different, and the mean diameters of the skin reactions were identical in the two groups. Other immunologic end points were not evaluated in the study, and none of the workers had clinical manifestations typical of PCB poisoning.

Contaminated Fish Consumption. Immunological parameters were compared in a group of 23 Swedish men with high consumption of fatty fish species from the Baltic Sea and 20 men with virtually no fish consumption (Svensson et al. 1994). Evaluation of white cell counts, numbers of total lymphocytes and their subsets, and serum immunoglobulin levels showed indications of reduced natural killer (NK) cell activity. The proportions and numbers of NK cells were marginally lower in the fisheaters than in the nonconsumers, although the differences were not statistically significant ($p>0.05$), and the weekly intake of fatty fish was negatively correlated with NK cell activity ($r= -0.32$, $p<0.04$). Concurrent measurements of blood PCBs were not performed. Data from some of the subjects obtained 3 years prior to the study showed weak negative correlations between numbers of NK cells and blood levels of PCB 126 and

PCB 118, but a similar correlation was also found for *p,p'*-DDT. Information on the presence and incidence of infections was not reported.

Lymphocyte subsets were also evaluated in 68 Latvian fisherman who consumed fatty fish from the Baltic Sea (Hagmar et al. 1995). The study group was divided into groups of 19, 24, and 25 subjects with low, intermediate, or high fish consumption (average 0.3, 3.3, and 12 meals/month, respectively). PCBs were not measured in the subjects or fish. High fish consumption was correlated positively with B cell numbers (r=0.41, p=0.0008) and $CD4^+/CD8^+$ ratios (r=0.40, p=0.001), but negatively with levels of cytotoxic ($CD8^+$) T cells (r= -0.38, p=0.002).

Information has been reported on infectious illnesses in breast-fed infants whose mothers consumed contaminated Great Lakes fish (Smith 1984). Seventy-three mother/infant pairs from Sheboygan, Wisconsin were divided into three groups: women who breast-fed and ate Lake Michigan or Sheboygan River fish at least twice a month for $3 years (Group 1, 23 pairs); women who breast-fed and ate Lake Michigan or Sheboygan River fish not more than twice a year for #3 years (Group 2, 39 pairs); and women who bottle-fed and ate Lake Michigan or Sheboygan River fish at least twice a month for $3 years (Group 3, 11 pairs). Mean PCB concentrations in maternal serum (5.48–5.76 ppb) and breast milk fat (1.13–1.14 ppm) were similar among the three exposure groups and at two postnatal sampling times (during the second month and at 4 months of age); PCB levels in maternal serum during pregnancy or in umbilical cord blood were not determined. There were no significant group differences in the mean number of infectious illnesses (colds, earaches, and flu symptoms) during the first 4 months of life. The number of infectious illnesses in the infants (r=0.33, p=0.03) was positively and significantly associated with maternal serum PCB level, although infant illnesses had a weak but significantly negative association with breast milk PCBs. Possible associations between infectious illnesses and other chemicals in the fish were not investigated.

Susceptibility to infections and immune status was studied in 98 breast-fed and 73 bottle-fed Inuit (Eskimo) infants from Arctic Quebec, Canada (Dewailly et al. 2000). The Inuits have high body burdens of various organochlorine compounds (2–10 times higher than those of southern Quebec populations) due to high consumption of marine foods, particularly sea mammal fat. Concentrations of PCBs and other chlorinated pesticides or metabolites were measured in early breast milk fat and used as an index of prenatal exposure to these substances; *p,p'*-DDE showed the highest mean concentration (962 ppb), followed by PCBs (621 ppb; sum of congeners 138, 153, and 180), hexachlorobenzene (107 ppb), dieldrin (30 ppb), and mirex (14 ppb) (Dewailly et al. 1993). Prenatal organochlorine exposure was not

determined in the bottle-fed infants. The number of infectious disease episodes and status of immunologic parameters (WBCs, total lymphocytes and lymphocyte subsets, serum immunoglobulins) were evaluated during the first year of life. Acute otitis media was the most frequent health problem during the first year of life, with 80.0% of ever breast-fed and 81.3% of bottle-fed infants experiencing at least one episode. Relative risk (RR) analysis by follow-up period and number of episodes showed associations between increasing prenatal exposure to organochlorine compounds and otitis media that were more consistent for hexachlorobenzene and *p,p'*-DDE than PCBs. For example, although RRs of experiencing at least one episode of otitis media during the first year of life were similar for hexachlorobenzene (RR, 1.49; 95% CI, 1.10–2.03), *p,p'*-DDE (RR, 1.52; CI, 1.05–2.22), and PCBs (RR 1.28; CI, 0.92–1.77) for the highest tertile of prenatal exposure compared to the lowest tertile, the RR of recurrent otitis media ($3 episodes) was 1.49 (95% CI, 1.10–12.56), 3.48 (CI, 0.86–14.11), and 1.65 (CI, 0.49–5.57), respectively. However, because these and other detected organochlorine compounds originated from the same few food items and have concentrations in breast milk that are correlated with each other due to similar properties such as lipid solubility and persistence, the results precluded identification of which compounds could be responsible for the increased susceptibility to otitis media. Immunologic parameters that were significantly lower in the breast-fed babies compared to the bottle-fed group included numbers of WBCs and lymphocytes (CD4 subtype) at 3 months of age, and serum IgA concentrations at 7 and 12 months of age; CD4/CD8 lymphocyte ratios (helper T-cells/cytotoxic T-cells) were also reduced in the breast-fed infants at 7 and 12 months of age, although the change did not reach statistical significance. None of the immune parameters were associated with prenatal organochlorine exposure.

Yusho and Yu-Cheng Exposures. Clinical observations strongly suggest that *Yusho* and *Yu-Cheng* patients experienced frequent or more severe skin and respiratory infections and lowered resistance to illness (Kuratsune 1989; Nakanishi et al. 1985; Rogan 1989; Shigematsu et al. 1971). Children born to mothers who had *Yu-Cheng* disease had higher prevalence of bronchitis or pneumonia at 6 months of age, respiratory tract infections at 6 years of age, and middle ear infections at 6–14 years of age (Chao et al. 1997; Yu et al. 1998). Total serum levels of IgA and IgM, but not IgG, were reduced in *Yusho* and *Yu-Cheng* patients (Chang et al. 1981; Shigematsu et al. 1971). Other assessments of *Yu-Cheng* patients found various other immunologic changes, including lower percentages of monocytes and PMN leukocytes with immunoglobulin and complement receptors, reduced T lymphocytes apparently due to reduced T-helper/inducer cells, and suppressed dermal delayed-type hypersensitivity responses to streptokinase/streptodornase antigen mixtures tested 1 year after exposure and tuberculin antigen tested 4 years after exposure (Chang et al. 1981, 1982a, 1982b; Lu and Wu 1985). Lymphoproliferative

responses of peripheral lymphocytes to T-cell mitogens (PHA, pokeweed mitogen [PWM], and tuberculin) were significantly enhanced in *Yu-Cheng* patients (Lu and Wu 1985).

General Population Exposures. Immunologic effects of pre- and postnatal environmental exposure to PCBs and dioxins were assessed in a subgroup of 55 infants (Weisglas-Kuperus et al. 1995) from the Dutch Mother-Child study summarized in Section 3.2.4.2.1.2 (Neurological Effects). Prenatal PCB/dioxin exposure was estimated by the sum of PCB congeners 118, 138, 153, and 180 in maternal blood during the last month of pregnancy and the total TEQ level in maternal milk (17 dioxin and 8 dioxin-like PCB congeners), and postnatal exposure was calculated as a product of the total TEQ level in human milk multiplied by the weeks of breast-feeding. No correlation was found between pre- or postnatal exposure and the number of episodes of rhinitis, bronchitis, tonsillitis, and otitis during the first 18 months of life, or with humoral immunity as evaluated by antibody levels to mumps, measles, and rubella at 18 months of age (infants were immunized at 14 months of age). Determination of monocyte, granulocyte, and lymphocyte counts in cord and venous blood at 3 and 18 months of age showed that a higher prenatal as well as postnatal PCB/dioxin exposure was associated with lower monocyte and granulocyte counts at 3 months of age, and that a higher prenatal exposure was associated with increased total numbers of T-lymphocytes and several T-cell subpopulations ($CD8^+$, $TcR\alpha\beta^+$, and $TcR\gamma\delta^+$) at 18 months of age. There were no significant associations between postnatal PCB/dioxin exposure and T cell markers at 18 months of age. Although there were differences in the leukocyte subpopulation between high and low PCB/dioxin-exposed infants, all values were within the normal range (Weisglas-Kuperus et al. 1995). Follow-up evaluations at 42 months of age, reported as a study abstract, found that prenatal PCB exposure was associated with increased T cell numbers and lower antibody levels to mumps, measles, and rubella (Weisglas-Kuperus 2000). Additionally, a higher prevalence of recurrent middle ear infections and chicken pox and a lower prevalence of allergic reactions was reported to be associated with PCB body burden at 42 months of age.

Evaluation of Human Studies. Limited information on immunological effects of PCBs in humans is available from studies of people exposed in the workplace, by consumption of contaminated fish and other marine foods, by consumption of contaminated rice oil in the *Yusho* and *Yu-Cheng* poisoning incidents, and via general environmental exposures. A comparison of PCB levels in blood and breast milk in some of these studies is included in Appendix A.

One study of PCB-exposed workers found no effects on delayed-type hypersensitivity skin reactions to the mumps and trichophyton antigens (Emmett et al. 1988a, 1988b). Other occupational studies reported

no changes in serum albumin, globulin, and/or total proteins, although a transient effect on total and differential WBC counts has been observed (Chase et al. 1982; Lawton et al. 1985a; Maroni et al. 1981b; Smith et al. 1982). Functional and other immunologic end points were not evaluated in any of the worker studies, precluding an assessment of the potential for adverse immune effects following occupational exposure.

The number of infant infectious illnesses (colds, earaches, and/or flu symptoms) during the first 4 months of life were positively correlated with maternal serum PCB levels in a study of women who consumed contaminated Great Lakes fish (Smith 1984), although other immunological end points and possible associations with other chemicals in the fish were not investigated. Susceptibility to infections was also studied in infants of Inuit women who had elevated body burdens of PCBs and other organochlorine chemicals due to high consumption of sea mammal fat (Dewailly et al. 2000). Associations between risk of acute otitis media and increasing organochlorine exposure (levels in breast milk) during the first year of life were found, although the data are insufficient for identifying whether the effect may be due to PCBs, hexachlorobenzene, *p,p'*-DDE, or other chemicals. No statistically significant changes in immunological indices were observed, although there were indications of reduced total serum IgA levels and altered T-lymphocyte subpopulations in breast-fed Inuit infants at 7 and 12 months of age.

Immunotoxic effects have been documented in the *Yusho* and *Yu-Cheng* populations and include changes consistent with those reported in the Inuit and Great Lakes populations, particularly increased middle ear and respiratory tract infections in children of exposed mothers and changes in T lymphocytes and their subsets (Chang et al. 1981, 1982a, 1982b; Chao et al. 1997; Kuratsune 1989; Lu and Wu 1985; Nakanishi et al. 1985; Rogan 1989; Shigematsu et al. 1971; Yu et al. 1998). The Dutch environmental exposure study (Weisglas-Kuperus et al. 1995) also found some changes in lymphocyte T cell subpopulations in infants (although all values were within the normal range), but the clinical significance of these alterations is unclear because there was no significant correlation between the incidence of infection (otitis, rhinitis, bronchitis, or tonsillitis) or antibody levels to common childhood vaccines (mumps, measles, or rubella) during the first 18 months of life and pre- or postnatal exposure to PCBs and dioxins. The human populations that have been studied differ greatly with respect to sources of PCB exposure and consequently are likely to vary with respect to both organochlorine contaminants and nutrient contents which may affect susceptibility to infections. Although the studies are insufficient for determining which specific chemical(s) may be responsible for the observed alterations, the available data support a possible association between PCBs and immune effects in humans that may be manifested as compromised ability to overcome infections, particularly in infants exposed *in utero* and/or by breast-feeding.

3.2.3.3 Animal Studies

The highest NOAEL values and all reliable LOAEL values for immunological and lymphoreticular effects for each study are recorded in Tables 3-2 and 3-3, and plotted in Figure 3-2.

3.2.3.3.1 Inhalation Exposure

No studies were located regarding immunological or lymphoreticular effects in animals following inhalation exposure to PCBs.

3.2.3.3.2 Oral Exposure

Commercial PCB Mixtures. Information on the immunotoxicity of commercial PCBs in orally-exposed animals is available from intermediate- and chronic-duration studies in various species. Findings in nonhuman primates are emphasized in the following summary because monkeys appear to be more sensitive than other species and provide a better animal model due to phylogenetic and biologic similarities to humans (Tryphonas 1994, 1995).

Aroclor 1260 and Similar Mixtures. Immunological effects of 60% chlorinated PCB mixtures were investigated in several guinea pig studies. Dietary exposure to Aroclor 1260 for 8 weeks caused decreases in gamma globulin-containing cells in popliteal lymph nodes following foot pad stimulation with tetanus toxoid at estimated doses of 0.8 and 4 mg/kg/day (lower doses not tested), although the magnitude of response was not dose-related. Increased mesenteric lymph node weights were also observed at $0.8 mg/kg/day, but there were no consistent changes in cervical lymph node weights or serum levels of albumin or globulins. Leukocyte counts and histology of the lymph nodes, thymus, and spleen were unaffected (Vos and de Roij 1972). Effects in guinea pigs that were fed 4 mg/kg/day Clopen A-60 or Aroclor 1260 for 6 weeks included decreases in antibody titers (IgM and IgG) to tetanus toxoid, skin (footpad) reactivity to tuberculin, leukocyte, and lymphocyte counts, and relative thymus weight, with no effects occurring at 0.8 mg/kg/day of Clopen A-60 (low dose of Aroclor 1260 not tested) (Vos and Van Driel-Grootenhuis 1972).

No changes in total or differential WBC counts or histology of the thymus, spleen, or lymph nodes were found in male and female rats that were exposed to Aroclor 1260 at dietary doses as high as 4.1 and 5.8 mg/kg/day, respectively, for 24 months (Mayes et al. 1998).

Aroclor 1254 and Similar Mixtures. Information on immunotoxicity of Aroclor 1254 is available from oral studies in rats, mice, rabbits, and monkeys. A number of significant effects on humoral and cell-mediated parameters were found in rats. Dietary exposure to Aroclor 1254 at estimated doses of 4.3 or 43 mg/kg/day for 10 weeks caused decreased serum total immunoglobulin G (IgG) antibody response to keyhole limpet hemocyanin (KLH) antigen, decreased NK cell activity, and increased interleukin 2 (IL-2) production by concanavalin A (ConA)-stimulated splenocytes in Sprague-Dawley male rats (Exon et al. 1985; Talcott et al. 1985). Male F344 rats treated with Aroclor 1254 by gavage for 5–15 weeks had reduced thymus weight and NK cell activity at 10 and 25 mg/kg/day, and increased PHA mitogen-induced lymphocyte proliferation at 25 mg/kg/day; no significant effects were seen at #1 mg/kg/day. (Smialowicz et al. 1989). Enhanced responses to ConA, PWM, or *Salmonella typhimurium* mitogen (STM) were not observed. Decreased thymus weight and enhanced lymphoproliferative activity in response to stimulation by PHA, but not PWM, were also observed in male Sprague-Dawley rats that were fed Aroclor 1254 at an estimated dose of 21.5 mg/kg/day for 7 days (Bonnyns and Bastomsky 1976). Thymus weight, WBC, and neutrophil counts were reduced in Sprague-Dawley rats fed an estimated dose of 50 mg/kg/day Aroclor 1254 for 6 weeks (Allen and Abrahamson 1973); immune function was not evaluated. No changes in total or differential WBC counts or histology of the lymph nodes, spleen, or thymus were found in male and female rats that were exposed to Aroclor 1254 at dietary doses as high as 4.3 and 6.0 mg/kg/day, respectively, for 24 months (Mayes et al. 1998).

Susceptibility to Moloney leukemia virus (MLV) was increased in male BALB/c mice that ingested $4.9 mg/kg/day estimated dietary doses of Aroclor 1254 for 6 months; no effect was found at 0.5 mg/kg/day (lowest tested dose) (Koller 1977). Similarly, susceptibility to mortality from herpes simplex virus was increased in male ICR mice that ingested Kanechlor 500 in the diet for 21 days at $33 mg/kg/day, but not at the lowest tested dose of 18 mg/kg/day (Imanishi et al. 1980). Swiss-Webster mice were fed Aroclor 1254 at doses of 1.2, 11.7, or 29.2 mg/kg/day for 12 weeks prior to mating with exposure continuing throughout gestation and lactation (Talcott and Koller 1983). Immunologic evaluation of the offspring at 8 weeks of age showed no significant effects on antibody titers to bovine serum albumin (BSA), phagocytosis of SRBC (measured by ingestion by peritoneal macrophages *in vitro*), or delayed-type hypersensitivity to oxazolone, although relative spleen weights were reduced at 29.2 mg/kg/day.

Male New Zealand rabbits that were exposed to 0.18–6.44 mg/kg/day dietary doses of Aroclor 1254 for 8 weeks had no effects on several immunological end points, including hemolysin and hemagglutination

titers against SRBC, gamma-globulin/transferrin ratio, and skin sensitivity to tuberculin, although significant atrophy of the thymus occurred at all doses (Street and Sharma 1975).

Immunological effects of Aroclor 1254 in monkeys were first indicated in pilot studies of general toxicity (Truelove et al. 1982; Tryphonas et al. 1986a). Dietary ingestion of Aroclor 1254 in apple juice-gelatin-corn oil emulsion at doses of 0.1 mg/kg/day (2 Cynomolgus monkeys) or 0.4 mg/kg/day (1 monkey) for 238–267 days, beginning at approximately day 60 of gestation, caused a decreased antibody response to SRBC in all treated animals compared to one control monkey (Truelove et al. 1982). No effect on antibody titers to tetanus toxoid was observed. Both monkeys exposed to 0.1 mg/kg/day delivered stillborn infants, and the 0.4 mg/kg/day monkey delivered a live infant which was nursed, but failed to respond to SRBC and died at 139 days postpartum with acute confluent bronchopneumonia.

Groups of four Cynomolgus and four Rhesus monkeys ingested 0 or 280 µg/kg/day Aroclor 1254 in apple juice-gelatin-corn oil emulsion on 5 days/week for 12–13 months and 27–28 months, respectively (Tryphonas et al. 1986a). Immunologic parameters that were evaluated included serum protein levels, total serum IgG, IgA, and IgM, and antibody titers to SRBC. Total serum IgM levels and anti-SRBC (IgM) titers were reduced in both species.

A subsequent series of tests on Aroclor 1254 was conducted in Rhesus monkeys because they appeared to be more sensitive than Cynomolgus monkeys based on relatively greater severity of clinical signs and higher blood and adipose PCB levels (Tryphonas et al. 1986a). Groups of 16 female Rhesus monkeys were orally administered Aroclor 1254 in capsules at doses of 0, 5, 20, 40, or 80 µg/kg/day, with immunological assessments performed after 23 months (Tryphonas et al. 1989) when blood PCB steady-state was established, and at 55 months (Tryphonas et al. 1991a, 1991b). Average concentrations of PCBs in the 0, 5, 20, 40, and 80 µg/kg/day dose groups around the time of immunologic testing were 0.1, 10.2, 34.0, 74.9, and 112 ppb, respectively, in blood and 0.4, 2.7, 9.0, 15.7, and 31.2 ppm, respectively, in adipose tissue (Tryphonas et al. 1989). Significant dose-related decreases in IgM (all doses except 0.02 mg/kg/day) and IgG (all doses) antibody titers to SRBC were found after 23 months. Secondary challenge with SRBC after 55 months showed decreasing dose-related trends in the IgM and IgG anamnestic responses, although only IgM was significantly lower than controls at all dose levels. Other effects included alterations in lymphocyte T-cell subsets characterized by a significant decrease in the ratio of T-helper/inducer (CD4) cells to T-suppressor/cytotoxic (CD8) cells, due to reduced CD4 and increased CD8 cells, after 23 months at 80 µg/kg/day (not tested at lower doses). No effects on total lymphocytes or B-cells were found, indicating that T-cells were preferentially affected by the PCBs,

although there were no exposure-related changes in T-cell subsets after 55 months suggesting that adaptation had occurred. Statistically significant dose-related trends, but no significant differences between exposed and control groups, were observed after 55 months for decreasing lymphocyte proliferation in response to mitogens (PHA and ConA, but not PWM), increasing NK cell activity, increasing levels of serum thymosin alpha-1, decreasing phagocytic activity of peripheral blood monocytes following activation with phorbol myristate acetate (PMA), and increasing total serum complement activity. End points that were not affected by PCB exposure included IgG antibody response to pneumococcal antigens, total serum immunoglobulins (IgG, IgM, and IgA) levels, and other serum proteins as well as serum hydrocortisone levels.

Offspring from the Rhesus monkeys studied by Tryphonas et al. (1989, 1991a, 1991b) were also evaluated for immunological changes (Arnold et al. 1995). Females were mated after 37 months of exposure to 0, 5, 20, 40, or 80 µg/kg/day of Aroclor 1254. The maternal dosing was continued throughout pregnancy and into lactation until nursing infants were approximately 7 weeks old, and treatment was restarted in the infants at weaning (22 weeks). Immunological testing was initiated at 20 weeks of age although statistical evaluation was limited by small numbers of animals due to fetal and postpartum deaths (see Section 3.2.5.3). IgM and IgG antibody levels were determined 1–3 weeks following immunization with SRBC at 20 and 60 weeks of age. Significant reductions in IgM titers were found at 5 and 40 µg/kg/day at weeks 22 and 23, and 5 µg/kg/day at weeks 61–63; IgM levels were insignificantly reduced in the 40 µg/kg/day group at weeks 61–63. IgG titers were significantly reduced only in the 40 µg/kg/day group at week 22. Other immunological tests were performed at 20, 28, and 60 weeks of age and included assays for lymphocyte proliferation (in response to stimulation by PHA, ConA, or PWM mitogens or leucocyte stimulator cells) and NK cell activity; the only significant finding was a decreased lymphocyte proliferation response at 40 µg/kg/day at weeks 28 and 60.

Aroclor 1248. Immune responses to Aroclor 1248 were investigated in oral studies with mice, rabbits, and monkeys. Female mice (Albino outbred) that were fed Aroclor 1248 for 5 weeks had increased endotoxin sensitivity at estimated doses of 13 and 130 mg/kg/day and decreased resistance to challenge by *S. typhimurium* at 130 mg/kg/day (not tested at lower dose), but no effects on spleen and thymus weight or histology at #130 mg/kg/day (Thomas and Hindill 1978). In a study with New Zealand rabbits, females were exposed to 3.6, 28, or 91 mg/kg/day dietary doses of Aroclor 1248 from 4 weeks before mating until offspring were weaned at 4 weeks of age (Thomas and Hinsdill 1980). Testing at 7 weeks of age showed that skin contact sensitivity response to dinitrofluorobenzene was reduced in the offspring of the 91 mg/kg/day rabbits. This effect was accompanied by reduced body weight, making it unclear

whether the effect was directly due to PCBs or secondary to some other form of toxicity. No exposure-related changes in spleen and thymus weights, plaque forming cell (PFC) response and antibody titers to SRBC, or mitogenic response of peripheral blood lymphocytes to PHA or ConA were observed in the rabbits at any dose level. Thymus weight and WBC and neutrophil counts were reduced in Sprague-Dawley rats fed Aroclor 1248 at an estimated dose of 50 mg/kg/day for 6 weeks (Allen and Abrahamson 1973); these effects were similar in severity to those induced by Aroclor 1254.

Increased susceptibility to bacterial infections was reported in two monkeys after dietary exposure to approximately 0.1–0.2 mg/kg/day Aroclor 1248 (Barsotti et al. 1976). The monkeys, which died after 173 and 310 days of treatment, had clinical signs of PCB toxicity and developed, terminally, a severe enteritis from which *Shigella flexneri* type IV was isolated.

Immunologic changes were investigated in groups of eight Rhesus monkeys that were immunized with SRBC and tetanus toxoid following dietary exposure to 0.1 or 0.2 mg/kg/day Aroclor 1248 for 11 months (Thomas and Hinsdill 1978). Comparison with a control group of five monkeys showed effects at 0.2 mg/kg/day that included reduced anti-SRBC antibody titers at weeks 1 and 12 after primary immunization (i.e., at 2 of 6 postimmunization times), and decreased percent gamma-globulin after 20 weeks. Antibody responses to SRBC were not significantly affected at 0.1 mg/kg/day. The response to tetanus toxoid was not significantly modified at either dose level.

Pathological changes in lymphoid tissues occurred in offspring of Rhesus monkeys that were fed 0.1 or 0.2 mg/kg/day estimated dietary doses of Aroclor 1248 for a 15-month period that included breeding, gestation, and lactation (Allen and Barsotti 1976). The offspring were exposed for approximately 46 weeks from beginning of gestation until they were weaned. The doses were both fetotoxic (early abortions occurred in 5 of 8 low-dose and 4 of 6 high-dose animals) and postnatally lethal (3 of 6 infants died of PCB intoxication between days 44 and 329). Gross and microscopic changes in the deceased infants included reduced cortical and medullary areas in the thymus, reduced lymph nodes and absence of germinal centers in the spleen, and hypocellularity of the bone marrow. The females from the Allen and Barsotti (1976) study were bred again after 1 year on the control diet (Allen et al. 1980). Early infant mortality was observed (2 of 4 in the former 0.1 mg/kg/day group and 2 of 7 in the former 0.2 mg/kg/day group), and histological examinations showed thymus, spleen, and bone marrow effects similar to those described above, as well as findings of hypocellular lymph nodes devoid of germinal centers. Regression of the cortical areas of the thymus and hypoplastic bone marrow were similarly observed in 5 infant

(1-month-old) Rhesus monkeys that were intubated with 35 mg/kg/day Aroclor 1248 for 30 days (Abrahamson and Allen 1973).

Aroclors 1242, 1221, and 1016. Effects of Aroclors 1242, 1221, and 1016 on immune function have been studied in male BALB/c mice. Mice that were exposed to an estimated dietary dose of 22 mg/kg/day Aroclor 1242 for 3 or 6 weeks caused decreased primary and secondary PFC responses to SRBC antigens with concurrent reductions in total serum IgG_1, IgM, and IgA levels (Loose et al. 1977, 1978a, 1978b, 1979). There were no effects on thymus and spleen weights or histological alterations in the thymus, spleen, or mesenteric lymph nodes, and morphometric analysis of the spleens did not show changes in the number, size, or cellular composition of the germinal follicles. Mice that were exposed to 22 mg/kg/day Aroclor 1242 for 3 or 6 weeks also had increased susceptibility to challenge by *Salmonella typhosa* endotoxin or the malarial parasite *Plasmodium berghei* which resulted in increased mortality (Loose et al. 1978a, 1979), although exposure to the same dose for up to 18 weeks did not affect macrophage function (*in vitro* phagocytic capacity and activity or microbiocidal activity) or resistance to challenge by EL-4 lymphoma or kidney ascites tumor cells (Loose et al. 1981). Susceptibility to Moloney leukemia virus was increased in male BALB/c mice that ingested dietary Aroclor 1242 for 6 months at estimated doses of $4.9 mg/kg/day, but not in mice that were similarly exposed to Aroclor 1221 at doses as high as 48.8 mg/kg/day (Koller 1977).

Male C57BL/6 mice that were exposed to Aroclor 1016 in the diet at an estimated dose of 22 mg/kg/day for up to 40 weeks had no consistent effects on thymus and spleen weights, lymphocyte counts, or lymphocyte function as evaluated by the splenic graft-versus-host (GVH) response, mixed lymphocyte response, mitogenic response to stimulation by PHA or LPS, or cytotoxic activity of sensitized lymphocytes to target tumor cells (Silkworth and Loose 1978).

No changes in total or differential WBC counts or histology of the lymph nodes, spleen, or thymus were found in male and female rats following 24 months of dietary exposure to Aroclor 1242 at doses as high as 4.0 and 5.7 mg/kg/day, respectively, or Aroclor 1016 at doses as high as 8.0 and 11.2 mg/kg/day, respectively (Mayes et al. 1998).

Defined Experimental Mixtures. The toxicity of a mixture of PCB congeners analogous to that in human breast milk (Canadian women) was studied in monkeys (Arnold et al. 1999). Groups of infant Cynomolgus monkeys (6 control males, 10 treated males) and Rhesus monkeys (2 control and 3 treated males, 1 control and 3 treated females) were administered the congener mixture in a total daily dose of

0 or 7.5 µg PCBs/kg/day from birth until 20 weeks old (i.e., without *in utero* exposure), and were observed until they were at least 66 weeks old. The dose was divided into thirds and administered prior to the first three daily feedings via syringe to the back of the mouth. The dose represented the approximate daily intake of a nursing human infant whose mother's milk contained 50 ppb PCBs (the Health Canada guideline for maximum concentration in breast milk). Immunological assessment of the infants was started at 22 weeks of age and included IgM and IgG antibody production following immunization with SRBC, lymphoproliferative activity of peripheral leucocytes in response to mitogens (PHA, ConA, and PWM), numbers of peripheral leucocytes and their subsets, and NK cell activity. Few statistically significant changes were observed. Anti-SRBC titers were reduced in the treated Rhesus and Cynomolgus monkeys, but were not significantly different from controls, although antibodies were significantly reduced over postimmunization time (p#0.025 for IgM and IgG in Cynomolgus monkeys, p=0.002 for IgM in Rhesus monkeys). Other changes included reduced absolute mean numbers of B lymphocytes in the treated Cynomolgus monkeys (no change in mean percent); the effect was not observed when re-evaluated in the monkeys at 1 year of age. The investigators concluded that, overall, the effects on the infant immune system were mild and of unclear biological significance due to large inter-animal variability and the small numbers of animals.

Single Congeners. A series of toxicity studies was performed in which groups of 10 male and 10 female Sprague-Dawley rats were exposed to diets containing four dose levels of various single congeners for 13 weeks (Chu et al. 1994, 1995, 1996a, 1996b, 1998; Lecavalier et al. 1997). End points relevant to the immune system included total and differential WBC counts, spleen weight, and histology of the spleen, thymus, mesenteric lymph nodes, and bone marrow. Data on these end points were reported for seven congeners: PCB 28, 77, 105, 118, 126, 128, and 153. Effects were essentially limited to thymic changes, generally reductions in cortical and medullary volume and atrophy, which were observed following exposure to PCB 126 ($0.74 µg/kg/day), PCB 153 ($3,534 µg/kg/day), PCB 28 ($3,783 µg/kg/day), and PCB 105 ($3,960 µg/kg/day). No changes in the immunologic end points were induced by PCB 77 (#892 µg/kg/day), PCB 118 (#170 µg/kg/day), or PCB 128 (#4,125 µg/kg/day).

Contaminated Fish Consumption. Effects on the immune system were investigated as part of a two-generation reproduction study of Sprague-Dawley rats that were fed diets containing 0, 5, or 20% (w/w) of lyophilized protein from chinook salmon from Lake Huron or Lake Ontario (Arnold et al. 1998; Feely and Jordan 1998; Feeley et al. 1998; Tryphonas et al. 1998a, 1998b). Daily intakes of total PCBs in the female F1 rats fed diet containing 0, 5, or 20% lyophilized Lake Ontario salmon flesh were calculated to be 0.22, 23.20, and 82.37 µg/kg/day, respectively (Feely and Jordan 1998). PCB intakes were

qualitatively similar, but generally were somewhat smaller, for males compared with females and for F0 rats compared with F1 rats, although intakes from the Lake Huron diet were about 35–40% lower than from the Lake Ontario diet. The DDT complex (*p,p'*-DDT, *p,p'*-DDE, and *p,p'*-DDD) accounted for 75 and 60% of organochlorine pesticide residues in the Lake Huron and Lake Ontario fish, respectively, and other major contaminants included CDDs and CDFs, mirex, chlordane, cadmium, lead, mercury, and arsenic. No consistent exposure-related effects were found across generations on various immunological end points, including numbers of splenic leukocytes and T-lymphocyte subsets, PFC response to SRBC antigen, NK cell activity, lymphocyte transformation in response to mitogens (ConA, PHA, and LPS), phagocytic activity of peritoneal exudate cells, and resistance to infection by *Listeria monocytogenes* (Tryphonas et al. 1998b). The most notable finding was an increase in absolute leukocyte and lymphocyte levels in the spleen of the F2 male rats fed the Lake Huron fish compared to controls and to F2 males fed Lake Ontario fish, with higher cell numbers in the 20% group compared to the 5% group in each fish source. Additional data suggested that the increases in splenic leukocyte and lymphocyte levels were due to changes in T-lymphocyte subsets, particularly the T-helper/inducer cells. The changes in spleen cellularity paralleled changes in peripheral WBC and lymphocyte levels (Tryphonas et al. 1998a).

Another study assessed immunological effects in juvenile C57Bl/6 mice that were fed diets containing no fish or 33% coho salmon from Lake Ontario or the Pacific Ocean for 2–4 months (Cleland et al. 1989). Intakes of persistent toxic substances were not reported although the halogenated aromatic hydrocarbons with the highest concentrations in the control chow, Pacific salmon diet, and Lake Ontario salmon diet were total PCBs (0.4, 20, and 2,900 ppb, respectively) and *p,p*-DDE (0.1, 10, and 670 ppb, respectively). Levels of PCDDs and PCDFs, mercury, tin compounds, and other metals were not examined. Evaluations included IgM, IgG, and IgA PFC responses to SRBC and numbers of spleen total lymphocytes, total T-lymphocytes, and T-lymphocyte subsets following 2 months of exposure. Cellular immunity was assessed after 4 months of exposure by the cytotoxic T-lymphocyte response to allogeneic tumor target cells. The only significant finding was a reduced PFC response to SRBC for all three immunoglobulin classes in the mice that consumed the Lake Ontario diet compared to responses in the mice fed the Pacific Ocean salmon or control diets.

3.2.3.3.3 Dermal Exposure

Limited data are available on immunological effects in animals after dermal exposure to PCBs. Dermal application of an estimated 44 mg/kg/day Aroclor 1260, 5 days/week for 4 weeks resulted in moderate atrophy of the thymus in rabbits (Vos and Notenboom-Ram 1972). No treatment-related histological effects were observed in the spleen and lymph nodes. Application of an estimated 42 mg/kg/day of the same Aroclor for 38 days to rabbits produced histological atrophy of the thymus cortex and a reduction in the number of germinal centers in the spleen and lymph nodes (Vos and Beems 1971). No treatment-related effects were observed in control rabbits in either study. These studies tested small numbers of animals and used Aroclor 1260 that had undetectable levels (<1 ppm) of CDFs.

3.2.3.3.4 Other Routes of Exposure

The relative potencies of five Aroclor mixtures and an experimental congener mixture resembling an extract from human milk were evaluated using the splenic plaque-forming cell response to SRBC in C57BL/6 mice treated by single intraperitoneal injection (Davis and Safe 1989). Comparison of ED_{50} values showed that the higher chlorinated Aroclors 1260, 1254, and 1248 (ED_{50} of 104, 118, and 190 mg/kg, respectively) were more potent than the lower chlorinated Aroclors 1242, 1016, and 1232 (ED_{50} of 391, 408, and 464 mg/kg, respectively). The experimental milk mixture contained an average chlorine percentage resembling Aroclor 1254, but did not significantly decrease the number of plaque-forming cells to SRBC, although the tested doses (5–50 mg/kg) were less than the ED_{50} values for Aroclor 1254 and the other mixtures.

A large number of acute intraperitoneal and *in vitro* studies have investigated congeneric structure-activity relationships for the purpose of elucidating mechanisms of immunotoxicity and relative potencies of individual congeners and their potential interactive effects. As summarized in Section 3.4.2 (Mechanisms of Toxicity), there is evidence from various test systems that noncoplanar as well as coplanar and mono-*ortho*-coplanar congeners are immunologically active, indicating that both Ah receptor-dependent and receptor-independent mechanisms are involved in the immunotoxicity of PCB mixtures (e.g., Brown and Ganey 1995; Brown et al. 1998; Davis and Safe 1989, 1990; Ganey et al. 1993; Harper et al. 1993a, 1993b, 1995; Schulze-Stack et al. 1999; Tithof et al. 1995).

3.2.3.3.5 Evaluation of Animal Studies

The immunotoxicity of PCBs has been evaluated in various species of animals that were exposed to commercial mixtures, mixtures of congeners analogous to human breast milk, Great Lakes fish, or single congeners. Studies in rats, mice, guinea pigs, and rabbits have conclusively shown that intermediate-duration oral exposure to $4 mg/kg/day doses of commercial PCB mixtures can induce both morphological and functional alterations in the immune system. Effects in lymphoid tissues were commonly observed, although no generalizations can be made across species. Decreases in thymus weight occurred in rats exposed to Aroclors 1254 or 1248 and rabbits exposed to Aroclor 1254 (Allen and Abrahamson 1973; Smialowicz et al. 1989; Street and Sharma 1975), but not in guinea pigs exposed to Aroclor 1260, mice exposed to Aroclors 1248, 1242, or 1016, or rabbits exposed to Aroclor 1248 (Loose et al. 1978b; Silkworth and Loose 1978; Thomas and Hinsdill 1978; Vos and de Roij 1972). Spleen weight was reduced in mice exposed to Aroclor 1254, but not in mice exposed to Aroclors 1248, 1242, or 1016, rabbits exposed to Aroclor 1248, or guinea pigs exposed to Aroclor 1260 (Allen and Abrahamson 1973; Loose et al. 1978b; Silkworth and Loose 1978; Talcott and Koller 1983; Thomas and Hinsdill 1980; Vos and de Roij 1972). Histological examinations showed no PCB-related changes in the thymus, spleen, and lymph nodes of guinea pigs exposed to Aroclor 1260 or mice exposed to Aroclors 1242, but histopathology data are not available for other orally-exposed species and mixtures (Loose et al. 1978b; Vos and de Roij 1972). Repeated dermal applications of Aroclor 1260 (42–44 mg/kg/day for 4–5 weeks), however, caused histopathologic changes in the thymus (cortical atrophy) and spleen and lymph nodes (reduced germinal centers) in rabbits (Vos and Beems 1971; Vos and Notenboom-Ram 1972).

Effects on immune function, as indicated by altered responses in humoral and cell-mediated immunity assays and host resistance tests, were also induced by intermediate-duration oral exposure to commercial mixtures. Studies in nonprimate species showed reduced antibody responses to tetanus toxoid in guinea pigs exposed to Clopen A-60 (4 mg/kg/day for 3–5 weeks), keyhole limpet hemocyanin in rats exposed to Aroclor 1254 (4.3 mg/kg/day for 10 weeks), and SRBC in mice exposed to Aroclor 1242 (22 mg/kg/day for 3–6 weeks) (Exon et al. 1985; Loose et al. 1977, 1978a, 1978b, 1979; Vos and Van Driel-Grootenhuis 1972). Commercial PCBs also increased susceptibility to infection by foreign antigens, including Moloney leukemia virus in mice exposed to Aroclor 1254 or Aroclor 1242 ($4.9 mg/kg/day for 6 months), herpes simplex virus in mice exposed to Kanechlor 500 ($33 mg/kg/day for 21 days), and *S. typhosa* endotoxin and the malarial parasite *Plasmodium berghei* in mice exposed to Aroclor 1242 (22 mg/kg/day for 3–6 weeks) (Imanishi et al. 1980; Koller 1977; Loose et al. 1979). Proliferative responses of splenic mononuclear leukocytes to PHA, but not to other mitogens (ConA, STM, or PWM),

was enhanced in rats exposed to Aroclor 1254, although no effects on mitogen-induced proliferation of lymphocytes were observed in rabbits exposed to Aroclor 1248 or mice exposed to Aroclor 1016 (Bonnyns and Bastomsky 1976; Silkworth and Loose 1978; Smialowicz et al. 1989; Thomas and Hinsdill 1980). Skin reactivity to tuberculin was reduced in guinea pigs exposed to Clopen A-60, but not in rabbits exposed to Aroclor 1254, and there was no effect on delayed-type hypersensitivity to the skin sensitizer oxazolone in mice exposed to Aroclor 1254 (Street and Sharma 1975; Talcott and Koller 1983; Vos and Van Driel-Grootenhuis 1972). NK cell activity was reduced in rats following intermediate oral exposure to Aroclor 1254 (Smialowicz et al. 1989; Talcott et al. 1985).

Immunological assessments of rodents fed Great Lakes fish that contained PCBs and other chemicals produced some changes that are similar to those observed in the studies of commercial PCB mixtures. Although no consistent exposure-related effects were found on several immunological variables (thymus weights, PFC response to SRBC, mitogen-induced lymphocyte proliferation, NK cell activity, and susceptibility to challenge with *Listeria monocytogenes*) in a multigenerational study of rats fed Lake Huron or Lake Ontario salmon, increases in splenic leukocyte and lymphocyte levels were increased in F2 male rats due to changes in T-lymphocyte subsets (Tryphonas et al. 1998a, 1998b). In addition, juvenile mice that consumed salmon from Lake Ontario for 2–4 months had reduced antibody responses to SRBC compared to mice fed Pacific Ocean salmon or control diets, but no changes in T-lymphocytes or their subsets were observed (Cleland et al. 1989).

Intermediate-duration oral studies of Aroclors in monkeys confirm the observations of PCB immunotoxicity in rats, mice, guinea pigs, and rabbits and further indicate that nonhuman primates are more sensitive than the other species. Early studies found decreased antibody responses to SRBC, increased susceptibility to bacterial infections, and/or histopathological changes in the thymus, spleen, and lymph nodes in adult monkeys and their offspring at 0.1–0.2 mg/kg/day doses of Aroclor 1254 and 1248, although these findings are limited by small numbers of animals and dose levels (Abrahamson and Allen 1973; Allen and Barsotti 1976; Allen et al. 1980; Barsotti et al. 1976; Thomas and Hinsdill 1978; Truelove et al. 1982; Tryphonas et al. 1986a). The most extensive characterization of immunological effects in nonhuman primates involved assessments on groups of 16 monkeys performed after 23 and 55 months of oral exposure to 5 dose levels of Aroclor 1254 ranging from 5 to 80 µg/kg/day (Tryphonas et al. 1989, 1991a, 1991b). The immune parameters that were most consistently affected in the monkeys were IgM and IgG antibody responses to SRBC, which showed significant dose-related decreases at levels as low as 5 µg/kg/day (lowest tested dose). Other effects were either transient (e.g., alterations in T-cell subsets occurring after 23 but not 55 months of exposure) or showed dose-related trends after

55 months without significant differences between exposed and control groups (e.g., decreasing lymphoproliferative responses to mitogens, increasing NK cell activity, increasing levels of serum thymosin alpha-1, decreasing phagocytic activity of peripheral blood monocytes following activation with PMA, and increasing total serum complement activity).

Results of studies in infant Rhesus monkeys are consistent with the data in adults showing immunosuppressive effects of PCBs at doses as low as 5 µg/kg/day. Evaluation of *in utero* and lactationally-exposed offspring from the monkeys in the Tryphonas et al. (1989, 1991a, 1991b) studies indicated exposure-related reductions in antibody levels to SRBC and mitogen-induced lymphocyte transformation that paralleled the findings in the maternal animals (Arnold et al. 1995). Although assessment of the data is limited by small numbers of infants in the exposed groups, statistical significance was achieved for some end points and evaluation times, including reduced IgM titers at 22–23 and 61–63 weeks of age (following gestational/lactational and/or postweaning dietary exposure) in the infants exposed to 5 µg/kg/day. Infant Rhesus and Cynomolgus monkeys that were orally administered a PCB congener mixture simulating the congener content of human milk at a dose level of 7.5 µg/kg/day for the first 20 weeks of life (i.e., from parturition without *in utero* exposure) had minimal immunological changes (Arnold et al. 1999). More specifically, anti-SRBC titers (IgM and IgG) were uniformly reduced in the treated compared to control monkeys, although group differences were not statistically significant due to small numbers of animals. In addition, B lymphocyte numbers in the exposed Cynomolgus monkeys were decreased compared to controls, although the levels were similar when evaluated again at 1 year of age. These results provide further evidence that monkeys are sensitive to low doses of PCBs administered either as commercial mixtures or as a mixture of congeners representative of those commonly found in breast milk.

As summarized above, oral immunotoxicity studies have shown that suppressed antibody response to SRBC is the parameter most consistently affected by PCBs in adult and infant monkeys and that effects on antibody responses have also been demonstrated in other species. Reductions in antibody responses to SRBC were also observed in juvenile mice that ingested diet containing fish from Lake Ontario. The immunologic response to SRBC antigens, as measured using the PFC assay, is a validated sensitive indicator for detecting potentially immunotoxic chemicals (Luster et al. 1992). Because antibody responses to SRBC antigens were suppressed in monkeys at dose levels of Aroclor 1254 as low as 0.005 mg/kg/day, the lowest tested dose of any PCB mixture in any species, this effect is used as the main basis for deriving the chronic MRL for oral exposure as indicated in the footnote to Table 3-2 and discussed in Chapter 2 and Appendix A.

3.2.4 Neurological Effects

3.2.4.1 Summary

The neurological effects of PCBs have been extensively investigated in humans and in animals. The main focus in humans studies has been on the effects in neonates and young children, although studies of adults have also been conducted. A great deal of concern exists that even low levels of PCBs transferred to the fetus across the placenta may induce long-lasting neurological damage. Because PCBs are lipophilic substances, there is also concern that significant amounts might be transferred to nursing infants via breast milk. Studies in humans who consumed high amounts of Great Lakes fish contaminated with environmentally persistent chemicals, including PCBs, have provided evidence that PCBs are important contributors to subtle neurobehavioral alterations observed in newborn children and that some of these alterations persist during childhood. Some consistent observations at birth have been motor immaturity and hyporeflexia and lower psychomotor scores between 6 months and 2 years old. There is preliminary evidence that highly chlorinated PCB congeners, which accumulate in certain fish, are associated with neurobehavioral alterations seen in some newborn children. Subtle neurobehavioral alterations have also been observed in children born to mothers in the general population with the highest PCB body burdens. Due to the limitations of epidemiological studies, these effects cannot be attributed entirely to PCB exposure. In one general population study, there was strong evidence that dioxins as well as PCBs were contributors to the neurobehavioral effects seen in exposed children. Children born to women who accidentally consumed rice oil contaminated with relatively high amounts of PCBs and CDFs during pregnancy also had neurodevelopmental changes. Studies in animals support the human data. Neurobehavioral alterations have been also observed in rats and monkeys following pre- and/or postnatal exposure to commercial Aroclor mixtures, defined experimental congener mixtures, single PCB congeners, and Great Lakes contaminated fish. In addition, monkeys exposed postnatally to PCB mixtures of congeneric composition and concentration similar to that found in human breast milk showed learning deficits long after exposure had ceased. A few other generalizations can be made from the data in animals. It appears that *ortho*-substituted PCB congeners are more active than coplanar PCBs in modifying cognitive processes. In addition, one effect observed in both rats and monkeys—deficits on delayed spatial alternation—has been known to be induced by exposure to *ortho*-substituted PCBs, defined experimental mixtures, and commercial Aroclors. Both dioxin-like and non-dioxin-like PCB congeners have been shown to induce neurobehavioral alterations in animals. Changes in levels of neurotransmitters in various brain areas have also been observed in monkeys, rats, and mice. Of all the observed changes, the most consistent has been a decrease in dopamine content in basal ganglia and

prefrontal cortex, but further research is needed before specific neurobehavioral deficits can be correlated with PCB-induced changes in specific neurotransmitters in specific brain areas.

3.2.4.2 Human Studies

3.2.4.2.1 Neurobehavioral Effects

3.2.4.2.1.1 Contaminated Fish Consumption

The Michigan Cohort. Indices of neurological development were evaluated in 313 newborn infants (Fein et al. 1984a, 1984b). Of these infants, 242 were born to mothers who had consumed moderate to large quantities of Lake Michigan fish sometime during their lives, and 71 were born to mothers who did not consume Lake Michigan fish. In the exposed group, mean fish consumption, estimated by recall and duration of consumption, was 6.7 kg/year and 15.9 years, respectively; this rate is equivalent to 2 or 3 salmon or lake trout/month (Fein et al. 1984a, 1984b). Consumption during pregnancy was 4.1 kg/year. The mean PCB level in maternal serum among those eating Lake Michigan fish was 6.1 ppb (SD=3.7), while the mean among those reporting no fish consumption was 4.1 ppb (SD=2.7). The mean PCB residues also were significantly higher in breast milk samples from the fisheaters as compared to the nonfisheaters, 865.6 ppb (fat basis) versus 622.2 ppb (Fein et al. 1984a). No relationship was found between cord serum PCB levels and maternal fish consumption possibly because of detection problems in cord serum analysis. A list of 68 potential confounders was collected from the maternal interview and medical record. The list contained data pertaining to demographic background, reproductive health history including pregnancy and delivery, anesthesia during delivery, and exposure to other substances such as caffeine, nicotine, and alcohol (Fein et al. 1984a, 1984b). Because many of these mothers had been exposed to polybrominated biphenyls (PBBs), cord serum PBB level also was used as a control variable. Potential confounders were included only if the frequency in each category exceeded 15%. Consequently, data on approximately 37 potential confounders were available for inclusion in the study analyses (Fein et al. 1984a, 1984b).

Gestational age was evaluated by both the Ballard Examination for Fetal Maturity and the mother's report of her last menstrual period. The Ballard Examination was administered at 30 to 42 hours after birth to 209 of 313 (67%) infants with maternal permission granted during the limited time frame available for assessment. The Ballard estimate is based on an assessment of the newborn's neuromuscular and physical maturity. The Neonatal Behavioral Assessment Scale (NBAS) was administered to 284 of 313 (91%)

newborns on day 3 after birth. Three other infants also were tested sometime after their third day of life. In order to enhance the reliability of measures, the 44 NBAS items were reduced to seven summary clusters; response decrement, orientation, tonicity, range of state, regulation of state, autonomic maturity, and reflexes. These reduced clusters were derived by synthesizing the results of factor analyses from studies of six independent samples (Fein et al. 1984a; Jacobson et al. 1984a).

The results of the tests conducted on the newborns showed that decreased neuromuscular maturity, as measured on the Ballard Scale was significantly associated with consumption of contaminated fish (Fein et al. 1984b). However, when the non-fisheater and fisheater populations were divided according to cord serum level (<3 and $3 ppb, respectively), there was no significant difference in neuromuscular maturity outcome. The relationship between seven clusters from the NBAS and contaminated fish consumption was evaluated using linear regression with control for caffeine and alcohol consumption both before and during pregnancy. The potential confounders were chosen based on their statistical significance in prior correlation analyses. Infants of mothers eating contaminated fish were more likely to exhibit hypoactive reflexes, more motor immaturity, poorer lability of states, and a greater amount of startle (Jacobson et al. 1984a).

A follow-up of 39% (92 fisheating mothers, 31 controls) of the children in the Michigan Mother-Child study occurred at 7 months of age (Jacobson et al. 1985). Infants were administered Fagan's test of visual recognition to assess the effect of pre- or postnatal PCB exposure on fixation to familiar and novel stimuli. Cord serum PCB level was a better, but only moderate, predictor of poorer mean visual recognition memory than overall contaminated fish consumption. Recognition memory performance was not related to postnatal exposure from breast-feeding. According to the investigators (Jacobson et al. 1985), there was an inverse relationship between preference for novelty and PCB levels in cord serum (Fein et al. 1984a, 1984b). The investigators further indicated that visual recognition was unrelated to neonatal variables such as birth size, gestational age, and neurobehavioral performance.

Approximately 75% of the children were re-examined at age 4 (Jacobson et al. 1990a, 1990b). Neurobehavioral testing showed that prenatal exposure (maternal exposure before and during pregnancy), assessed by cord serum PCB levels was associated with poorer performance on both the Verbal and the Memory scales of the McCarthy Scales of Children’s Abilities. There was no indication of perceptual motor deficits or alterations of long-term memory. Activity level was inversely related to 4-year serum PCB level in a dose-dependent manner and also to maternal milk PCB level. Multivariate analysis of variance indicated that the effect of maternal milk was strongest in children of women with higher-than-

average milk PCB levels ($780 ppb) who breast-fed for at least 12 months. Correlations with fish consumption were not examined. Cognitive performance was unrelated to exposure from breast-feeding, which, according to the investigators (Jacobson et al. 1990a), suggested that the neurobehavioral deficits were due to fetal exposure. Jacobson et al. (1990a) indicated that the deficits found in these studies were not attributable to exposure to PBBs, lead, or seven other organochloride pesticides since these variables were controlled for.

A second evaluation of 226/313 children, 3 months after the McCarthy Scales assessment, was undertaken using adaptions of the Sternberg visual search and recognition memory test, the Kagan's Matching Familiar Figures Test, and the Streissguth vigilance paradigm (Jacobson et al. 1992). Regression analyses were performed with control for statistically selected potential confounders. The exposure variables employed were cord serum and maternal milk PCB levels as well as the duration of breast feeding. Less efficient visual discrimination processing and increased errors in short term memory scanning were associated with prenatal exposure to PCBs, but sustained attention was not. Cognitive performance was unrelated to postnatal exposure via breast milk (Jacobson et al. 1992).

A reanalysis of the assessment at 4 years of age was undertaken using the average of the standardized scores for cord serum, maternal serum, and milk PCB values. All values below the detection level (66.9% of cord and 22.5% maternal serum values) were discarded (Jacobson and Jacobson 1997). Results using this composite score as the exposure and the McCarthy Scales, height, and weight as outcomes were similar to those reported by Jacobson et al. (1990a, 1990b,1992). Potential confounders in these analyses were not delineated. Additional findings were reported using the composite score which indicated that the McCarthy Memory Scale and the General Cognitive Index declines were associated with prenatal PCB exposure only in the most highly exposed children.

An 11-year follow-up was undertaken to assess the relationship between prenatal exposure to PCBs and intellectual impairment. The outcomes studied were the Wechsler Intelligence Scales, the Wide Range Achievement tests, and the Woodcock Reading Mastery tests (Jacobson and Jacobson 1996a). The exposure variable consisted of a standardized average of the cord serum, maternal serum, and breast milk PCB values. These values were available for approximately 178/313 (57%) of the original group of children in the study. Linear regression modeling with confounder control, indicated that prenatal exposure to PCBs was significantly associated with lower full-scale and verbal IQ scores. On the academic achievement tests, prenatal exposure to PCBs was associated with poorer word comprehension and overall reading comprehension. Covariates included in all the models were SES, maternal education

and vocabulary, and the Home Observation for Measurement of the Environment (HOME) inventory. Additional confounders selected on the basis of their statistical relationship to the particular outcome were also included in several models. Mercury was included in two of the Woodcock Reading Mastery test models, while lead was not included in any of the multivariate analyses assessing PCBs and intellectual performance (Jacobson and Jacobson 1996a).

The associations of intellectual performance to lead and mercury were evaluated in separate multivariate linear regression models lacking terms for PCB exposure. Lower verbal IQ scores, lower verbal-comprehension scores, and poorer word, passage, and reading comprehension were significantly associated with higher lead levels at 4 years of age. Poorer spelling was significantly associated with a higher mercury concentration at 11 years of age (Jacobson and Jacobson 1996a).

The Oswego Cohort. A study similar to the one conducted in Michigan was initiated in Oswego County (New York) based on babies born between 1991 and 1994 (Lonky et al. 1996). Pregnant women were recruited from the office of one obstetric practice and, following interviews, were divided into three groups based on their estimated fish consumption. The high fish consumption group was composed of women who reported having eaten $40 PCB-equivalent pounds of Lake Ontario fish in their lifetime (n=152) (the same as Michigan's high fish consumption group). The low consumption group reported eating <40 PCB-equivalent pounds (n=243), while the no fish consumption group had never eaten Lake Ontario fish (n=164). The mean PCB-equivalent pounds consumed in the high fish consumption group was 388.47 (SD=859.0), while the mean among those in the low fish group was 10.14 (SD=17.8). The exposure in the high fish consumption group corresponds to a mean of 2.3 salmon or trout meals per month (belly fat trimmed and skin fat removed). The three groups did not differ with regard to demographic, health and nutritional data, maternal substance use, and infant birth characteristics. The high fish consuming group had a significantly heavier pre-pregnancy weight than the nonfisheating group.

The end points evaluated in the study were based on the NBAS. The NBAS behavioral and reflex items were reduced to seven clusters nearly identical to the clusters used in the Michigan Mother-Child pairs study (Jacobson et al. 1984a). The NBAS was administered twice to each infant, once at 12–24 hours and again at 25–48 hours after birth. A total of 58 potential confounding variables were submitted to principal components analysis. Three sets of analyses were performed; the first set contained demographic, nutrition, and stress variables while the second was composed of substances consumed during pregnancy, chronic medical conditions, and other toxic exposures including the type of plumbing

in the woman's home (lead). The third group of variables included labor and delivery complications as well as birth characteristics. A total of 24 components were derived from these three sets of variables.

Statistical analyses were performed using the change scores from the NBAS evaluation (Time 2–Time 1). Multivariate analysis of covariance (MANCOVA) was performed for each of the NBAS clusters with group membership (high, low, and no fish consumption) as the independent variable and the 24 components representing potential confounders as covariates. Approximately 75% of each fish consumption group was included in the analysis (n=416). The loss of subjects occurred because only subjects with data for all variables were included. Multiple regression was also performed for each of the NBAS clusters with inclusion of component covariates for confounder control.

The results of the MANCOVA analyses indicated that newborns exposed to high concentrations of fish demonstrated a greater number of abnormal reflexes and less mature autonomic responses than newborns in the other two exposure groups. Change scores for the Habituation cluster were analyzed in a separate analysis of covariance due to the large number of subjects with missing data (n=285 in the analysis). For that cluster, infants in the high fish group showed a worsening performance from Time 1 to Time 2. The regression analyses showed that infants in the high fish group had a significantly smaller decrease in the number of abnormal reflex scores from Time 1 to Time 2 than the low and no fish groups. In the Autonomic cluster, the high fish group demonstrated a significant worsening in performance between the first and second testing. Performance for the remaining clusters was not significantly associated with fish consumption in the regression analyses. In this study, birth weight, head circumference, and gestational age were unrelated to fish consumption. The differences in the birth weight and head circumference findings of the Michigan and Oswego studies could be due to the differences in PCB exposure levels.

In a later publication, the Oswego group of investigators examined the validity of using fish consumption as a surrogate for PCB exposure (Stewart et al. 1999). The study included 279 women with complete fish consumption histories, PCB cord blood levels, and demographic and covariate information. The sample included 145 women who reported never having consumed Lake Ontario fish (controls) and 134 who reported consuming at least 40 PCB-equivalent pounds over their lifetime (high fish consumption as defined earlier).

Total PCB levels were divided into three PCB homologue clusters representing the lower, middle, and upper tail of the distribution of all PCB homologues. The lower tail corresponded to lightly chlorinated PCB homologues with one to three chlorines per biphenyl (C1 1–3); the middle, to moderately

chlorinated PCBs with four to six chlorines per biphenyl (C1 4–6); and the upper tail, to heavily chlorinated PCB homologues with seven to nine chlorines per biphenyl (C1 7–9). In a previously conducted study in rats fed Lake Ontario salmon, the highly chlorinated PCB homologues accounted for a greater proportion of the PCBs detected (mole percent) when the fish was fed longer or the absolute concentration of PCBs was higher (Stewart et al. 2000a). The authors predicted the same type of results from the validity study using human cord blood. The average concentration of total PCBs in human cord blood was extremely low, 0.8 ppb among high fisheaters and 1.03 ppb among nonfisheaters (controls) ($p=0.36$). The relative percent (mole percent) of low- and medium-chlorinated congener PCB clusters from cord blood were similar for fisheaters and controls across each level of total PCB. The mole percent of highly chlorinated congeners was significantly greater in the cord blood of women who ate Lake Ontario fish as compared to the controls who reported no fish consumption ($p=0.006$). The difference between fisheaters and controls increased significantly ($p=0.02$) as the total PCB concentration increased.

Eighty-three women in the study also provided breast milk samples within 6 months of the birth of their child. The C17–C19 homologues in breast milk and cord blood were moderately correlated (Pearson's $r=0.29$; $p<0.05$), while no correlation was found for the light- and moderately-chlorinated homologues. Actual values of PCBs in milk were not provided.

Based on their findings, the authors concluded that maternal consumption of Great Lakes fish increases the risk of prenatal exposure to the most heavily chlorinated PCB homologues.

A subset of women from the Lonky et al. (1996) study also had cord blood samples collected for total PCB and congener distribution pattern analysis (Stewart et al. 1999). The study group was comprised of mothers who had consumed Lake Ontario fish (n=141) and those who had not (n=152). Each cord blood sample was analyzed for the presence of 69 PCB congeners and several coeluters (e.g., hexachloro-benzene [HCB], mirex, DDE). Exposure was divided into four groups based upon the distribution of heavily chlorinated PCBs (Cl 7–9) in each sample. The exposure variable was an ordinal level measure with the following categories: nondetectable (n=173); bottom 33rd percentile of detectable (n=39); middle 33rd percentile (n=40); and upper 33rd percentile (n=40). The actual lipid-adjusted PCB levels represented by these tertiles among those with detectable PCB levels were: >0–23.2 ng/g fat; 23.3–132.7 ng/g fat; and $132.7 ng/g fat. The heavily chlorinated PCB congeners were used as the measure of PCB exposure since the validity study results support the position that these congeners are the most valid index of fish-borne PCB exposure from Lake Ontario (Stewart et al. 1999, 2000a).

The end points evaluated were based on the seven clusters of the modified NBAS as evaluated by Lonky et al. (1996). The modified NBAS was also used in the Michigan Mother-Child Study (Fein et al. 1984b; Jacobson et al. 1984a). Potential confounders included in the models were selected if preliminary analyses using one-way analysis of variance or linear regression of each covariate in relation to exposure resulted in a p value of <0.20. Those meeting the p<0.20 criterion included: education, SES score, HOME score, maternal prepregnancy weight and weight gain, child gender, birth weight and head circumference, cigarettes/day, and caffeine consumption. Unlike the analyses employing fish consumption as the exposure, the outcomes (i.e., NBAS performance clusters at Time 1 [12–24 hours] and Time 2 [25–48 hours after birth]) were analyzed separately rather than as a change score (Time 2–Time 1) (Stewart et al. 2000b). No associations were noted between PCB cord levels and the NBAS clusters at Time 1. These findings are similar to those described using fish consumption as the exposure variable (Lonky et al. 1996). In looking at Time 2 NBAS cluster indices, significant linear trends were observed between poorer Habituation and poorer Autonomic scores and exposure to heavily chlorinated PCBs. The suggestion of a trend for abnormal reflexes with PCB exposure was observed, but the p value was 0.10 (Stewart et al. 2000b). Linear trend analysis also revealed a significant association between the proportion of poor NBAS clusters and heavily chlorinated PCBs. None of the NBAS performance scores were associated with non-PCB contaminants (i.e., HCB, DDE, lead, mercury, mirex) in linear regression modeling (Stewart et al. 2000b).

Lake Michigan Aging Population Study. This study was designed to assess the neuropsychological functioning of a group of 50–90-year-old fisheaters exposed to PCBs through Great Lakes fish consumption compared to a group of age- and sex-matched nonfisheaters (Schantz et al. 1996a, 1999). Fisheaters were defined as those who regularly consumed one or more meals of Lake Michigan sportsfish/week (>24 pounds/year); nonfisheaters consumed <6 pounds/year. Four classes of control variables were evaluated: a comprehensive list of demographic, life-style, psychological, and health-related variables. Fisheaters and nonfisheaters had very similar demographic characteristics, reported similar patterns of smoking and alcohol consumption, and had comparable scores on measures of intellectual functioning and affect (Schantz et al. 1996a).

The final analysis was conducted on 101 fisheaters and 78 nonfisheaters. Blood samples of the participants were analyzed for PCBs and 10 other contaminants included PBBs, DDE, HCB, oxychlordane, dieldrin, mirex, mercury, and lead. Serum levels of PCBs and DDE were significantly elevated in the fisheaters (PCBs=16.0 ppb) relative to the age- and sex-matched nonfisheaters (PCBs=6.2 ppb), and also relative to the population at large. Lead and mercury were low in both groups,

but were slightly higher in the fisheaters. Because of the high correlation between serum PCBs and DDE, the effects of PCBs and DDE were assessed jointly using a single derived exposure variable categorized as low, intermediate, or high (Schantz et al. 1999). A great majority of the high exposure group were fisheaters and a large majority of the low exposure group were nonfisheaters. However, 15% of nonfisheaters had elevated PCB/DDE exposure and 15% of fisheaters had low PCB/DDE exposure. Based on this, Schantz et al. (1999) stressed the importance of quantitating contaminant levels rather than relying on fisheating status as a surrogate measure for exposure.

Each subject was tested on two fine-motor tasks, the Grooved Pegboard Test (GPT), which assesses visual-motor coordination, and the Static Motor Steadiness Test (SMST), which assesses hand steadiness. Each subject performed the task first with the dominant hand and then with the nondominant hand. The final multivariate model for GPT included age, gender, income, diabetes and use of angiotensin-converting enzyme (ACE) inhibitors, sympatholytic agents, and cardiac glycosides. PCB/DDE exposure was not a significant factor affecting the GPT score; age and gender were the strongest predictors of performance followed by sympatholytics and income. Performance on the SMST was not related to PCB/DDE exposure in initial unadjusted analyses and in the final model, scores on the SMST improved slightly as PCB/DDE exposure increased.

3.2.4.2.1.2 General Population Exposure

The North Carolina Breast Milk and Formula Project. The North Carolina Breast Milk and Formula Project (NCBMFP) is a cohort study designed to assess the relationship between exposure to prenatal and postnatal PCBs and growth and development in infants and children. The NCBMFP was initiated in 1978 and included a cohort of 931 children born between 1978 and 1982. Mothers planning to deliver at one of three participating institutions were recruited from hospital familiarization tours, Lamaze classes, and from both private and public prenatal clinics. No attempt was made to assemble a random sample of women (Rogan et al. 1986a, 1986b, 1987). The participants were administered a questionnaire while in the hospital following delivery. Maternal serum, cord blood, and placenta samples were collected as well as colostrum, breast milk, or formula. The first follow-up visit occurred at 6 weeks with subsequent evaluations at 3 and 6 months postpartum. Breast milk or formula was collected at each of these visits. A second maternal serum specimen also was collected at the 6-week assessment. Subsequent follow-up evaluations occurred at 12, 18, and 24 months, with yearly visits until the age of 5. The children were examined and a health history was taken at each exam. The mothers also were queried about weaning. Breast milk was collected until the mother ceased lactation (Rogan et al. 1987). All biological samples

and a 10% sample of formula specimens were analyzed for PCBs. Because most of the PCB levels from the cord blood and placenta were below the quantitation limits, these two samples were not used as measures of exposure. The median PCB maternal serum level at birth was 9.06 ppb. PCB levels in milk at birth averaged around 1.8 ppm (fat basis). In lactating women, the levels of PCBs in breast milk declined about 20% over six months and about 40% over 18 months (Rogan et al. 1986a).

The participating mothers were not a representative sample of the North Carolina population. Ninety percent of the participants were white, with an age range of 16–41 (median=27). The women were well educated with 53% having a college education. Occupations among the participants were listed as housewife for 16%, while 41% were professionals. Eighteen percent smoked and 40% drank alcohol at least once a week. Twenty-one percent reported eating sportfish at least once during pregnancy. Forty-three percent of the women were primiparous and most (88%) breast-fed their study child to some extent (Rogan et al. 1986a).

The assessment at birth comprised 912 children with at least partial neonatal information. The outcomes evaluated in the neonatal period included birth weight, head circumference, and the presence of jaundice as recorded in the medical record. The NBAS was also administered to the newborns by a trained staff member in the presence of the parents. Fifty-nine percent of the NBAS exams were conducted during the first week of life, 20% in the second, and 16% in the third. The seven cluster scores used in the Michigan Mother-Child Study (Jacobson et al. 1984a) were also employed in this project (Rogan et al. 1986b). The relationships of birth weight, head circumference, and the NBAS clusters to PCB levels were assessed by multiple regression. The covariates (potential confounders) included in the analyses of birth weight and head circumference were infant race, sex, mother's age, education, occupation, smoking, alcohol consumption, prior pregnancies, maternal weight, and center enrolling the participant. The analysis of head circumference also included the birth weight variable (Rogan et al. 1986b). The covariates included in the analyses of the NBAS clusters included mother's age, education, occupation, smoking, alcohol consumption, sportfish consumption, general anesthesia during delivery, infant race and sex, birth weight, presence of jaundice, number of hours since eating, one term for the center, and one for the examiner (Rogan et al. 1986b).

The multiple regression analyses found no associations between birth weight or head circumference and PCB level. For the NBAS assessment, only the cluster scores for tonicity and reflexes were significantly associated with PCB levels. The authors looked at the four scales that make up the tonicity cluster score and found that exposure to PCBs affected the general tone and activity scales; less muscle tone and

activity were associated with higher PCB levels, but only at the highest levels of PCBs. The reflex cluster score was also significantly affected by PCB exposure. When the abnormal reflex scores were separated into high and low, it became apparent that only hypo-reflexia, (not hyper-reflexia), was associated with PCB levels. Since the NBAS were carried out over the first 3 weeks of life rather than during the first 3 days of life (considered to be the best time for this exam according to several investigators), the authors also repeated the same analysis with the population restricted to those whose exams were conducted on day 3 or earlier. The effect of PCB levels on hyporeflexia remained significant while the effect on tonicity was unchanged in size but no longer statistically significant. The authors interpreted the lack of significance to the decrease in sample size rather than confounding due to the age the exam was administered (data were not shown, Rogan et al. 1986b).

The follow-up evaluations at both 6 and 12 months included the administration of the Bayley Scales of Infant Development (Gladen et al. 1988). This exam yields a mental development index (MDI) score and a psychomotor development index (PDI) score, both of which are scaled like a standard IQ test. There were 858 infants (92%) from the original cohort who participated in the study past the neonatal period. Of these, 788 had Bayley scores available at 6 months while 720 had 12-month scores (706 children had scores at each time period). The exposure variable representing prenatal exposure used in the analyses at 6 and 12 months was the estimated PCB levels in milk at birth. The exposure variable representing postnatal exposure used in these follow-up assessments was a combination of the concentration of PCB in breast milk fat and the duration of breast feeding. In addition, milk was assumed to average 2.5% fat over the entire lactation. The authors also assumed that children consumed 700 grams of milk daily, if mostly breast fed, and half of that amount until breast-feeding stopped (Rogan et al. 1987). Children who were not breast fed were counted as having no postnatal exposure (Gladen et al. 1988). Potential confounders included maternal age, race, education, occupation, smoking, alcohol consumption and the infant's sex, gestational age, birth weight, head circumference, jaundice, duration of breast feeding, number of older siblings, number of abnormal reflexes from the NBAS exam, age Bayley administered, and center or examiner.

Linear regression analyses indicated that the psychomotor index scores declined with increasing prenatal PCB exposure at both 6 and 12 months. At 6 months, the PDI was estimated to decrease 0.96 points for every increase of 1 ppm in PCBs. This would mean a drop of 2.6 points if a child moved from the 5th to the 95th percentile of PCB exposure. At 12 months, the drop was estimated at 1.34 points/ppm. Neither the 6-month nor the 12-month mental index scores were related to transplacental PCB exposure (Gladen

et al. 1988). Similar analyses were run to examine postnatal exposure in breast-fed children. Postnatal exposure to PCBs was not associated with the PDI or MDI scores at either time period.

The children also were evaluated by the Bayley Scales of Infant Development at 18 and 24 months. Scores were available for 676 (73%) children at 18 months and for 670 (72%) children at 24 months (Rogan and Gladen 1991). Linear regression modeling was used to assess the relationship between prenatal the Bayley scores and exposure to PCBs. Covariate adjustment included sex, race, age of exam, number of older siblings, maternal age, education, and occupational grouping. Maternal smoking, alcohol consumption, and a term for the examiner were also included. The effects of prenatal PCB exposure on the PDI score at 18 and 24 months were similar to those seen at 6 and 12 months; however, neither were significant. Scores at the age of 18 months, declined 0.38 for every increase of 1 ppm in PCBs. The decline at 24 months of age was 1.16 points for every 1 ppm increase in transplacental PCB exposure. As the score pattern was not linear, the authors also conducted an analysis of variance in which the transplacental exposures were broken into categories. Each category of PCB exposure was then compared to the lowest with adjustment for covariates. At 18 and 24 months, adjusted scores on the psychomotor scales were 4–9 points lower among children in the two highest exposure groups (top 5th percentile of prenatal PCB exposure), significantly so at 24 months ($p < 0.05$). There was no evidence of an effect through postnatal PCB exposure in breast milk. An additional report in this series found that the deficits observed in children through 2 years of age were no longer apparent at ages 3, 4, and 5 years as determined by evaluation with the McCarthy Scales of Children's Abilities (Gladen and Rogan 1991). Finally, evaluation of third and higher grade children showed no significant relationship between the child's work habit or conduct grades and PCB exposure either prenatally or through breast milk, or between hyperactivity reported by parents and exposure (Rogan and Gladen 1992).

The Dutch Mother-Child Study. The Dutch Mother-Child Study was designed as a prospective study to assess the possible adverse health effects of prenatal and postnatal PCB and dioxin exposure. The initial study group consisted of 489 healthy mother-infant pairs recruited between June 1990 and June 1992 during the last month of pregnancy by their obstetrician or midwife (Koopman-Esseboom et al. 1994b). The entry criteria included first or second-born term infants (37–42 weeks gestation) without serious illnesses or complications during pregnancy and delivery. All participants were caucasian (Huisman et al. 1995a). Among the volunteers, 50% of the mothers were planning to breast feed for at least 6 weeks (for postnatal exposure assessment), while the other 50% were planning to use formula from a well characterized batch. This was part of the study design in order to compare breast-fed infants with bottle-fed infants. Seventy-one mother-infant pairs were lost because of the inability to breast feed for 6 weeks

leaving 418 pairs in the study population. Two hundred seven pairs (105 breast-fed and 102 formula-fed) were from Rotterdam, a highly industrialized area, while 211 pairs (104 breast-fed and 107 formula-fed) were from Groningen, a semi-urban area in northern Holland (Koopman-Esseboom et al. 1994b).

The exposure variables used in this study were maternal serum and milk samples as well as cord blood specimens. Maternal serum was collected during the last month (weeks 36–40) of pregnancy while milk samples were collected at 2 and 6 weeks post delivery. Data on the duration of breast feeding in weeks were also collected (Koopman-Esseboom et al. 1996). PCB levels in maternal serum and cord blood were assumed to be a direct measure of prenatal PCB exposure while the breast milk values in the second week after delivery were assumed to reflect the extent of intrauterine and neonatal exposure during the first 2 weeks after birth (Huisman et al. 1995a).

The focus of the authors was to investigate if one of the more easily measurable PCB congener levels could predict the PCB and dioxin exposure of the developing fetus and breast-fed infant. In order to express the potency of the mixture of dioxins and dioxin-like PCBs in breast milk, the authors used the toxic equivalency factor (TEF) approach (Huisman et al. 1995a; Koopman-Esseboom et al. 1994b; Patandin et al. 1999). As discussed in Section 3.5.2, the TEF approach compares the relative potency of individual congeners with that of 2,3,7,8-TCDD, such that the TEF for 2,3,7,8-TCDD is 1. TEQs were calculated by multiplying the concentration of each congener by it's TEF. These values were then multiplied by the number of weeks of breast feeding reported by the mother to obtain a measure of postnatal PCB exposure (Patandin et al. 1998). In breast milk, of the total TEQ value, dioxins contributed 46%, coplanar PCBs 24%, mono *ortho*-substituted PCBs 23%, and di-*ortho*-substituted PCBs 7%.

Because dioxin measurements are time-consuming, expensive, and require large volumes of blood, the authors chose four nonplanar PCB congeners (PCB 118, 138, 153, and 180) as indicators of PCB and dioxin exposure of the developing fetus and breast–fed infant (Koopman-Esseboom et al. 1994b). Although the correlation coefficients between these congeners and congener levels in maternal plasma and PCB levels in cord plasma or PCB and dioxin levels in human milk were highly significant, the 95% predictive interval was too wide to accurately predict the PCB and dioxin levels to which an individual infant is exposed *in utero* or postnatally by breast feeding, from the PCB levels in maternal plasma (Koopman-Esseboom et al. 1994b). The sum of these four congeners in maternal plasma and cord plasma amounted to 2.21 and 0.45 ppb, respectively. A total of 26 PCB congeners, including the four mentioned above, were measured in breast milk and the total PCB concentration in milk was approximately 620 ppb (fat basis). The sum of PCB 118, 138, 153, and 180 in milk totalled 430 ppb.

Data from the obstetrical optimality list were collected in this study as potential confounders and covariates of interest. The list included 72 items that measure SES (demographic, educational, and occupational variables) and pre-, intra-, and immediate postpartum conditions (Huisman et al. 1995a). Other potential confounders were maternal smoking and alcohol consumption (Koopman-Esseboom et al. 1996). The obstetrical optimality score was calculated by counting the number of items that fulfilled preset criteria for optimality. Data on the 5th, 50th, and 95th percentiles of the PCB distributions have been presented in these reports for the biological samples. The PCB levels were logarithmically transformed (natural logarithm). Comparisons of participant levels from Groningen to Rotterdam were made using the chi-square and Wilcoxen rank sum test. Both univariate and logistic regression analyses were conducted to evaluate the relationship between exposure variables and outcomes while controlling for covariates.

Several outcomes were evaluated in the newborn period. The neonatal neurological examination was administered to evaluate age appropriate neurological behavior. Sixty-three percent of the newborns were examined in the second week of life, 31% in the third week, and 6% in the fourth week of life. The examination used in this study included performance on a 10 item reflex cluster and an 11 item postural tone cluster. The scores for each item (0=low, 1=intermediate, and 2=high) were summed for each cluster. A score of #9 was considered to reflect low muscle tone on the postural cluster and a score of #10 was classified as low responsiveness on the reflex score. A neurological optimality score (NOS) was also calculated using a 60 item scale. The NOS score was dichotomized at the median of the pooled population scores (<57=not optimal, $57=optimal) (Huisman et al. 1995a).

Logistic regression analyses with NOS as the dependent variable and maternal serum or cord blood as the measure of prenatal exposure, were conducted with adjustment for maternal age, study center, alcohol, and the interaction of age and alcohol. Models for each of the four nonplanar PCB congeners (118, 138, 153, 180) alone and the sum of the four resulted in odds ratios (ORs) around 1.0 (no association). An OR of greater than 1.0 indicates an increased risk in the exposed group (see Chapter 10, Glossary). The prenatal exposure variables (PCB levels in maternal and cord plasma) were not associated with either the reflex or postural cluster scores. Another logistic regression analysis, with the postural tone cluster as the dependent variable, and adjusting for study center, showed a significantly higher percentage of hypotonia with an increase in planar PCB TEQ in milk. No effect on the reflex cluster was found.

At 18 months of age, the neurological condition of the infants was assessed using an age-specific neurological examination which focuses on the observation of motor functions (grasping, sitting,

crawling, standing, and walking) in a standardized free field situation (Huisman et al. 1995b). Based on this examination, each infant was classified as normal, mildly abnormal, or abnormal. The neurological findings were also evaluated in terms of optimality. Huisman et al. (1995b) also state that special attention was given to the quality of movements in terms of fluency since fluency of motility has been shown to be an indicator for the integrity of brain function in fetuses and prematures. The effect of PCB and dioxin exposure was investigated by a multiple linear regression analysis in which the dependent variables were the neurological optimality score and the fluency cluster score at 18 months. After adjusting for covariates, the results showed that prenatal PCB exposure had a small negative effect on the neurological condition of 18-month-old infants whose fathers did not smoke; no such effect was observed in children of fathers who smoked. Neurological condition was unrelated to exposure to PCBs and dioxins via breast milk.

To assess the mental and psychomotor development of infants exposed to PCBs both pre- and postnatally, the Dutch standardized version of the Bayley Scales of Infant Development were administered at 3, 7, and 18 months of age. Both the MDI and the PDI were included in the assessments. The tests were performed at the infant's home in the presence of the parent(s) (Koopman-Esseboom et al. 1996). The evaluations of the infants using the Bayley Scales of Infant Development were undertaken only for the 207 children from Rotterdam. Rotterdam is an urban area thought to have higher exposures to PCBs than Groningen, a semi-urban area in northern Holland.

Multiple regression analysis assessing the effects of prenatal PCB exposure on the psychomotor scale revealed that prenatal exposure to PCBs was significantly associated with a decrease in the PDI score at 3 months of age. A doubling of the PCB-plasma-sum resulted in a decrease in the psychomotor score of three points. The covariates included in the model were gestational age, parity, the HOME inventory score, education of the mother, and duration of breast feeding. At both 7 and 18 months of age, there was no significant effect of prenatal PCB exposure on the PDI scores (Koopman-Esseboom et al. 1996). Decreased PDI scores at 7 months among infants who were breast fed for longer periods and had higher TEQ scores were associated with postnatal total TEQ (PCB plus dioxin) exposure. The PDI score at 18 months was not associated with postnatal PCB-dioxin exposure. The MDI scores were not significantly associated with either prenatal or postnatal PCB exposure. Higher MDI scores at 7 months of age was positively associated with breast feeding *per se*. Finally, neither the psychomotor nor the mental development scales (at any age) were associated with an exposure variable created with the PCB-milk-sum multiplied by the duration of breast feeding.

At the age of 42 months, follow-up evaluations included both neurological and cognitive outcomes (Lanting et al. 1998c; Patandin et al. 1999). The neurological evaluation was comprised of an age-appropriate clinical exam, which focused on the observation of motor functions (i.e., prehension, sitting, crawling, standing, and walking) as well as the calculation of age appropriate NOS. A movement fluency score also was tabulated (not described).

A preliminary analysis summarizing the plasma PCB levels among children in the Rotterdam group at 42 months (n=173), found that median plasma levels were 3.6 times higher in breast-fed children (0.75 µg/L) than in their formula-fed peers (0.21 µg/L). Breast feeding period and breast milk PCB levels were important predictors of plasma levels in breast-fed children at 42 months, while plasma levels in formula-fed children were significantly related to maternal serum levels during the last month of pregnancy (Patandin et al. 1997). These results were obtained using multivariate linear modeling. The neurological assessment included 394 mother-infant pairs (94% of total participants) from both Rotterdam and Groningen. The clinical exam yielded a diagnosis of "neurologically normal" in 97% of the children.

Linear regression analyses using the NOS as the dependent variable and either maternal PCB-cord sum, maternal PCB-serum sum, or the child's PCB level at 42 months as the exposure found no associations between this outcome and any of these exposure variables. (Each exposure variable was modeled separately). Potential confounders in each model included the study center, the type of feeding during early life, the duration of breast feeding, and several items from the obstetrical optimality score (i.e., SES, obstetrical and perinatal conditions) (Lanting et al. 1998c). A similar model with PCB breast milk levels (TEQ method) as a measure of postnatal PCB exposure with NOS as the outcome, also found no association between the dependent and independent variables. In the last set of four models, fluency score, the dependent variable, was not found to be significantly associated with any of the four exposure variables.

Cognitive abilities also were evaluated at 42 months using the Kaufman Assessment Battery for Children (KABC), an 11 sub-test exam standardized for a large sample of preschool children in the 2.5–4.5 year old range (Patandin et al. 1999). The KABC is constructed to assess two types of mental functioning, sequential problem solving, and simultaneous problem solving. Both the Rotterdam and the Groningen children were administered this test battery. The Rotterdam children also were evaluated for verbal comprehension using the Dutch version of the Reynell Developmental Language Scales (RDLS). Logistical difficulties was stated as the reason for the omission of the Groningen children from this

evaluation. The exposure variables included the two measures of prenatal exposure (maternal serum and cord blood congener sums), postnatal exposure via breast milk (TEQ method), and current PCB body burden based on the serum sample levels in the children at 42 months of age.

The effects of prenatal, postnatal, and current body burden of PCBs on the cognitive outcomes were studied using multivariate linear regression. Potential confounders were chosen based on previous research, clinical expertise relative to the developmental outcomes, and beta coefficient changes observed when adding new variables to the linear regression model. Covariates included in the final regression models were: maternal age at the child's birth; parity; gender; feeding type; duration of breast feeding; HOME score; paternal and maternal educational levels; parental verbal IQ scores; smoking and alcohol use during pregnancy; and study center. Analyses were conducted for the entire group, for breast-fed children, and for formula-fed children.

In the group as a whole, a significant decline ($p<0.05$) in scores on the KABC for the overall scale, the sequential processing scale and the simultaneous processing scale were observed in adjusted regression models with maternal serum PCB levels as the independent variable. When the groups were divided into breast-fed and formula-fed, only formula-fed children showed a significant association between declines in the scores for the same three KABC scales, as well as the RDLS verbal comprehension scale, and maternal serum PCB levels.

Adjusted regression analyses conducted with a categorized version of maternal plasma PCB levels found the mean overall score on the KABC to be four points lower in the group with the highest PCB exposure ($3 ppb) as compared to children in the lowest (#1.5 ppb). Four point deficits in both the simultaneous and sequential scales also were calculated for the highest exposure group as compared to the lowest. Six to eight point deficits were observed for the formula-fed group on the KABC scales while a nonsignificant decline of two points was observed in the breast-fed group. Cognitive performance at 42 months was not related to either lactational exposure or current exposure to PCBs and dioxins.

European Background PCB Study - German Sample. This multicenter European study was designed as a prospective study to assess developmental outcomes associated with prenatal exposure to PCBs in Germany, the Netherlands, and Denmark (Winneke et al. 1998b). This study is very similar in design to the Dutch Mother-Child Study. The German cohort included 171 mother-infant pairs consecutively recruited from the obstetrical wards of three hospitals in Dusseldorf. All infants were term, from German speaking families, with an Apgar score of $7, first or second children, with no serious illnesses or

complications during pregnancy and delivery. Exposure to PCBs was based on the sum of PCB congeners 138, 153, and 180 in cord blood (0.55 ppb). A second measure of PCB exposure in milk was obtained from samples collected at 2 and 4 weeks of age and analyzed for these same three PCB congeners (427 ppb, fat basis). From 171 mother-infant pairs, 169 cord blood and 131 breast milk samples were obtained.

Outcomes measured at 7 months of age included the Bayley Scales of Infant Development (BSID) and the Fagan Test of Infant Intelligence (FTII). The Bayley Scales are comprised of the MDI, the PDI, and the Behavior Rating Scale; only the MDI and the PDI were used in this study. The MDI and PDI were used in the North Carolina Breast Milk and Formula Project (Gladen et al. 1988) and in the Dutch Mother-Child Study (Koopman-Esseboom et al. 1996). A test by Fagan also was used in the Lake Michigan Mother-Child Study (Jacobson et al. 1985). A test of the reliability of the mobile test version of the FTII in the Dusseldorf cohort for 2 observers and 10 children was close to zero (lack of reliability). Confounder selection procedures included a combination of *a priori* selection and statistical significance with the outcome. Linear regression modeling was used to assess the effects of PCB exposure on outcome with adjustment for other covariates.

After adjusting for confounders, there was a significant inverse association between MDI scores and PCBs in milk. There was no association between MDI, PDI, or FTII scores and blood PCBs.

3.2.4.2.1.3 Occupational Exposure

Reports of neurological effects in workers exposed to PCBs are limited. Approximately 49% of workers (64 males, 94 females) exposed to 0.07–11 mg/m^3 mean area concentrations of various Aroclors (early exposure to Aroclors 1242 and 1254; recent exposure to Aroclors 1016 and 1221) at a capacitor manufacturing plant for more than 5 years complained of headache, dizziness, depression, fatigue, memory loss, sleeplessness, somnolence, and nervousness (Fischbein et al. 1979). The prevalence of these symptoms was not compared to a control group. Routine neurological examination did not reveal any remarkable prevalence of abnormalities; extensor weakness was observed in six individuals (1.8%), whereas only one worker presented tremor at physical examination. No further relevant information was provided in this study. In a study by Smith et al. (1982) of three groups of workers occupationally exposed to Aroclors 1242, 1016, 1254, and/or 1260 significant positive correlations of symptoms suggestive of altered peripheral sensation were noted with increasing concentration of serum PCBs. However, there was no overt clinical dysfunction identifiable on physical examinations. Geometric mean

serum levels of up to 500 ppb of low-chlorinated PCBs (#4 chlorines/molecule) and up to 44 ppb of high-chlorinated PCBs were reported for workers in some jobs. Frequent headaches, sleeping difficulties, and memory problems were reported in switchgear workers exposed to Aroclors 1260 and 1242 (0.00001–0.012 mg/m^3) compared to unexposed workers (Emmett et al. 1988a). The geometric mean serum PCBs in the exposed group was 9.7 ppb, and was significantly higher than in the comparison group, 4.6 ppb. Emmett et al. (1988a) stated that the reported symptoms were probably not related to PCBs because they were not consistent with toxic effects ascribed to PCBs in the published literature.

3.2.4.2.1.4 Accidental Exposure

Children from Chinese women accidentally exposed to PCBs and other related chemicals through consumption of contaminated rice (*Yu-Cheng* incident) have been evaluated for cognitive development. Evaluations were conducted when the children were 4–7 years old (Stanford-Binet test and Wechsler Intelligence Scale) and were compared to controls matched for neighborhood, age, sex, mother's age, parent's combined educational level, and parent's occupation. The results showed that at each age, and for each scale other than the WISC-R at the age of 6 years, there was a consistent 5-point difference between the *Yu-Cheng* children and the control children (Chen et al. 1992). Results of the evaluation of the behavior and activity level of these children were published by Chen et al. (1994). Emotional or behavioral disorders were evaluated with the Rutter's Child Behavior Scale A and activity level with a modified Werry-Weiss-Peters Activity Scale. At each year, *Yu-Cheng* children scored 7–43% worse (more disorders) than control children in the Rutter scale. At any fixed age, *Yu-Cheng* children scored 11–63% worse than control children. Furthermore, there was no consistent trend toward decreased differences in scores of *Yu-Cheng* and control children as the interval between the exposure and year of birth increased. Similar results were observed for the activity scores, although the differences between *Yu-Cheng* children and controls were less marked (*Yu-Cheng* children had increased activity levels). The authors also found that children with physical signs had a higher mean score in the Rutter's and activity scores at some age and a lower score at others. There were no consistent relationships between either Rutter or activity scores and cognitive scores of PCB detectability, maternal serum PCB levels, or breast-feeding mode. *Yu-Cheng* children also scored significantly lower than controls in MDI and PDI tests between the ages of 6 months and 2 years (Lai et al. 1994) and in Raven's Colored Progressive Matrices and at ages 6, 7, or 9, and in Standardized Progressive Matrices at age 9 (Guo et al. 1995).

3.2.4.2.2 Neurophysiological Effects

Various neurological symptoms, including numbness, weakness and neuralgia of limbs, hypesthesia, and headaches, are common in *Yusho* and *Yu-Cheng* victims (Chia and Chu 1984, 1985; Kuratsune 1989; Rogan 1989). It is important to mention, however, that the findings from the studies of these groups cannot be attributed solely to exposure to PCBs since the victims also were exposed to CDFs and other chlorinated chemicals (ATSDR 1994). Conduction velocities were reduced in sensory nerves (radial and/or sural) in 9 of 23 *Yusho* patients examined soon after poisoning (Kuroiwa et al. 1969). Sensory fibers may have been preferentially affected as conduction velocities in motor nerves (ulnar and tibial) were reduced in only two cases and motor functions were normal. Follow-up studies were not performed on the *Yusho* patients, but disappearance of related symptoms and signs indicated that the effects on nerve conduction did not persist. Reduced sensory and motor nerve conduction velocities also occurred in *Yu-Cheng* patients (Chen et al. 1985; Chia and Chu 1984, 1985). Evaluation of 110 patients within 1 year of *Yu-Cheng* exposure showed significantly reduced sensory nerve (median and ulnar) and motor nerve (tibial and peroneal) conduction velocities in . 44 and 22% of the patients, respectively (Chen et al. 1985). All of the subjects had developed eye and skin manifestations of toxicity, but there were no significant correlations between nerve conduction values and blood levels of PCBs, CDFs, or PCQs. Electroencephalographic examination of *Yu-Cheng* patients did not show any abnormalities potentially indicative of central nervous system damage (Chia and Chu 1984, 1985). Additional information on the *Yusho* and *Yu-Cheng* poisoning episodes can be found in the toxicological profile for chlorodibenzofurans (ATSDR 1994).

3.2.4.2.3 Evaluation of Human Studies

Several studies are available that evaluated the relationship between prenatal PCB exposure (and postnatal exposure in some instances) and neurobehavioral parameters in infants and children. These studies are the Michigan Mother-Child Study (Fein et al. 1984a, 1984b; Jacobson and Jacobson 1996a, 1997; Jacobson et al. 1984a, 1985, 1990a, 1990b, 1992), the Oswego Newborn and Infant Development Project (Lonky et al. 1996, Stewart et al. 1999, 2000a), the North Carolina Breast Milk and Formula Project (Gladen et al. 1988; Rogan and Gladen 1991, 1992; Rogan et al. 1986a, 1986b, 1987), the Dutch Mother-Child study (Huisman et al. 1995a, 1995b; Koopman-Esseboom et al. 1994b, 1996; Lanting et al. 1998c; Patandin et al. 1999; Weisglas-Kuperus et al. 1995), and the German Study (Winneke et al. 1998b). A comparison of PCB levels in blood and breast milk in some of these studies is included in Appendix A. Related information is also available from the *Yu-Cheng* accidental poisoning incident in

Taiwan (Chen et al. 1992, 1994; Guo et al. 1995; Lai et al. 1994). Data from adults exposed to PCBs are available from studies by Schantz et al. (1996a, 1996b, 1999) and from evaluations of victims from the Taiwan poisoning episode (Chen et al. 1985; Chia and Chu 1984, 1985).

The association between consumption of Great Lakes contaminated sportfish (i.e., PCB exposure) and neurodevelopmental alterations in children has been examined in the Michigan series of studies and in the Oswego series. Despite concerns about the design and analysis of the data from the Michigan Mother-Child Study (Expert Panel 1994; Paneth 1991; Schantz 1996; Seegal 1996a, 1996b), many of the findings of Jacobson and colleagues in the Michigan cohort have been replicated in studies of other cohorts. Jacobson et al. (1984a) found that newborn children exposed to PCBs from mothers who ate PCB-contaminated sportfish were more likely to exhibit hypoactive reflexes, more motor immaturity, poorer lability of states, and greater amount of startle. In the Oswego study, the high PCB exposure group was defined as those who consumed a mean of 2.3 salmon or lake trout meals/month, as done in the Michigan study, and children born to mothers from this group demonstrated a greater number of abnormal reflexes and less mature autonomic responses than those born to low PCB-exposed or non-exposed mothers (low-fisheaters or nonfisheaters) (Lonky et al. 1996). However, Lonky et al. (1996) found no significant association between fish consumption and birth weight, head circumference, and gestational length, as Fein et al. (1984a) had found in the Michigan cohort. Taking advantage of improved analytical techniques available at the time of the study, researchers from the Oswego study observed a significant linear trend between poorer Habituation and Autonomic scores and heavily chlorinated PCBs (Stewart et al. 2000b). The suggestion of a trend for abnormal reflexes with PCB exposure was observed, but the finding was not statistically significant. Linear trend analysis also revealed a significant association between the proportion of poor NBAS clusters and heavily chlorinated PCBs. No significant association was seen for the lightly and moderately chlorinated PCBs, DDE, lead, HCB, and mercury. It is worth noting that in the Oswego cohort, the average concentration of total PCBs in cord blood was 0.8 ppb among high fisheaters and 1.03 among nonfisheaters, such that no association could have been found had total PCBs in cord blood been used as surrogate for exposure.

Neonatal evaluations also were conducted in the North Carolina study (Rogan et al. 1986b). This is a study of women from the general population with no known high exposure to PCBs. PCBs in milk at the time of birth (approximately 1.8 ppm, but may have been overestimated by a factor of 2) was used as indicator of prenatal exposure; the median PCBs in maternal serum at birth was 9.06 ppb. In the Michigan cohort, the mean concentration of PCBs in maternal milk and serum in high fisheaters were approximately 0.9 ppm and 6.1 ppb, respectively. The NCBMFP also found that less muscle tone and

activity were associated with higher PCB levels. The reflex cluster score was also significantly affected by PCB exposure. When the abnormal reflexes were separated into high and low, it became apparent that hypo-reflexia, not hyper-reflexia, was associated with PCB levels.

Evaluations of various cohorts at later ages provide the opportunity to compare results of similar tests conducted at similar ages. For example, the BSID has been administered to infants in the North Carolina cohort, the Dutch children, and the German study. This group of tests yields a MDI and a PDI score, both of which are scales like a standard IQ test. In the North Carolina cohort, a significant decrease in PDI scores at the ages of 6 and 12 months was associated with prenatal exposure to PCBs (assessed by PCBs in maternal milk at birth, 1.8 ppm) (Gladen et al. 1988), although the association lost statistical significance at the ages of 18 and 24 months (Rogan and Gladen 1991). No significant association was observed between PDI scores at 6–24 months of age and postnatal exposure to PCBs (PCBs in milk factored by duration of breast feeding). There was no significant association between MDI scores and either prenatal or postnatal exposure to PCBs. The latter is consistent with a lack of significant association between MDI scores at 7 or 18 months of age and prenatal or postnatal exposure, also observed in the Dutch children (Koopman-Esseboom et al. 1996). *Yu-Cheng* children also had lower PDI and MDI scores when tested between the ages of 6 months and 2 years old (Lai et al. 1994).

Both the Dutch and the German studies assessed prenatal and postnatal exposure by measuring the concentration of a limited number of PCB congeners in cord blood and in breast milk. In the Dutch study, the researchers measured PCBs 118, 138, 153, and 180 in cord blood and the sum of the mean concentration of these congeners was 0.45 ppb (Koopman-Esseboom et al. 1994b). The German study measured PCBs 138, 153, and 180 in cord blood and the sum amounted to 0.55 ppb (Winneke et al. 1998b). The added concentration of these four PCB congeners in breast milk in the Dutch study was 430 ppb, whereas in the German study, the concentration of PCBs 138, 153, and 180 in breast milk was essentially the same at 427 ppb. Both studies evaluated MDI and PDI scores at 7 months of age. In the Dutch study, at this age, neither PDI nor MDI scores were significantly associated with prenatal exposure to PCBs; however, lower PDI scores, but not MDI scores were significantly associated with postnatal exposure. In the German study, also no significant association was found between MDI or PDI scores and prenatal exposure to PCBs, but in contrast with findings from the Dutch children, lower MDI scores, but not PDI scores, was significantly associated with postnatal exposure to PCBs. Thus, it would appear that practically the same exposure assessments in the two studies (including the concentration of marker PCBs) and tests conducted at the same age provided apparently opposite results. This may indicate that more thorough analyses are necessary especially given the importance of ‘minor’ congeners in animal

studies. Also, the results from the German cohort on background PCB exposure in Europeans appear preliminary with few details described.

Evaluations at the age of 4 years have been done on the Michigan (Jacobson and Jacobson 1997; Jacobson et al. 1990a; 1992) and Dutch children (Lanting et al. 1998c; Patandin et al. 1999). Jacobson et al. (1990a) showed that poorer performance on both the Verbal and the Memory scales of the McCarthy Scales of Children's Abilities was associated with prenatal exposure to PCBs. Jacobson et al. (1992) also found that less efficient visual discrimination processing and more errors in short-term memory scanning, but not sustained attention, were associated with prenatal exposure to PCBs. Dutch children evaluated at 42 months of age on neurological optimality scores showed no decrement on performance as a result of exposure to PCBs either prenatally or postnatally (Lanting et al. 1998c). Cognitive abilities evaluated in these children using the KABC showed a significant decreased performance associated with prenatal exposure to PCBs (Patanding et al. 1999). Since the tests from the Patandin et al. (1999) and Jacobson et al. (1990a) are both designed to provide a measure of general intelligence, the data are comparable and the effects observed in the two studies on these end points are consistent.

Evaluation of the children from the Michigan cohort at 11 years of age showed that lower full-scale and verbal IQ scores and poorer reading word comprehension were significantly associated with prenatal exposure to PCBs (Jacobson and Jacobson 1996a). The mean maternal serum PCB concentration among fisheaters in the Michigan study was 6.1 ppb (Fein et al. 1984a). Decreased IQ was also observed among *Yu-Cheng* children (Chen et al. 1992), but exposure levels in this group were significantly higher than in the Michigan cohort and there was also significant exposure to CDFs, dioxins, and other related chemicals.

Evaluation of an adult population (50–90-years old) on a visual-motor coordination test and a hand steadiness test revealed no significant effect from exposure to PCB/DDE through long-term consumption of Lake Michigan fish (>24 pounds/year) (Schantz et al. 1999). Results from cognitive assessment of this cohort have not yet become available. Workers exposed to PCBs have reported adverse neurological symptoms, but routine examination of these workers did not reveal any clinical dysfunction (Emmett et al. 1988a; Fischbein et al. 1979; Smith et al. 1982). There is no indication that cognitive function or fine motor behavior was evaluated in any way in the workers. Neurological examination of *Yusho* and *Yu-Cheng* victims showed reduced both motor and sensory nerve conduction velocities (Chen et al. 1985; Chia and Chu 1984, 1985; Kuroiwa et al. 1969), but due to the mixed chemical nature of the rice oil exposure, the results cannot be attributed specifically to PCBs.

In summary, there is mounting evidence that PCBs can be important contributors to subtle neurodevelopmental alterations in neonates and infants of women who consume high-amounts of sport-caught fish from the Great Lakes and also of women in the general population with the highest PCB body burdens. Most of the studies summarized above have quantitative exposure estimates, associations between the outcome and exposure which are likely free from confounding, and minimal biases, making their findings more convincing. Data from *Yu-Cheng* can only be considered supportive due to the known exposure to other chlorinated aromatic hydrocarbons. Data from the Dutch Mother-Child Study strongly suggest that exposure to dioxins in general, not solely PCBs, may be related to altered developmental effects in neonates and children. The Oswego validity study to assess fish consumption as a measure of PCBs, as well as the reanalysis of the Oswego neurodevelopmental outcomes using cord blood C17–C19 PCB homologue levels as measure of exposure makes this series of studies important in establishing biomarkers of exposure for quantitative risk assessment. Unlike the North Carolina study, the outcomes of the Oswego study are limited to NBAS within the first 2 days of life, although it is expected that these children will be the subject of follow-up evaluations for years to come.

3.2.4.3 Animal Studies

The highest NOAEL values and all reliable LOAEL values for neurological effects for each study are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.4.3.1 Neurobehavioral Effects

Oral Exposure

Neurobehavioral effects of PCBs have been examined in several species exposed for various durations. Experiments have been conducted with commercial mixtures, defined experimental mixtures, contaminated fish, and single congeners.

Commercial PCB Mixtures. Spontaneous motor activity was significantly decreased in male CD_1 mice (unspecified age) 15 minutes to 3 hours following a single gavage dose of 500 mg Aroclor 1254/kg (Rosin and Martin 1981). However, dosing with 30 or 100 mg/kg for 14 days had no significant effect on this end point. Assessment or motor coordination with two tests (screen test and rotor rod) did not reveal any significant effect 15–120 minutes after treatment with a single dose of 500 mg Aroclor 1254/kg. Similar results regarding a decrease in spontaneous motor activity were reported in adult male Long-

Evans rats following single gavage doses of $300 mg Aroclor 1254/kg; no significant effect was seen with 100 mg/kg (Nishida et al. 1997). Incomplete recovery to pretreatment levels of activity occurred over a 9-week period. Repeated administration of Aroclor 1254 resulted in dose-related decreases in activity at doses $30 mg/kg/day, the NOEL was 10 mg/kg/day. In this case, complete recovery of activity occurred 3 weeks after exposure. Kodavanti et al. (1998) also reported decreased motor activity in adult male rats treated with 30 mg Aroclor 1254/kg/day for 4 weeks. Nishida et al. (1997) also conducted flavor aversion conditioning tests and reported that the acute and repeated NOELs for this behavioral test were 15 and 7.5 mg/kg, respectively.

Freeman et al. (2000; General Electric Co. 1995a, 1995b) conducted a 52-week feeding study in rats with various Aroclor mixtures (1016, 1242, 1254, 1260) and found no significant treatment-related effects on a comprehensive number of neurological end points. PCB intakes ranged from 1.3 to 14.1 mg/kg/day depending on the Aroclor mixture. The functional observational battery assessed autonomic function, muscle tone and equilibrium, sensorimotor function, and central nervous system function. Motor activity tests and histopathological examination of the central and peripheral nervous system were also performed.

Open field activity on PND 14 was significantly suppressed in offspring from rats treated by gavage with 2 mg/kg/day Fenclor 42 (a non-Aroclor PCB, similar in composition to Aroclor 1242) on PND days 1–21, but not in offspring from rats treated with 2–4 mg/kg/day on gestation days 6–15 (Pantaleoni et al. 1988). Neurobehavioral alterations including impaired swimming behavior and acquisition of one-way avoidance response were also observed in the pups exposed *in utero* and also following postnatal exposure.

Neurobehavioral alterations were also reported in the offspring of rats treated with 2.4 mg/kg/day Clophen A30 (technical mixture with 42% chlorine) premating and during gestation (Lilienthal et al. 1990). Offspring, which continued on the PCB diet after weaning, were tested for open field activity on PND 22 and 120, active avoidance learning on PND 65–75, and operant conditioning on a fixed interval on PND 380. Spontaneous activity was increased on PND 22, but not on PND 120. Avoidance responses and intertrial responses were increased, as were the responses in the operant conditioning test. No significant behavioral alterations were seen in rats treated with about 0.4 mg Clophen 42/kg/day. In a subsequent cross-fostering experiment, Lilienthal and Winneke (1991) reported that exposure *in utero* resulted in alterations in active avoidance learning and retention of a visual discrimination task similar to those seen in rats exposed *in utero* plus through mother's milk, whereas postnatal-only exposure caused no detectable behavioral changes. Lilienthal and Winneke (1991) also observed that brain levels of

higher chlorinated congeners peaked at weaning in groups with postnatal or postnatal plus prenatal exposure, whereas the concentration of a low-chlorinated congeners was lower at weaning than at birth or at later ages. This suggests that transfer of low-chlorinated congeners may be more pronounced during gestation, while preferential transfer of high-chlorinated PCBs occurs via the milk.

Overmann et al. (1987) reported that pups from rats exposed in the diet to approximately 1.3 mg Aroclor 1254/kg/day during gestation and lactation were significantly slower than controls in the negative geotaxis test when tested on PND 7 and 8, but not on PND 5 or 6. Also, the appearance of auditory startle was slightly delayed on PND 12, but not PND 11, 13, or 14, in pups from dams treated with 0.13 and 1.3 mg/kg/day. The development of air righting ability also was slightly delayed at the 1.3 mg/kg/day dose level on PND 18, but not PND 16, 17, or 19.

Suggested evidence of memory impairment in a radial arm maze in rats resulting from perinatal exposure to Aroclor 1254 was presented by Corey et al. (1996). The pups, tested at age 42–54 days, were exposed *in utero* and via mothers' milk. The dams received 8 or 17.8 mg Aroclor 1254/kg/day during gestation and exposure continued until postpartum day 28. Rats exposed to PCBs made significantly more maze errors than control rats regardless of whether exposure of the pups ceased at weaning or had continued by direct feeding. In a more recent study, the same group of investigators reported that offspring from rats fed approximately 1 mg Aroclor 1254/kg/day during gestation and lactation and tested between 25 and 29 days of age performed significantly worse than controls in a Morris water maze during trials 8, 9, and 10 (Provost et al. 1999). No differences were seen in earlier trials, and interestingly, during the first four trials, control rats performed much worse than treated rats.

Neurobehavioral studies also have been conducted in monkeys born to exposed mothers. In a study by Bowman et al. (1978), the assessment was conducted in three offspring of mothers fed a diet providing approximately 0.1 mg/kg/day Aroclor 1248 for 16–21 months; PCB feeding terminated at the end of 3 months of nursing. All monkeys were tested on a sequence of 11 tasks between the age of 6 and 24 months; four untreated monkeys served as controls. Relative to controls, exposed monkeys showed hyperlocomotor activity at 6 and 12 months of age, which correlated with peak PCB body burdens; they also showed decreased performance in five out of nine discriminating learning tasks. According to the investigators (Bowman et al. 1978), the learning deficits appeared to represent residual toxicity, since they could be observed after almost total clearance of PCBs from the body. The same monkeys tested at 44 months of age appeared to exhibit hypoactive behavior relative to controls (Bowman and Heironimus 1981).

Neurobehavioral deficits reflected as impaired performance on a spatial learning and memory task were seen in the progeny of monkeys fed 0.08 mg/kg/day Aroclor 1248 for 18 months and allowed to breed 32 months postexposure (Levin et al. 1988). The deficit did not appear to be due to memory impairment, but rather to impairment in associational or attentional processes. Aroclor 1016, tested at a dose level of 0.008 mg/kg/day, did not significantly alter performance on that task (Levin et al. 1988), but impaired the monkeys' ability to learn a simple spatial discrimination problem at 0.03 mg/kg/day (Schantz et al. 1989). These long-term studies in monkeys showed that doses of $0.03 mg/kg/day of some PCBs can alter performance in neurobehavioral tests.

Defined Experimental Mixtures. Rice and Hayward (1997) studied the effects on learning in monkeys of postnatal exposure to a PCB mixture representative of the PCBs typically found in human breast milk. Eight male monkeys were dosed from birth to 20 weeks of age with 0.0075 mg/kg/day of PCBs. Five monkeys served as controls. At 20 weeks of age, the levels of PCBs in fat and blood in treated monkeys were 1.7–3.6 ppm and 2–3 ppb, respectively; corresponding values for controls were 0.05–0.2 ppm and 0.30–0.37 ppb. Beginning at 3 years of age, the monkeys were tested on a series of nonspatial discrimination reversal problems followed by a spatial delayed alternation task. Treated monkeys showed decreased median response latencies and variable increases in mean response latencies across three tasks of nonspatial discrimination reversal. There was no difference in overall accuracy of the tests. There was no correlation between performance and tissue levels of PCBs. Treated monkeys also displayed retarded acquisition of a delayed alternation task and increased errors at short delay task responses. These finding were interpreted as a learning/performance decrement rather than an effect on memory *per se.* In a separate portion of this study (Rice 1997), treated monkeys displayed shorter mean interresponse times when compared with controls. The increase in pause time for fixed-interval performance emerged more slowly across the 48 sessions in treated monkeys. For fixed-ratio performance tasks, the control monkeys decreased their mean pause time across 10 sessions, whereas the treated monkeys did not. Rice (1997) interpreted these results as suggesting learning deficit, perseveration, and/or inability to inhibit inappropriate responding as a result of postnatal PCB exposure. Testing of these monkeys at 4.5–5 years of age showed that treated animals performed in a less efficient manner than controls under a differential reinforcement of low rate (DRL) schedule of reinforcement (Rice 1998). There were no differences between groups on the accuracy of performance on a series of spatial discrimination reversal tasks, although some treated monkeys made more errors than others on certain parts of the experiment. Further tests conducted at about 5 years of age did not find treatment-related effects on a series of concurrent RI-RI (random interval) schedules of reinforcement (Rice and Hayward 1999a). This schedule was designed to study behavior in transition (learning) as well as at steady state. However, there was a

difference between treated and control monkeys on performance on a progressive ratio (PR) schedule. Rice and Hayward (1999a) stated that the interpretation of this finding is not straightforward, but may be indicative of retarded acquisition of the steady-state PR performance in treated monkeys.

Contaminated Fish Consumption. The neurobehavioral effects of exposure of rats to feed adulterated with 5 or 20% lyophilized salmon fillets from Lake Huron (LH) or Lake Ontario (LO) were examined by Pappas et al. (1998). The study was conducted in F_1- and F_2-generation 88-day-old male and female rats that had been exposed *in utero*, during lactation, and postnatally until they were tested. Exposure to the contaminated diet caused no observable effects on many behavioral parameters including activity, exploration, sensorimotor function, and stereotypy. Also, there was no diet-induced impairment of spatial learning or long-term memory, and no evidence of an exaggerated response to food reward reduction. The only significant effect found was decreased performance of the F_1 LO-20 and F_2 LH-20 rats in the reference/working memory version of the radial arm maze. Stewart et al. (2000a) examined the effects of feeding adult male rats a 30% diet of salmon from Lake Ontario, Pacific Ocean salmon, or a laboratory chow on performance on a multiple FR-PR reinforcement schedule. Rats were fed for 65 days. Lake Ontario diet contained 739 ppb PCBs, whereas the Pacific Ocean diet and the lab diet contained 45 and 64 ppb PCBs, respectively. Also, the average chlorines per biphenyl for the Lake, Ocean, and lab diets were 5.65, 4.58, and 4.54, respectively. Analysis of PCB homologues in the brain of rats showed that rats fed Lake fish had significantly higher concentrations of homologues with 6, 7, 8, or 9 chlorines per biphenyl than the other groups. These rats' brains also contained DDE and mirex, which were not detected in the Ocean or lab diet groups. Behavioral test results showed that Lake rats responded normally during FR schedules, but quit significantly sooner than control rats on a PR-5 schedule, when response costs were demanding. None of the response rates were significantly related to contaminants.

Single Congeners. Administration of 32 mg 3,3',4,4'-tetraCB/kg (PCB 77) to pregnant CD-1 mice on gestation days 10–16 resulted in motor and behavioral alterations in the offspring tested at 35 and/or 65 days of age (Tilson et al. 1979). Some, but not all, exposed mice exhibited a neurobehavioral syndrome consisting of intermittent stereotypic circling activity (PCB-spinners). Relative to controls and to PCB-nonspinners, PCB-spinners were hyperactive during the dark phase of the diurnal phase, showed decreased muscular strength and impaired ability to traverse a wire rod, altered visual placement responding, and impaired acquisition of one-way avoidance. However, reflex activity and orientation to environmental stimuli were not affected by exposure to PCB 77. No PCB-derived radioactivity could be detected in $28-day-old mice born to dams administered radioactive PCB 77 on gestation days 10–16. Microscopical examination of tissues from PCB-spinners (up to 8 months old) showed cylindrical

peninsulas of central nervous system parenchyma in the cranial and spinal nerve roots as well as alterations in synapses of the nucleus accumbens (Chou et al. 1979). Increased motor activity in PCB-spinners was still evident at 1 year of age (Agrawal et al. 1981). Altered spontaneous motor activity was seen in 4-month-old male NMRI mice given a single gavage dose of 0.41 mg PCB 77/kg at 10 days of age (Eriksson et al. 1991). When tested during three 20-minute blocks, treated mice were hypoactive during the first block, but hyperactive during the third block relative to controls. Similar results were obtained with PCBs 28, 52, and 126, but no such effect was seen with PCBs 118, 156, or 105 (Eriksson and Fredricksson 1996a, 1996b, 1998). There was also a marginal effect of PCB 52 (4.1 mg/kg) on learning and memory assessed by performance on a swim-maze and a radial-arm maze and of PCB 126 (0.46 mg/kg) also on the swim-maze.

Several PCB congeners have been evaluated for neurobehavioral alterations in rats. The most widely studied appears to be PCB 126, a dioxin-like congener, with an estimated dioxin-like toxicity potency 1/10 that of 2,3,7,8-TCDD. Barnhoft (1994) found reduced onset of spontaneous movements and delayed neuromuscular maturation in pups from Lewis rats administered six gavage doses of 10 or 20 µg PCB 126/kg on gestation days 9–19; evaluations were conducted during the first 4 weeks of life. Spontaneous activity level was not affected by treatment. Tests for visual discrimination learning (age 5–18 weeks) did not reveal any significant differences in performance between PCB-treated rats and controls. However, in a later paper, the same group of investigators reported that administration of six doses of 2 µg PCB 126/kg on gestation days 10–20 resulted in both poorer visual discrimination and hyperactivity in treated rats relative to controls; similar, but less marked effects were seen with 2,3',4,4',5-pentaCB (PCB 118) (Holene et al. 1995). In two more recent studies, it was reported that male pups from dams treated with PCB 153 from day 3 to 13 after delivery were hyperactive and had impaired performance in a visual discrimination test (Holene et al. 1998), but female pups showed no significant differences compared with controls (Holene et al. 1999).

Rice and coworkers conducted a series of behavioral studies in offspring from Long-Evans rats dosed with 0, 0.25, or 1 µg PCB 126/kg/day beginning 5 weeks before and continuing through gestation and lactation (Bushnell and Rice 1999; Rice 1999a; Rice and Hayward 1998, 1999b). Exposure to PCB 126 did not significantly alter performance of male or female pups on a multiple fixed interval fixed-ratio schedule of reinforcement or on a DRL schedule at about 200 days of age (Rice and Hayward 1998). Exposure to PCB 126 did produce developmental toxicity as evidenced by reduced birth weight, reduced serum thyroxine, and changes in hematology and serum biochemistry parameters. Assessment of performance on a spatial delayed alternation task also revealed no significant differences between treated

and control rats (Rice 1999a, 1999b); no PCB was detected in pups brain at age 60 days. Further tests conducted in males to assess visuospatial attention and sustained attention showed no significant treatment-related deficits (Bushnell and Rice 1999). Finally, male and female pups were tested under a series of three concurrent RI-RI schedules of reinforcement at about 400 days of age followed by assessment under a PR schedule (Rice and Hayward 1999b). Although there was some indication of less accurate performance in high-dose rats in the RI-RI schedule, there was no difference between treated and control rats in the PR performance.

Schantz and colleagues assessed learning and memory in an 8-arm radial maze and in a T-maze in male and female offspring from Sprague-Dawley rats treated with PCB congeners on gestation days 10–16 (Schantz et al. 1995, 1996b, 1997). Testing started at 60 days of age. The first study tested the *ortho*-substituted congeners PCBs 28, 118, and 153 (Schantz et al. 1995). No treatment-related effects were seen on a memory task on a radial maze test, but female offspring were less accurate than controls on a T-maze spatial delayed alternation task; PCB 118 caused the smallest deficit of the three congeners. Subsequent studies examined 2,3,7,8-TCDD, the dioxin-like congeners PCB 77 and PCB 126, and PCB 95 and found that performance on the radial arm maze task was facilitated by treatment with each of the four compounds, but mostly by 2,3,7,8-TCDD (Schantz et al. 1996b, 1997). No significant group differences were seen on the T-maze test. An additional observation was that exposure to PCB 95 did not alter spontaneous activity in rats tested as juveniles (35 days old), but induced hypoactivity in tests conducted in adulthood (100 days old). In these rats exposed to PCB 95, Schantz et al. (1997) also observed decreased density of ryanodine receptor binding proteins in the hippocampus, increased binding in the cerebral cortex, and a biphasic response in the cerebellum.

Other Routes of Exposure. Altmann et al. (1995) examined the effect of 3,3',4,4'-tetraCB (PCB 77) on long-term potentiation (LTP), a measure of neuronal functional plasticity, in rats. Pregnant Wistar rats were treated with daily subcutaneous injections of 1 mg/kg of PCB 77 on gestation days 7–18. At the age of 180–220 days, offspring were sacrificed and slices were prepared from the visual cortex and hippocampus. Treatment with PCB 77 resulted in inhibition of LTP in visual cortex slices, but not in the hippocampus. A follow-up study reported the same result in slices from 11–19-day-old pups (Altmann et al. 1998). In the latter study, PCB 47 was also tested and was much less effective than PCB 77. Using the same exposure protocol, the effects of these two congeners also were assessed on locomotor activity in the open field, spatial learning in the radial arm maze, catalepsy induced by the dopamine receptor blocker haloperidol, and passive avoidance learning at PND 25, 95, 180, and 220, respectively (Weinand-Harer et al. 1997). Of all of these end points, exposure to PCB 77 altered the haloperidol-induced

catalepsy and impaired performance in passive avoidance behavior; PCB 47 produced changes in the same direction, but the differences relative to controls did not achieve statistical significance.

3.2.4.3.2 Neurochemical Effects

Neurochemical effects of PCBs have been examined in rats, mice, and monkeys exposed to commercial PCB mixtures and to individual PCB congeners. Some studies have assessed both neurochemical and neurobehavioral effects of PCBs in an attempt to link a biochemical alteration to a particular neurobehavioral deficit.

Oral Exposure

Commercial PCB Mixtures. Administration of single, high doses (500 and 1,000 mg/kg) of a mixture of Aroclor 1254 and 1260 to adult male rats reduced serotonin levels in the frontal cortex and hippocampus, increased serotonin in the lateral olfactory tract, and had no effect in the hypothalamus and brainstem (Seegal et al. 1986a). A correlation between the direction of the changes (increase or decrease) and changes in PCB levels in the different areas could not be made. In a similar study, there was a dose-dependent decrease in the levels of dopamine in the caudate nucleus, but not in the lateral olfactory tract, of adult rats treated with 500 or 1,000 mg/kg of a mixture of Aroclor 1254 and 1260 (Seegal et al. 1986b). In a subsequent study, a dose of 1,000 mg/kg of Aroclor 1016 increased dopamine turnover in peripheral neurons of rats, whereas the same dose of an Aroclor 1254/1260 mixture increased dopamine turnover in central neurons (Seegal et al. 1988), suggesting that PCBs with different degrees of chlorination can alter dopaminergic functions in different locations of the nervous system. Treatment of adult male rats with a diet that provided approximately 0, 39, or 79 mg Aroclor 1254/kg/day for 30 days resulted in significant decreases in dopamine concentrations and metabolism in the striatum and the lateral olfactory tract, but not in other brain areas (Seegal et al. 1991a). Analysis of PCB congeners in striatum, lateral olfactory tract, and hippocampus showed that the hippocampus contained the highest PCB concentration and that the major PCB congeners were penta and hexabiphenyls mono- or di-*ortho*-substituted. In contrast to the finding of decreased dopamine concentration by Seegal et al. (1991a), Kodavanti et al. (1998) found no alterations in the concentrations of dopamine, norepinephrine, or serotonin in the striatum or cortex of adult male rats treated with up to 30 mg Aroclor 1254/kg/day for 4 weeks; in addition, tyrosine hydroxylase activity in striatal minces was not significantly altered by PCB treatment. However, treatment with Aroclor 1254 significantly reduced the Ca buffering capacity of microsomes and mitochondria in the cerebellum and of microsomes in the frontal cortex and striatum. Aroclor 1254 also

decreased total protein kinase C (PKC) activity and increased membrane-bound PKC activity in the cerebellum, but not in other brain areas. In this study, treated rats were hypoactive compared to controls.

Doses between 0.8 and 3.2 mg/kg/day Aroclor 1016 in the diet for 20 weeks did not alter the concentrations of noradrenaline, adrenaline, or serotonin in several areas of the brains of monkeys (Seegal et al. 1990). A similar exposure protocol with Aroclor 1016 or 1260 resulted in dose-dependent decreases in dopamine contents in several areas of the brain (Seegal et al. 1991b). Dopamine continued to be depressed 24 weeks after the exposure period ceased (Seegal et al. 1992, 1994); at this time, the concentration of PCBs had greatly decreased. After the 20-week exposure, only PCBs 28, 47, and 52 congeners were detected in the brains of the monkeys treated with Aroclor 1016, and mainly hexa- and hepta-chlorinated di-*ortho*-substituted congeners were detected in the brains of monkeys treated with Aroclor 1260. Seegal et al. (1991b) concluded that the neurochemical changes were caused by a mechanism different than that involved in other toxic responses to PCBs. Because the concentration of total PCBs was higher in the brains of monkeys treated with Aroclor 1260 than in those treated with Aroclor 1016, Seegal et al. (1991b) concluded that lightly-chlorinated congeners were more effective in reducing central dopamine levels than highly-chlorinated ones.

Exposure of rats *in utero* to 0, 5, or 25 mg Aroclor 1254/kg (gestation days [Gd] 10–16) resulted in a significant increase in levels of 5-hydroxyindole acetic acid (5-HIAA), and in the ratio of 5-HIAA/5-hydroxytryptamine in the lateral olfactory tract, prefrontal cortex, and hippocampus from 90-day-old offspring (Morse et al. 1996a). Dopamine, 3,4-dihydroxyphenylacetate, norepinephrine, and homovanillic acid were not affected. In a study of similar design, Morse et al. (1996b) observed significant increases in the glial cell marker GFAP in the lateral olfactory tract and the cerebellum and significant decreases in the brain stem of offspring at 21 and 90 days old. The neuronal marker synaptophysin was significantly decreased in the lateral olfatory tract, prefrontal cortex, and striatum of 90-day-old offspring. These changes were interpreted as reactive gliosis following direct damage to the neurones or glia, or alteration in the regulation of the proliferation or protein expression of specific subpopulations of neural cells.

Choline acetyltransferase (ChAT) activity was significantly decreased in the basal forebrain, but not in the hippocampus, from 15-day-old rats exposed to Aroclor 1254 (125 or 250 ppm in diet) during gestation and via mother's milk (Corey et al. 1996). However, ChAT activity returned toward control levels by 60 days of age. These rats made significantly more maze errors than control rats regardless of whether exposure to PCBs ceased at weaning or had continued by direct feeding. In a follow-up paper,

the authors reported that treatment with much smaller amounts of Aroclor 1254 (1.25 or 12.5 ppm in the diet) resulted in a significant increase in ChAT activity relative to controls in the hippocampus and forebrain from 15-day-old low-dose pups, but not high-dose pups (Provost et al. 1999). In 30-day-old pups, ChAT activity was significantly decreased in both the hippocampus and basal forebrain with the two dietary PCB levels. At this age, high-dose pups performed significantly worse than controls on a Morris water maze.

Contaminated Fish Consumption. The neurochemical effects of exposure of rats to feed adulterated with 5 or 20% lyophilized salmon fillets from Lake Huron (LH) or Lake Ontario (LO) were examined by Seegal et al. (1998). The study was conducted in 88-day-old male and female rats that had been exposed *in utero*, during lactation, and postnatally until they were tested. Dopamine, serotonin, norepinephrine, and their metabolites, as well as ChAT activity were assayed in the frontal cortex, nucleus accumbens, caudate nucleus, hippocampus, and substantia nigra. Significant treatment-related effects included (1) decreased dopamine in the frontal cortex of the high-dose rats, (2) decreased dopamine in the caudate nucleus from all groups, (3) decreased dopamine in the substantia nigra from the high-dose LO rats, (4) reduced epinephrine in all groups except for low-dose LO rats, and (5) no significant effect on ChAT concentration in any experimental group. No specific contaminants were assayed in the fish in this report.

Single Congeners. Mice exposed to 3,3',4,4'-tetraCB (PCB 77) *in utero* (maternal dose 32 mg/kg/day), which exhibited spinning behavior and hyperactivity at 1 year of age, had decreased dopamine levels and dopamine receptor binding sites in the corpus striatum (Agrawal et al. 1981). However, Seegal et al. (1997) reported that *in utero* and lactational exposure of rats to PCB 77 (0.1 or 1 mg/kg/day) resulted in significant and persistent elevations in dopamine concentrations in the frontal cortex and the substantia nigra, but not in the caudate nucleus. In contrast, similar treatment with PCB 47 (1–20 mg/kg/day) significantly decreased dopamine concentrations in the frontal cortex and caudate nucleus (Seegal et al. 1997). Administration of a single dose of 0.41 or 41 mg PCB 77/kg to 10-day-old NMRI mice resulted in a significant decrease (not dose-related) in density of muscarinic cholinergic receptors in the hippocampus 7 days after dosing, but not 24 hours after dosing relative to controls; there was no significant effect on receptor density in the cerebral cortex (Eriksson 1988). In mice similarly exposed but tested at the age of 4 months, muscarinic cholinergic receptor density was slightly but significantly increased (high-dose only) in the hippocampus; no significant changes were detected in the cortex, striatum, or midbrain and thalamus (Eriksson et al. 1991). As previously mentioned, these mice had abnormal spontaneous motor activity at that age. Treatment with PCB 28 and PCB 52 did not alter

muscarinic receptor density in the hippocampus, but PCB 52 increased the density of nicotinic cholinergic receptors in the cortex (Eriksson and Fredriksson 1996a, 1996b). Neither dopamine, serotonin, or metabolites were significantly altered in the striatum by treatment with PCB 28 or PCB 52. At this age (6 months old), the mice showed altered spontaneous activity. Neonatal exposure to 3,3',4,4',5-PentaCB (PCB 126) resulted in increased density of nicotinic cholinergic receptors in the hippocampus at 5 month of age and in impaired learning on a swim maze; no neurochemical or behavioral alterations were seen in mice treated with 2,3,3',4,4'-pentaCB (PCB 105) (Eriksson and Fredriksson 1998).

Treatment of female and male weanling Sprague-Dawley rats for 90 days with PCB 28 in the diet ($0.04 mg/kg/day) resulted in a significant decrease in dopamine concentration in the substantia nigra in females, but not in other brain areas (Chu et al. 1996a); neither norepinephrine, serotonin, or their metabolites were altered in any brain area. Other significant effects in similarly conducted experiments included decreased dopamine in the caudate nucleus and substantia nigra and decreased serotonin in the substantia nigra with PCB 118, $0.2 mg/kg/day in females, decreased dopamine in the caudate nucleus in males and increased serotonin in the substantia nigra in females with PCB 105, $4 mg/kg/day, decreased dopamine and serotonin in the frontal cortex with PCB 153, $0.01 mg/kg/day in males, and decreased dopamine in the frontal cortex with $0.005 mg/kg/day PCB 128 and in the hippocampus with 4.4 mg/kg/day PCB 128 in females; no significant changes were seen with PCB 126 (up to 0.009 mg/kg/day) (Chu et al. 1994, 1995, 1996b, 1998a, 1998b; Lecavalier et al. 1997).

3.2.4.3.3 Other Neurological Effects

Oral Exposure

Commercial PCB Mixtures. The effects of perinatal exposure to PCBs on auditory function has been studied in rats. Goldey et al. (1995) tested the hypothesis that hypothyroidism induced by developmental exposure to PCBs may cause permanent auditory dysfunction. Long-Evans rats were given 0, 1, 4, or 8 mg Aroclor 1254 from gestation day 6 through PND 21 and pups were evaluated at various ages up to 1 year old. Exposure to Aroclor significantly reduced circulating thyroxine concentrations up to PND 45. At PND 24, the high-dose pups showed reduced auditory startle amplitudes, but this was not seen when tested as adults. However, Aroclor induced permanent auditory deficits (20–30 dB threshold shift) at the frequency of 1 kHz in the mid- and high-dose rats. In a subsequent study, by monitoring brain stem auditory evoked responses, the authors concluded that the auditory alterations are consistent with peripheral auditory dysfunction (Herr et al. 1996). In a more recent study, Goldey et al. (1998) reported

that thyroxine replacement therapy significantly attenuated the effect of Aroclor 1254. It is important to note that high mortality occurred among the pups in the mid- and high-dose groups. By PND 12 and 21, 25 and 50% of the pups in the 8 mg/kg/day group had died, respectively. In the control and 4 mg/kg/day groups, 3 and 15% of the pups had died by PND 21, respectively.

Single Congeners. Offspring (76–90 days old) from rats that received 1 µg/kg/day of PCB 126 for 35 days prior to breeding and throughout gestation and lactation had elevated auditory thresholds for 0.5 and 1 kHz tones (Crofton and Rice 1999). There were no treatment-related effects in postnatal mortality or litter size.

Other Routes of Exposure

Single Congeners. Subcutaneous administration of 1.5 mg/kg of PCB 77 to pregnant Long-Evans rats on gestational days 7–18 resulted in altered electroretinogram (ERG) in female offspring recorded at about 200 days of age (Kremer et al. 1999). Specific alterations consisted of decreases in the scotopic b-wave as well as on the a-wave and maximum potential, the first two wavelets of the oscillatory potentials, and the flicker response at the beginning of light adaptation. No significant alterations were observed in male offspring or in male or female offspring from rats injected subcutaneously 1.5 mg/kg of PCB 47.

Numerous *in vitro* studies have been conducted with both commercial PCB mixtures and single congeners in efforts to elucidate the mechanisms of neurotoxicity of these compounds, to possibly discern patterns among structurally similar types of congeners, and to establish toxic potency rankings. These studies are discussed in Section 3.5.2 Mechanisms of Toxicity.

3.2.4.3.4 Evaluation of Animal Studies

Neurobehavioral Effects. For the purpose of this appraisal, the neurobehavioral effects of PCBs in animals are divided into (1) effects on motor activity and (2) effects on higher functions (e.g., learning and memory). Motor activity (spontaneous or open field) has been evaluated in mice, rats, and monkeys exposed to commercial mixtures and single PCB congeners in a variety of experimental designs leading, not unexpectedly, to a wide range of results from which few generalizations can be made. Single or repeated administration of relatively high doses of Aroclor 1254 to adult mice or rats generally decreased spontaneous motor activity (Kodavanti et al. 1998; Nishida et al. 1997; Rosin and Martin 1981). Postnatal (via breast milk) exposure to Fenclor 42 decreased open field activity in pups on PND 14, but

exposure *in utero* did not (Pantaleoni et al. 1988). Exposure to similar doses of Clophen A30 (congener composition similar to Fenclor 42) during gestation and lactation increased open field activity on PND 22 but not on PND 120 (Lilienthal et al. 1990). Exposure of monkeys to Aroclor 1248 during pregnancy and for 3 months after giving birth resulted in hyperlocomotor activity in the offspring at 6 and 12 months of age (Bowman et al. 1978), but these monkeys exhibited hypoactive behavior at the age of 44 months (Bowman and Heironimus 1981). A somewhat similar finding was reported by Schantz et al. (1997) in rats; in this case, offspring from Sprague-Dawley rats treated with the di-*ortho*-substituted PCB 95 on gestation days 10–16 had control levels of spontaneous activity at 35 days of age (juveniles), but were hypoactive as adults (100 days old). A series of studies by Eriksson and coworkers (Eriksson and Fredricksson 1996a, 1996b, 1998; Eriksson et al. 1991) observed altered spontaneous activity (increased followed by decrease in trials separated by 20 minutes) in 4–5-month-old mice administered a single gavage dose of a variety of PCB congeners at the age of 10 days. These effects were observed with both coplanar congeners (dioxin-like) PCB 77, PCB 126, and with the mono *ortho*-substituted congener PCB 28 and the di-*ortho*-substituted PCB 52. No effects were observed with three mono-*ortho*-substituted congeners (PCB 105, 118, or 156). No pattern is apparent from these findings.

The effects of PCBs on higher cognitive functions (i.e., learning, memory, attention) have been examined in rats and monkeys exposed mostly perinatally, but in some cases, testing was done long after exposure occurred. Exposure of rats to Fenclor 42 during gestation or via mother's milk impaired acquisition of one-way active avoidance (Pantaleoni et al. 1988). A different study reported decreased active avoidance learning and retention of a visual discrimination task in rats exposed during gestation to Clophen A30, but not in rats exposed postnatally (Lilienthal and Winnecke 1991). Rats exposed during gestation and lactation made more errors than controls in a radial arm maze (Corey et al. 1996) and performed worse than controls in a Morris water maze (Provost et al. 1999). Studies in monkeys exposed to Aroclors during gestation and lactation, and tested after exposure ceased, showed decreased performance in discriminating learning tasks (Bowman et al. 1978), impaired associational or attentional processes and ability to learn a simple discrimination problem, and failure to learn the irrelevancy of a shape cue (Levin et al. 1988; Schantz et al. 1989). Monkeys that were treated from birth to 20 weeks of age with a defined PCB mixture analogous to the congener composition of human breast milk (comprised mostly of mono- and di-*ortho*-substituted congeners), and tested beginning at age 3 years, had impaired performance in both nonspatial and spatial discrimination reversal tasks and exhibited inability to inhibit inappropriate responding (Rice 1997, 1998, 1999b; Rice and Hayward 1997, 1999a). Because these effects occurred at a level of 0.0075 mg/kg/day, the lowest tested intermediate-duration dose of any PCB mixture in any species, they are used as the basis for deriving the intermediate MRL for oral exposure as indicated in the

footnote to Table 3-2 and discussed in Chapter 2 and Appendix A. Perinatal exposure of rats to the dioxin-like PCB congener PCB 126 during gestation and lactation provided little evidence that it altered behavior (Bushnell and Rice 1999; Rice 1999a, 1999b; Rice and Hayward 1998, 1999a, 1999b). Of five tasks designed to assess a range of cognitive processes, only one provided any suggestive evidence of an effect. Other coplanar PCBs, as well as 2,3,7,8-TCDD, failed to alter the response of rats exposed *in utero* on a T maze test, but facilitated the response on a radial arm maze (Schantz et al. 1995, 1996b). In contrast, *ortho*-substituted congeners had no effect on performance on the radial maze test, but impaired performance of females on the T-maze test. From the data summarized above, few generalizations can be attempted. It appears that *ortho*-substituted PCB congeners are more active than coplanar PCBs in modifying cognitive processes. In addition, an effect observed in both rats and monkeys was a deficit on delayed spatial alternation, and was induced by exposure to *ortho*-substituted PCBs (Schantz et al. 1995), defined experimental mixtures (Rice and Hayward 1997), and commercial Aroclors (Levin et al. 1988).

Neurochemical Effects. The most consistent result from studies that examined the neurochemical effects of PCBs is a decrease in dopamine concentrations in different areas of the brain. This was seen in adult rats and monkeys administered relatively high doses of Aroclor mixtures (Seegal et al. 1986b, 1991a, 1991b) and in 90-day dietary studies that used relatively low doses of single PCB congeners (Chu et al. 1994, 1995, 1996a, 1996b, 1998a, 1998b). Less studies reported alterations in serotonin levels, and for the most part, levels of norepinephrine were unaffected. No single brain region appeared to be a preferred target. Studies with single congeners in rats reported decreases in dopamine levels in the frontal cortex, caudate nucleus, substantia nigra, and striatum. Studies with Aroclors in rats and monkeys observed decreases in dopamine in the caudate, striatum, substantia nigra, putamen, hypothalamus, and olfactory tract. The lowest effective doses in the 90-day single congeners studies were 0.01 and 0.005 mg/kg/day for PCB 153 and PCB 128, respectively, two di-*ortho*-substituted hexachlorobiphenyls. The dioxin-like PCB 126 was ineffective at the highest dose tested, 0.009 mg/kg/day. In contrast with the majority of the findings in adult animals, Seegal et al. (1997) reported an increase in dopamine concentration in the frontal cortex and substantia nigra from rats exposed to the coplanar PCB 77 *in utero* and via mother's milk. In the series of studies by Eriksson and colleagues (Eriksson and Fredricksson 1996a, 1996b, 1998; Eriksson et al. 1991), no significant alterations in biogenic amine levels were seen in brains from adult mice exposed to PCB 28 or PCB 52 at 10 days of age; these mice had altered spontaneous motor activity and those exposed to PCB 52 had impaired learning and memory functions. These investigators also described increases in density of cholinergic muscarinic and nicotinic receptors in certain brain areas in mice exposed to PCB 77, PCB 52, or PCB 126, but no such assays were conducted for PCB 118, PCB 105, or PCB 156. More information is necessary to speculate on patterns of effects among general

classes of PCB congeners or to try to associate specific behavioral alterations with neurochemical changes.

Other Neurological Effects. The findings of Crofton and Rice (1999) of auditory deficits in offspring from rats administered PCB 126 during gestation and lactation without evidence of general toxicity give credence to those of Goldey and coworkers (Goldey and Crofton 1998; Goldey et al. 1995; Herr et al. 1996). In the latter series, high mortality was observed among the exposed pups during the first 3 weeks of life suggesting that survivors may have been in less than optimal health conditions. Whether the effect seen with PCB 126 represents an Ah receptor-mediated effect remains unknown until additional both dioxin- and nondioxin-like PCB congeners are tested.

3.2.5 Reproductive Effects

3.2.5.1 Summary

Information is available on reproductive effects of PCBs in humans. Studies that examined reproductive end points found indications that exposure to PCBs is associated with menstrual disturbances in women and effects on male fertility. Increasing PCB levels have also been observed in women with late miscarriages. In addition, a reduction in the months of lifetime lactation was associated with increasing levels of PCBs in maternal breast milk. The reproductive toxicity of PCBs in animals has been well established. Effects in females have been observed in various species, including rats (prolonged estrus, decreased sexual receptivity, and reduced implantation rate in adults and/or their offpsring exposed via gestation and lactation), mice (decreased conception), minks (partial or total reproductive inhibition), and monkeys (prolonged menstruation, decreased fertility). Female minks and monkeys are particularly sensitive to reproductive effects of PCBs. There is limited evidence for reproductive effects in male adult animals, although it is well documented that gestational and lactational exposure to PCBs can adversely affect morphology and production of sperm and fertility in the male offspring of rats and mice.

3.2.5.2 Human Studies

3.2.5.2.1 Female Reproductive Effects

Occupational Exposure. There were no apparent effects on gravidity (number of pregnancies) in women capacitor manufacturing workers who were exposed to Aroclors 1254, 1242, and/or 1016 for a minimum of 3 months between 1946 and 1975 (Taylor et al. 1989). High-exposure workers were directly exposed to Aroclors during the manufacturing process for at least 1 year prior to birth of an infant, and workers with low exposure were employed in areas where Aroclors were not used directly. Area air samples collected in 1977 showed geometric mean air concentrations of 310 and 27 $\mu g/m^3$ in the high and low exposure groups, respectively. Evaluation of birth data on 172 high-exposure and 184 low-exposure workers showed no significant difference in the mean number of pregnancies (3.2±1.7 and 3.5±2).. As discussed in the Developmental Toxicity section (Section 3.2.6.2), decreased birth weights and gestational ages in the exposed women were associated with increased serum PCB levels. Other reproductive outcomes and well-designed reproductive epidemiologic studies have not been conducted in this highly exposed female occupational cohort.

Contaminated Fish Consumption.

The New York State Angler Cohort. The New York State (NYS) Angler Cohort is a population-based group of New York State anglers who were between 18 and 40 years of age and held fishing licenses for the 1990–1991 season. The cohort was compiled for the study of a variety of reproductive and other health end points (Mendola et al. 1995a, 1995b). Data from the entire cohort were collected from self-administered questionnaires mailed to anglers living in 16 counties in close proximity to Lake Ontario. Responses were received from 10,782 male anglers, 934 female anglers, and 6,579 wives/partners of male anglers for a total response rate, among the anglers, of about 40%. This cross-sectional survey included questions on sportfish consumption patterns (to estimate exposure to PCBs) and reproductive outcomes and associated data focusing on children born between June 1986 and June 1991. A telephone interview with 100 randomly selected nonrespondents revealed that nonresponders did not differ from respondents with respect to fishing habits, knowledge of fishing advisories, and fish consumption patterns, but had sociodemographic differences (were less likely to be married and had lower levels of education and income). Findings for reproductive or developmental end points from this study have been reported in several reports (Buck et al. 1997, 1999, 2000; Kostyniak et al. 1999; Mendola et al. 1995a, 1997).

Shorter menstrual cycles were associated with consumption of sportfish was associated with in women from the NYS Angler Cohort (Mendola et al. 1997). Menstrual cycle data were collected from 2,223 women (mean age 31.2 years) who stated in 1991 that they were considering becoming pregnant in the following 3 years. Lake Ontario fish consumption was measured in terms of exposure duration (total number of years eating fish) and monthly frequency of fish meals in 1991, and an index was developed to estimate cumulative lifetime PCB exposure through fish consumption (subjects were placed in no, low, and combined moderate/high exposure classes). Multiple regression analyses showed that consumption of more than one Lake Ontario fish meal/month was associated with statistically significant reductions in mean menstrual cycle length of >1 day in all 2,223 women (-1.11 days, 95% CI -1.87 to -0.35), and of about half a day for a subgroup of 2,080 women who reported having regular menstrual cycles (-0.51 day, 95% CI -0.92 to -0.10). Similar reductions were found in women in the highest cumulative exposure category (moderate/high); mean cycle length was about 1 day shorter for the main group (-1.03 days, 95% CI -1.88 to -0.19), and about half that reduction in the regular menstrual cycle group (-0.56 day, 95% CI -1.01 to -0.09). No significant differences in mean menstrual cycle length were found when the subjects were classified into groups based on the number of years during which fish were consumed. The strengths of the study include the use of trained nurses to obtain menstrual cycle information. Limitations include the reliance on self-reported exposure data (biological samples were not collected and analyzed for PCBs and other expected contaminants) and outcome data, and the lack of information on potential confounders such as current smoking status, stress, and the use of contraceptives. Mendola et al. (1997) noted that although the small decreases in menstrual cycle length are not likely to be clinically significant or of major public health concern, they may indicate potential endocrine effects on a population level.

No statistically significant association was found between time-to-pregnancy (TTP), a measure of fecundity and conception delay, and consumption of Lake Ontario sportfish in a preliminary analysis of 874 women from the NYS Angler Cohort who were pregnant between 1991 and 1993 (Buck et al. 1997). Exposure was estimated as the total number of years from 1955 to 1991 in which fish caught in Lake Ontario were consumed. The mean duration of fish consumption was 2.2 years (SD 4.5) for all women and 5.2 years (SD 5.6 years) for women who reported any fish consumption. No differences were observed in fish consumption between women who did and did not become pregnant, fish consumption between women with known and unknown TTP, or age distributions for women with and without a pregnancy. Multiple regression analysis was used to assess the linear relation between log-TTP and log-years eating fish (duration of exposure). Analyses were stratified by fish consumption (fish consumers/all women) and parity status (nulliparous/parous), and controlled for maternal age, smoking, gynecologic pathology (e.g., endometriosis), and history of sexually transmitted diseases. R^2 coefficients showed that

duration of fish consumption and maternal age accounted for only a small percentage of the explained variance in TTP (0.5%), even after the analysis was restricted to women who reported eating fish (0.6%). Beta coefficients, calculated to reflect the unit change in log-TTP for every unit change in log-years eating fish, were positive but not statistically significant. A larger beta coefficient was observed for log-years eating fish among nulliparous women (0.1030) in comparison to parous women (0.0095), suggesting that the effect of duration of fish consumption on TTP may have been greater among nulliparous women. Strengths of the study included the use of trained nurse interviews to obtain TTP information. As with other NYS Angler Cohort studies, limitations include the reliance on self-reported exposure data and outcome data, and the lack of information on potential confounding factors such as occupational exposures, alcohol and caffeine consumption, and current smoking status. In addition, women with unplanned pregnancies were necessarily ruled out from the analysis; Buck et al. (1997) noted that this may be a potential bias inherent in the study.

In further analysis of the Buck et al. (1997) study summarized above and the Buck et al. (1999) study summarized in Section 3.2.5.2.2 (Male Reproductive Effects), Buck et al. (2000) combined maternal and paternal fish consumption into one model looking at fecundability ratios as the outcome, rather than TTP (continuous variable used in 1997 paper) or conception delay (>12 months unprotected intercourse used in 1999 paper). The sample included 606 women with known and unknown TTP who discontinued birth control in order to become pregnant during 1991–1993 and for whom the partners' fish consumption data also were available. The exposure measures included the duration, frequency, and lifetime PCB index used in the previous studies. Separate analyses were run for each exposure measure as both paternal and maternal consumption measures were correlated. Statistical analyses included the use of a discrete-time analog of the Cox proportional hazards model to predict the probability of conception (i.e., fecundability-biological capacity for reproduction) at the jth cycle given the absence of conception at an earlier cycle. The natural logarithm of this conditional probability was modeled as a linear function of the covariates and potential confounders (i.e., maternal smoking, gynecologic history [e.g., endometriosis; pelvic inflammatory disease], parental ages, gravidity [number of pregnancies], and history of fertility drugs). The outcome measure used was the conditional fecundability ratio (CFR), the probability of conception during a given menstrual cycle for the exposed fisheaters divided by that for unexposed nonfisheaters. The 95% CI for the CFR was also calculated; exclusion of the value 1.0 from the CI indicates statistical significance at the p=0.05 level. This ratio is conditional upon becoming pregnant; a value <1 indicates reduced fecundability. Fish consumption was generally higher for men than women (68 and 42%, respectively) as was the mean number of years of fish consumption (5.9 and 2.1 for the entire sample whether they consumed fish or not). Men also had higher mean PCB indices than women in the study,

although partners' consumption patterns were related (e.g., both might be in the same category of fish consumption). The adjusted fecundability ratios for parental Lake Ontario fish consumption indicated that maternal consumption of 3–6 years was associated with significantly reduced fecundability (CFR=0.75, 95% CI 0.59–0.91), as was eating more than one monthly fish meal in 1991 (CFR = 0.73, 95% CI 0.54–0.98). Paternal consumption was associated with slightly elevated, but non-significant, CFRs for all three measures of fish consumption, suggesting that maternal but not paternal consumption of contaminated fish may reduce fecundability among couples attempting pregnancy. The investigators considered the findings preliminary, given the retrospective data collection on TTP and fish consumption, limited information on potential confounders, and potential sources of bias. In addition to the limitations indicated for the previous studies, Buck et al. (2000) commented on several biases associated with TTP, particularly pregnancy recognition bias (when or how women became aware of pregnancy). Another possible bias is the fact that the analytic strategy is dependent on a woman achieving a pregnancy; if fish consumption exerts a deleterious effect on fecundability, no pregnancy will be achieved and the women will not be in the sample in the first place. Hence, it is possible that women with the highest exposures were excluded from the study since they did not achieve a pregnancy. Due to the preliminary nature of the findings, the investigators could not speculate as to whether the effect on fecundability could be strong enough to reduce fertility, as measured by a reduction or absence of livebirths, or to impair fecundity, as measured by pregnancy loss.

No statistically significant associations between increased risk for spontaneous fetal death and dietary exposure to Lake Ontario fish were found among 1,820 multigravid, fertile women from the NYS Angler Cohort (Mendola et al. 1995a). Spontaneous fetal death histories (ever having a pregnancy end in miscarriage, spontaneous abortion, or stillbirth) were obtained from New York State live-birth certificates. Fish consumption histories were used to construct four measures of PCB exposure for each subject: (1) lifetime PCB exposure based on species-specific PCB levels (subjects were placed in no, low, moderate, or high exposure classes); (2) number of years of sportfish consumption from 1955 to 1991; (3) kilograms of sportfish consumed in the 1990–1991 season; and (4) lifetime kilograms of sportfish eaten (no, low, moderate, or high exposure classes). Women who never ate Lake Ontario sportfish comprised the referent group. Odds ratios and 95% CI were calculated in bivariate analyses to identify potential confounders including smoking and alcohol consumption, and parental ages, paternal sportfish consumption (none were statistically significant). Unconditional logistic regression models were used to calculate ORs and 95% CI for multivariate analyses. Analyses were stratified by number of prior pregnancies to better describe the relationship between maternal age and spontaneous death at different levels of prior gravidity, and controlled for smoking and maternal age. No consistent relationship was

seen between a history of spontaneous fetal death and any of the four measures of exposure. The ORs in the logistic regression models evaluating lifetime PCB level and lifetime sportfish consumption relative to spontaneous fetal death tended to be slightly above 1.0 (the null value) for the low exposure categories and below 1.0 for the moderate and high exposure categories. Odds ratios of approximately 1.0 also were observed in the models assessing the number of years of consumption and the mass (kg) of sportfish consumed in 1990–1991 (Mendola et al. 1995a). Strengths of the study included sufficient statistical power to detect fairly small increases in ORs and the reliability of reproductive history data on birth certificates. Limitations included the focus on clinically recognized fetal deaths which may not detect early pregnancy loss, the self-reported nature of both exposure and outcome data, and the lack of biomarker monitoring to validate the self-reported exposure data. Because the findings suggested that early fetal loss may be important, a prospective pregnancy study is currently underway (ongoing study by J. Vena, see Section 3.12.3).

Decreasing number of months of lifetime lactation were significantly associated with increasing levels of PCBs or DDE in breast milk (normalized for lipid content) in a group of 98 lactating women from the NYS Angler Cohort (Kostyniak et al. 1999). In this sample, PCB levels in breast milk were significantly associated with self-reported measures of fish consumption, but DDE levels were not. The observed association is likely to be important in estimating dose rates for these chemicals in nursing neonatal populations, but the relevance of the association to reproductive performance is not clear.

The Michigan Anglers Cohort. An association between conception delay and sportfish consumption was found in a survey of 626 married couples conducted between 1993 and 1995 (Courval et al. 1999). At least one person in each couple was a licensed angler residing in 1 of 10 Michigan counties bordering a Great Lake (Lake Erie, Lake Huron, or Lake Michigan). Subjects were categorized into four sex-specific exposure classes (none, low, medium, high) based on an index of lifetime fish consumption (estimated number of sportfish meals consumed in the past 12 months multiplied by the number of years since 1970 in which fish were caught and consumed): 0, 1–114, 115–270, and 271–1,127 for men, and 0, 1–54, 55–138, and 139–1,127 for women. Conception delay, defined as ever having failed to conceive a child after 12 months of trying, was essentially the same in both sexes (reported by 12.9% of the men and 13.3% of the women). Unadjusted logistic regression analysis showed that ORs for conception delay increased in women with increasing exposure class, although results of a trends test were not statistically significant (p=0.35); the OR in the high exposure category for women was 1.4 (95% CI 0.7–2.7). Adjustment for age, race, region of Michigan, household income, smoking, and alcohol consumption did not strengthen the associations in women. The OR in the high exposure category declined in the female

models after the addition of husbands' fish consumption, indicating no risk associated with female consumption after accounting for male partner consumption. In contrast to the findings in women, analysis of the male data provide suggestive evidence that frequent consumption of Great Lakes sportfish may be associated with an increased risk of conception delay for men (see Section 3.2.5.2.2). Although data analysis controlled for several potential confounders, no information was collected regarding the subjects' frequency or timing of sexual intercourse during the period of attempting to conceive, whether the partner providing fish consumption data also was the partner with whom the conception delay had occurred, or levels of PCBs and other persistent toxic chemicals in biological samples from the subjects. The researchers will be addressing many of these limitations in a prospective reproductive health study. Additionally, the participation rate (29%) was extremely low in this study, which could have resulted in nonresponse bias, a bias similar to selection bias.

General Population Exposures. A case-control study was conducted that compared mean plasma concentrations of 14 PCB congeners and 11 chlorinated pesticides in women with endometriosis and women without endometriotic lesions (Lebel et al. 1998). Cases (86) and controls (70) were selected among premenopausal women with no previous diagnosis of endometriosis who underwent laparoscopy for either pelvic pain, infertility, or tubal fulguration, and were matched according to the indication for laparoscopy. Cases and controls did not differ with respect to age, body mass index, history of breast feeding, use of organochlorines, smoking, mean number of fish meals/week, income, and education, although the proportion of women who had never been pregnant was higher in cases than controls. Analysis of covariance was used to adjust means for confounding variables, and ORs were estimated by logistic regression. Crude or adjusted mean concentrations of individual or summed congeners did not differ between the groups. Additionally, there was no significant linear trend in the adjusted ORs for endometriosis as PCB concentrations increased.

In a study of 89 women (87% German) with repeated ($2) miscarriages, Gerhard et al. (1998) found that blood concentrations of PCBs were higher than the reference level in 22% of the cases. The sum of congeners 101–180 was used for evaluation because they were the only congeners detected in significant concentrations. Blood levels of other organochlorine compounds (pentachlorophenol, DDE, β- and γ-hexachlorocyclohexanes, HCB) were higher than reference ranges in 7–15% of the cases. No significant differences in PCB levels were found between women with early or late miscarriages (after #12 or >12 weeks of gestation) and primary or secondary miscarriages (had never delivered or delivered at least one baby). Women with a history of at least four miscarriages (n=25) had significantly elevated blood levels of PCBs, although other organochlorine compounds (γ-hexachlorocyclohexane and HCB)

were also increased. Hormonal disorders were identified as the cause of repeated miscarriages in 31% of the women, including hyperprolactinemia in 9%, hyperandrogenemia in 7%, and luteal insufficiency in 14% of the cases. Correlations were found between increasing PCB concentrations and some hormonal parameters (e.g., increasing FSH and LH, decreasing TSH) and immunological parameters (e.g., increasing IgM, monocytes, and NK cells, decreasing interleukin 2 receptor-positive cells), but none of the associations were specific for PCBs. There were no significant associations between PCB concentrations and further conceptions or the outcome of further pregnancies.

Yusho and Yu-Cheng Exposures. Irregular menstrual cycles (60% of 81 patients) and abnormal basal body temperature patterns (85% of 81 patients) were observed female *Yusho* patients in 1970 (Kusuda 1971). Menstrual irregularities included changes in cycle intervals, duration, and flow that showed no consistent pattern and were unrelated to severity of *Yusho* poisoning as indicated by degree of dermal signs. These alterations were accompanied by decreased urinary excretion of estrogens, pregnanediol, and pregnanetriol. Fertility, fecundity, and rates of spontaneous abortion have not been studied in *Yusho* and *Yu-Cheng* patients (Hsu et al. 1994; Kuratsune 1989; Masuda 1994; Rogan 1989). Sex ratio was not altered in children born to 74 *Yu-Cheng* women during or after the poisoning began (Rogan et al. 1999). Of 137 live births occurring between 1978 and 1985, 69 were girls and 68 were boys.

3.2.5.2.2 Male Reproductive Effects

Occupational Exposure. Sperm counts, fertility history, and testicular abnormalities as determined by physical examination were normal in 55 transformer repairmen compared to 56 unexposed workers who were similar in age, race, and marital status (Emmett et al. 1988a, 1988b). The mean length of employment of the exposed workers was 3.75 years, most (38) of the workers were currently exposed to PCBs, and the predominant exposure was from Aroclor 1260 with lesser exposure to Aroclor 1242. Measurements of air PCB levels at four work areas showed 8-hour TWA concentrations of 0.0167–0.024, 0.0032–0.007, 0.00001–0.0004, and 0.0007–0.0124 mg/m^3. Geometric mean PCB concentrations in the current-exposed, past-exposed, and comparison workers were 2.08, 0.83, and 0.60 ppm, respectively, in adipose tissue and 12.2, 5.9, and 4.6 ppb, respectively, in serum. Interpretation of the negative results of this study is complicated by the similar PCB body burdens in the past-exposed and control groups.

Contaminated Fish Consumption.

The New York State Angler Cohort. Paternal exposure to Lake Ontario fish was not associated with an increased risk of conception delay, as indicated by TTP, in women from the NYS Angler Cohort (Buck et al. 1999). The study sample included 785 spouses of male anglers reporting one or more pregnancies between 1991 and 1993, known TTP, and complete paternal fish consumption histories. Female anglers were excluded from the study, as fish consumption data from their spouses and partners were not collected. Three measures of paternal fish consumption were used: (1) frequency of consumption (number of Lake Ontario fish meals consumed in 1991), (2) duration of consumption (number of years), and (3) an index of lifetime cumulative PCB exposure from fish consumption (categorized as low, moderate, and high). Conception delay was defined as requiring $12 menstrual cycles with unprotected intercourse to achieve pregnancy. Statistical analyses included descriptive methods and unconditional logistic regression modeling to calculate ORs and 95% CIs. Potential and known confounders included maternal age, age at menarche, menstrual regularity, education, income, cigarette smoking; history of prior pregnancy; and history of previous pregnancy loss. Adjusted ORs for paternal fish consumption and risk of conception delay were <1.0 for all categories of meal frequency and duration. For the PCB index measure, the ORs were <1.0 in all categories except moderate consumption. The confidence intervals included one in all analyses. The ORs of <1.0 and inclusion of the value 1.0 in the confidence intervals indicate that paternal fish consumption did not significantly increase the risk of conception delay among the women. When the analyses were restricted to spouses or partners with no Lake Ontario fish consumption (n=445), similar results were obtained for each of the three paternal fish consumption exposure variables. Selection bias is a potential study concern as the study did not include women who may have become pregnant accidentally, although there is no evidence to suggest that fish consumption is systematically related to pregnancy intentions (Buck et al. 1999). Other study limitations include possible underestimation of paternal fish consumption because data did not include the 2 years prior to the TTP assessment, and possible residual confounding as several potential confounders of female fecundity were not collected.

In further analysis of the Buck et al. (1999) study summarized above and the Buck et al. (1997) study summarized in Section 3.2.5.2.1 (Female Reproductive Effects), Buck et al. (2000) combined maternal and paternal fish consumption into one model looking at fecundability ratios as the outcome, rather than TTP (continuous variable used in 1997 paper) or conception delay (>12 months unprotected intercourse used in 1999 paper). The sample included 606 women with known and unknown TTP who discontinued birth control in order to become pregnant during 1991–1993 and for whom the partners' fish consumption

data also were available. The exposure measures included the duration, frequency, and lifetime PCB index used in the previous studies. As described in Section 3.2.5.2.1, Buck et al. (2000) used a discrete time analogue of Cox proportional hazards analysis to estimate conditional fecundability ratios and 95% CI for fish consumption among couples with complete exposure data who discontinued birth control to become pregnant. Fish consumption was generally higher for men than women (68 and 42%, respectively) as was the mean number of years of fish consumption (5.9 and 2.1 for the entire sample whether they consumed fish or not). Men also had higher mean PCB indices than women in the study, although partners' consumption patterns were related. The adjusted fecundability ratios for parental Lake Ontario fish consumption indicated that maternal consumption of 3–6 years was associated with significantly reduced fecundability, as was eating more than one monthly fish meal in 1991 (see Section 3.2.5.2.1). Paternal consumption was associated with slightly elevated, but nonsignificant, CFRs for all three measures of fish consumption (only duration of 1–2 years had a CFR below 1.0). The findings suggest that maternal but not paternal consumption of contaminated fish may reduce fecundability among couples attempting pregnancy.

The Michigan Anglers Cohort. An association between conception delay and sportfish consumption was found in a survey of 626 married couples conducted between 1993 and 1995 (Courval et al. 1999). At least one person in each couple was a licensed angler residing in 1 of 10 Michigan counties bordering a Great Lake (Lake Erie, Lake Huron, or Lake Michigan). Subjects were categorized into four sex-specific exposure classes (none, low, medium, high) based on an index of lifetime fish consumption (estimated number of sportfish meals consumed in the past 12 months multiplied by the number of years since 1970 in which fish were caught and consumed): 0, 1–114, 115–270, and 271–1,127 for men, and 0, 1–54, 55–138, and 139–1,127 for women. Conception delay, defined as ever having failed to conceive a child after 12 months or more of trying, was essentially the same in both sexes (reported by 12.9% of the men and 13.3% of the women). Unadjusted logistic regression analysis showed that ORs for conception delay increased in men with increasing exposure class: 1.2 (95% CI 0.5–2.9), 1.3 (0.6–3.1), and 2.0 (0.9–4.5); results of a trends test were marginally statistically significant (p=0.06). Adjustment for age, race, region of Michigan, household income, smoking, and alcohol consumption minimally increased the odds ratios for men. The addition of the partners' fish consumption in the adjustment further increased the odds ratios associated with fish consumption in the model for men; the high fish consumption category OR was 2.8 (1.0–8.0), indicating that men with the highest fish consumption were at nearly 3 times the risk of conception delay as nonconsumers. The findings provide suggestive evidence that frequent consumption of Great Lakes sportfish may be associated with an increased risk of conception delay for men. As discussed in Section 3.2.5.2.1, there was no evidence of increased risk of conception delay in the exposed

women. Although analysis of the data controlled for several potential confounders, no information was collected regarding the subjects' frequency or timing of sexual intercourse during the period of attempting to conceive, whether the partner providing fish consumption data also was the partner with whom the conception delay had occurred, or levels of PCBs and other persistent toxic chemicals in biological samples from the subjects. Additionally, the participation rate (29%) was extremely low in this study, which could have resulted in nonresponse bias, a bias similar to selection bias.

General Population Exposures. Semen samples from fertile men and those with low sperm counts (idiopathic oligospermia or azoospermia) were analyzed for 74 PCB congeners (Bush et al. 1986). Multiple linear regression analysis of combined sample data showed no association between concentration of any individual congener or total PCBs (summed congeners) and either sperm count, motility, or percentage of normal forms. Analysis of the data by fertility status (fertile, subfertile, infertile) indicated that in the infertile men (sperm count <20 million cells/mL), decreasing sperm motility was associated with increasing concentrations of three congeners (2,3',4,4',5-pentaCB [PCB 118], 2,2',3,4,4',5-hexaCB PCB 137], and 2,2',4,4',5,5'-hexaCB [PCB 153]). The proportion of total variance attributable to the regression (R^2) was 9–16% for these congeners. Another study found that blood concentrations of tetra-CBs and penta-CBs, but not hexa-CBs and total PCBs, were significantly higher in infertile males than in normal individuals (Pines et al. 1987). Levels of *p,p'*-DDT and other organochlorine compounds were also increased in the semen and blood of the men in these studies.

Yusho and Yu-Cheng Exposures. Sexual maturation was not delayed, and testicular and scrotal development was not altered in boys born to *Yu-Cheng* women, although the exposed boys had significantly shorter penises (Guo et al. 1993). Sex ratio was not altered in children born to 74 *Yu-Cheng* women during or after the poisoning began (Rogan et al. 1999). Of 137 live births occurring between 1978 and 1985, 69 were girls and 68 were boys.

3.2.5.2.3 Evaluation of Human Studies.

Information is available on reproductive effects of PCBs in humans from studies of people exposed by the general environment, consumption of contaminated rice oil in the *Yusho* and *Yu-Cheng* poisoning incidents, consumption of contaminated fish, and occupational exposures. A comparison of PCB levels in blood and breast milk in some of these studies is included in Appendix A.

Females. Gerhard et al. (1998) examined a number of university hospital female patients (n=89) with a history of miscarriages. Although other substances were also detected (e.g., hexachlorobenzene), PCB levels were found to be higher in the blood of patients with a history of three or more miscarriages. Another study of the general population found no association between endometriosis and concentrations of PCBs in the blood (Lebel et al. 1998).

Menstrual irregularities (i.e., altered intervals, duration, and flow) were observed in women exposed during the *Yusho* poisoning incident (Kusuda 1971). Heating of the PCB-contaminated rice oil also resulted in the formation of other contaminants of concern (i.e., dibenzofurans and ter-, and quarterphenyls) (Rogan 1989).

In a study of Native Americans, fish consumption has been shown to be a major risk factor for elevated PCB body burdens (Fitzgerald et al. 1996). The studies that examined reproductive end points in women whose diets contained Great Lakes fish found evidence that consumption of the fish may be associated with a slightly shorter length of menstrual cycle (Mendola et al. 1997), but not with increased risk of conception delay in females (Buck et al. 1997; Courval et al. 1999) or increased risk for spontaneous fetal death (Mendola et al. 1995a). Buck et al. (1997) examined time-to-pregnancy (i.e., after stopping birth control, the number of menstrual cycles before pregnancy) as the outcome measure of conception. However, in a more recent study (Buck et al. 2000), their outcome measure was a fecundability ratio (i.e., probability of conception during a given menstrual cycle for the exposed, divided by the same probability for the unexposed). Utilizing this outcome, the researchers found that maternal consumption of fish for 3–6 years was associated with a reduction in fecundability (i.e., biological capacity for reproduction). Significantly higher levels of several PCB congeners (e.g., 153 and 138) were also detected in the breast milk of fisheaters (Kostyniak et al. 1999). The number of months of lifetime lactation declined in these females with a rise in PCB concentration in breast milk.

Mendola et al. (1997) note that the effect on menstrual cycle length in the women fisheaters is a preliminary finding that needs to be interpreted cautiously because of certain limitations (e.g., lack of information on confounders such as stress, use of contraceptives, body mass index, and physical exercise). The decreases in menstrual length were small and were considered not likely to be clinically relevant. However, they may be indicative of potential endocrine effects to the population. At the highest exposure levels, the decrease was approximately 0.5 days for women who reported regular cycles and 1 day for all women who reported cycle length information. The effect did not appear to be mediated through irregular cycles since the fish consumption-based exposure levels were similar for women who

reported regular or irregular cycles. The human populations in which menstrual changes have been observed differ with respect to the sources of PCBs and exposures to other chemicals that may affect susceptibility to menstrual disturbances. Although the studies are insufficient for determining which specific chemical(s) may be responsible for the observed alterations, the available data support a possible association between PCBs and menstrual disturbances.

There was no apparent effect on mean number of pregnancies in women who were occupationally exposed to Aroclors 1254, 1242, and/or 1016 (Taylor et al. 1989). This study had limitations due to small numbers of subjects and the availability of only estimates of exposure based upon job descriptions, manufacturing process, and industrial hygiene data. Additionally, the mean number of pregnancies represented data not adjusted for potential confounders.

The human studies of reproductive effects in females have not always resulted in consistent findings. For example, two studies of fish consumption and conception demonstrated no effect (Buck et al. 1997; Courval et al. 1999). However, a more recent study by Buck et al. (2000) demonstrated that fish consumption of a 3–6 year duration was associated with a reduction in fecundity in females. Despite the variation in results between studies, an association can be observed between the documented reproductive effects (e.g., menstrual irregularities and conception failure), making these findings biologically persuasive.

Males. Analysis of semen for 74 PCB congeners showed that increasing concentrations of three congeners (PCBs 118, 137, and 153), but not total PCBs, were associated with decreasing sperm motility in infertile men (Bush et al. 1986). Another study found that blood concentrations of tetra-CBs and penta-CBs, but not hexa-CBs and total PCBs, were significantly higher in infertile males than in normal individuals (Pines et al. 1987). These results do not necessarily indicate a causative relationship between PCBs and infertility in men for a number of reasons, particularly because levels of *p,p'*-DDT and other persistent toxic chemicals were also increased in semen and blood. Bush et al. (1986) found that the PCB congeners only accounted for a small proportion of the total variance attributable to the linear regression analysis, and hypothesized that the increased levels of PCBs in the low sperm count samples could be due to other factors, such as biological malfunction in the sperm generation system causing lipid leakage. Associations between conception delay and consumption of PCB-contaminated Great Lakes sportfish were reported in exposed men, but not their wives, in the study of the Michigan Anglers Cohort (Courval et al. 1999). Although analysis of the data controlled for several potential confounders, no information was collected regarding the subjects' frequency or timing of sexual intercourse during the period of

attempting to conceive, or levels of PCBs and other persistent toxic chemicals in biological samples from the subjects. Additionally, there was no clear association between paternal exposure to consumption of contaminated fish and conception delay or reduced fecundability in the NYS Angler Cohort, another cohort of Great Lakes Anglers (Buck et al. 1999, 2000). Occupational studies of reproductive effects in men provide no clear indications of PCB-related effects. Sperm counts, fertility history, and testicular examinations were normal in a study of transformer repairmen who were occupationally exposed to Aroclors 1260 and 1242 for a mean duration of 3.75 years (Emmett et al. 1988a, 1988b). Although the overall evidence for associations between PCBs and effects on sperm and conception delay in males has not always been consistent, there are indications of possible reproductive effects in males which are supported by the findings for female study subjects with similar exposure patterns.

3.2.5.3 Animal Studies

The highest NOAEL values and all reliable LOAEL values for reproductive effects for each study are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.5.3.1 Female Reproductive Effects

Commercial Mixtures. Information on reproductive toxicity of commercial PCB mixtures in female animals is available from studies in rats, mice, rabbits, minks, and monkeys. As discussed below, effects on fertility and/or reproductive function have been observed in most of these species with minks and monkeys showing particular sensitivity.

Wistar rats that were administered 10 mg/kg/day Aroclor 1254 by gavage for 4–6 weeks had prolonged estrus cycle, decreased sexual receptivity, and a transient decrease in body weight gain, but no significant effect on the number of ovulations compared to unexposed controls (Brezner et al. 1984). Animals that were subsequently bred (duration of exposure at time of mating not specified) experienced treatment-related vaginal bleeding during gestation, delayed parturition, and decreased litter size. Evaluation of pups following gestational and lactational exposure showed decreased body weight gain, decreased preweaning survival, premature vaginal opening, and delayed first estrus, but there were no effects on sexual differentation, estrous cycle, mating, or pregnancy. There were no significant changes in number of implantation sites, litter size, or offspring sex ratio in Long-Evans rats that were exposed to 4 mg/kg/day Aroclor 1254 in the diet from 50 days prior to mating until birth (Hany et al. 1999b). There were no overt signs of maternal toxicity, and other dose levels were not tested. Body weight (average of

both sexes) was significantly reduced in the pups at birth and PND 7–21, and relative testes weights and serum testosterone levels were reduced in adult male offspring at PND 170.

Two-generation studies were performed in which groups of 20 female Sherman rats were exposed by diet to Aroclor 1254 in doses of 0, 0.06, 0.32, 1.5, or 7.6 mg/kg/day or Aroclor 1260 in doses of 0, 0.39, 1.5, or 7.4 mg/kg/day (Linder et al. 1974). Exposure to Aroclor 1254 caused significantly reduced litter sizes at 7.6 mg/kg/day in the F1a generation (14% smaller than controls) and $1.5 mg/kg/day in the F1b, F2a, and F2b generations (15–72% smaller than controls). No effects on litter size were found in either generation of rats fed 0.06 mg/kg/day of Aroclor 1254 or $0.39 mg/kg/day of Aroclor 1260. Insufficient information is available to determine whether the effect on litter size is due to reproductive or developmental toxicity because fertility and other reproductive end points were not evaluated in the study.

Reproductive effects were evaluated in female offspring of Holtzman rats following maternal exposure to 0, 8, 32, or 64 mg/kg/day of Aroclor 1254 by gavage on lactation days 1, 3, 5, 7, and 9 (Sager and Girard 1994). Young, mature, and older adult offspring were examined at 2–4.5, 5–8, and 8.5–13 months of age, respectively, and mated to untreated males at 112, 200, and 350 days of age, respectively. Effects included a dose-related reduction in preweaning weight gain that was statistically significant at $32 mg/kg/day, delayed puberty as indicated by late vaginal opening and first estrus at $32 mg/kg/day; reduced mating rate (sperm-positive females) in mature offspring at $8 mg/kg/day; reduced implantation rate and mean number of embryos in young and mature offspring at 64 mg/kg/day; reduced uterine weight during proestrus in young, mature, and older offspring at $8 mg/kg/day; and reduced uterine response to exogenous 17-beta estradiol in ovariectomized mature offspring at $8 mg/kg/day. Average estrus cycle length was not significantly different in any of the groups, although cycle patterns were altered in low- and high-dose young offspring and in mid-dose mature rats. Pregnancy and ovulation rates, reproductive aging, and ovarian weights were not affected by exposure Aroclor 1254.

Estrogenic effects of PCBs were also evaluated in *in utero* and lactationally exposed female offspring of Sprague-Dawley rats that were administered 0 or 30 mg/kg/day doses of Aroclor 1221, 1242, or 1260 by gavage on days 12–20 of gestation (Gellert and Wilson 1979). Evaluation of the offspring at approximately 6 months of age showed no exposure-related changes in ovarian weight, ovulatory status, or vaginal estrus cyclicity.

Female ICR Swiss mice that were exposed to Aroclor 1254 in the diet at a dose of 12.5 mg/kg/day for 90 days before mating had a conception rate that was reduced approximately 30% compared to lower

dose groups (#1.25 mg/kg/day) and controls (Welsch 1985). There were no exposure-related effects on fertilization rate or pre- and postimplantation embryonic losses in New Zealand rabbits that were administered 4 mg/kg of Aroclor 1260 (only treatment level) by gavage on 3 days/week for 12–15 weeks before artificial insemination and throughout gestation (Seiler et al. 1994). No other reproductive parameters were examined in these studies.

It is well established that oral exposure to low doses of PCBs causes reproductive failure in minks. For example, in minks that were exposed to Aroclor 1254 at an estimated dietary dose of 0.4 mg/kg/day for a 39-week period that began approximately 6 months before mating and ended when the kits were 4 weeks of age, only two of seven mated females produced offspring with a total of two kits (one alive and one dead) (Aulerich and Ringer 1977). No effects on numbers of females producing offspring or kits born were induced by similar exposure to Aroclor 1242, 1221, or 1016, although 0.4 mg/kg/day was the only dose level tested. Dietary exposure to 0.2 mg/kg/day Aroclor 1254 for a 21-week period that began approximately 4 months before breeding and ceased at the end of gestation had no effect on numbers of females producing offspring or kits born, although marked reductions occurred at 0.9 mg/kg/day, and there were no births at 2.8 mg/kg/day (Aulerich and Ringer 1977). Female minks that were exposed to 1.3 mg/kg/day Aroclor 1254 in the diet from approximately 5 weeks before breeding until 5 days after parturition had an increased frequency of interrupted pregnancies and 48% reduced litter size with no live births (Kihlstrom et al. 1992). Similar effects on reproduction were observed in female minks that were exposed to 0.5–1.7 mg/kg/day Aroclor 1254 or 1.8 mg/kg/day Clophen A50 from before mating until 5 days after parturition for total durations of approximately 3 months (Backlin and Bergman 1995; Backlin et al. 1997, 1998a, 1998b; Jones et al. 1997). No indications of PCB-induced impaired ovulation or implantation have been observed in minks, although histopathological studies of mid- to late-gestation placentae indicate that fetal death is mediated by degenerative changes in maternal (endothelial detachment and thrombosis in maternal vessels) and fetal (trophoblastic disintegration and loss of fetal capillary integrity) tissues (Backlin and Bergman 1995; Backlin et al. 1997, 1998a, 1998b; Jones et al. 1997; Kihlstrom et al. 1992).

Reproductive effects of low doses of commercial PCB mixtures have also been demonstrated in intermediate-duration studies in female monkeys. Exposure to 0.8 mg/kg/day Aroclor 1248 for 2 months caused a reduction in conception rate in monkeys (Allen et al. 1974a). Conception did not occur in two of five monkeys that were bred 3 months posttreatment, resorption and/or abortion occurred in two of the three pregnant monkeys, and the two nonpregnant monkeys were bred twice again during the subsequent 5 months without success. Groups of eight female Rhesus monkeys were exposed to 0.1 or

0.2 mg/kg/day Aroclor 1248 in the diet from 7 months prior to breeding and throughout pregnancy (Barsotti et al. 1976). Increased menstrual duration (5–7 days) and bleeding occurred at $0.1 mg/kg/day, and conception rate was decreased at 0.2 mg/kg/day. Resorptions or abortions occurred in 3/8 and 4/6 of low- and high-dose impregnated monkeys, compared with 0/12 in controls, and the remaining two high-dose animals were bred 5 times without success. Similar effects occurred in four Rhesus monkeys that were mated after 38 weeks of dietary exposure to 0.2 mg/kg/day Aroclor 1248 (Arnold et al. 1990). Following extended post-implant bleeding, all of the treated monkeys aborted within 30–60 days of gestation. Following recovery from the abortions, the monkeys were bred again up to a maximum of 7 times but none appeared to conceive, and the menstrual cycle lengths and durations became erratic and longer during and subsequent to the breeding period.

Information on reproductive effects of chronic exposure to PCBs is available from a study in which groups of 16 female Rhesus monkeys ingested capsules providing 0, 0.005, 0.02, 0.04, or 0.08 mg/kg/day doses of Aroclor 1254 for up to 72 months (Arnold et al. 1993a, 1993b, 1995, 1997). Evaluation during the premating phase of the study (first 37 months) found no exposure-related changes in serum levels of estrogen and progesterone (assessed during one menstrual cycle), menstrual duration (number of days of menstrual flow), or menstrual cycle length (number of days from first day of menses until the day preceding the next menses) (Arnold et al. 1993a, 1993b). The average cycle duration was slightly increased in the 0.04 and 0.08 mg/kg/day groups compared to controls and the average cycle length was slightly shortened in treated groups compared to controls, but none of the differences were statistically significant. There also were no apparent treatment-related effects on incidences of anovulatory cycles or temporal relationships between estrogen peak and menses onset, menses end, or progesterone peak. After 37 months of exposure, the females were mated with untreated males and dosing was continued throughout mating and gestation until the breeding phase of the study (29 months) was completed (Arnold et al. 1995). Incidences for impregnation success were 11/16, 10/16, 4/15, 6/14, and 5/15 in the control to high-dose groups. Statistical analysis of these conception rates, adjusted for either total number of matings or number of matings with positive sires, showed that there was a significant ($p=0.017$) decreasing trend in the rate of impregnation with increasing dose from 0 to 0.08 mg/kg/day. There was no evidence of such a trend when conception rates among only the treated groups were compared. Comparisons between the treated and control groups showed that the conception rates were significantly ($p<0.05$) reduced at doses $0.02 mg/kg/day. Age of the females did not appear to be a confounding factor. A significantly increasing dose-related trend in fetal mortality incidence rates ($p=0.040$) was also found in this study. Comparisons between the treated and control groups showed that fetal mortality was significantly ($p<0.05$) increased at 0.08 mg/kg/day and marginally ($p=0.077$) increased at 0.02 mg/kg/day, indicating that

0.02 mg/kg/day is the LOAEL for both reduced conception and fetal survival. Although the increased fetal mortality in the 0.02 mg/kg/day group was marginally nonsignificant, the number of animals was small; this group had three fetal deaths in four impregnated animals, and the infant that was born died within 2 weeks postpartum. Maternal treatment was discontinued after approximately 7 weeks of lactation to preclude infants from self-ingesting the mother's dosing capsule, and restarted in the adult monkeys when infants were weaned at 22 weeks of age and continued for the following 8 months (Arnold et al. 1997). Necropsies performed at the end of the postweaning exposure period showed no exposure-related histopathological changes in the uterus and other parts of the reproductive system or increased incidences or severity of endometriosis.

Defined Experimental Mixtures. There were no significant effects on number of implantation sites, litter size, or offspring sex ratio in Long-Evans rats that were exposed to 4 mg/kg/day of a PCB congener mixture simulating the congener content of human milk from 50 days prior to mating until birth (Hany et al. 1999b). Overt signs of maternal toxicity were not observed, and other dose levels were not tested. Body weight in the pups (average of both sexes) was significantly reduced at birth and PND 7–21, relative uterine weight was significantly increased in the female offspring on PND 21, and relative testes weights and serum testosterone levels were significantly reduced in adult male offspring at PND 170.

Contaminated Fish. No adverse reproductive effects were found in a 2-generation study in which Sprague-Dawley rats were fed diets containing 0, 5, or 20% (w/w) of lyophilized protein from chinook salmon from Lake Huron or Lake Ontario (Arnold et al. 1998; Feely and Jordan 1998; Feeley et al. 1998). The F0 rats (30 males and 30 females/group) were mated after 70 days on the test diet and the F1 rats (1 male and 1 female from 24 litters) were mated 70 days postweaning. Daily intakes of total PCBs in the female F1 rats fed diet containing 0, 5, or 20% lyophilized Lake Ontario salmon flesh were calculated to be 0.22, 23.20, and 82.37 µg/kg/day, respectively (Feely and Jordan 1998). PCB intakes were qualitatively similar, but generally somewhat lower, for males compared with females and for F0 rats compared with F1 rats, and intakes from the Lake Huron diet were about 35–40% lower than from the Lake Ontario diet. The DDT complex (*p,p'*-DDT, *p,p'*-DDE, and *p,p'*-DDD) accounted for 75 and 60% of organochlorine pesticide residues in the Lake Huron and Lake Ontario fish, respectively, and other major contaminants included CDDs and CDFs, mirex, chlordane, cadmium, lead, mercury, and arsenic. Comprehensive reproductive assessment, which included evaluation of conception rate and mating, fertility, viability, and lactation indices, showed no significant exposure-related adverse effects in either generation.

A 2-generation reproduction study of Lake Huron fish was conducted in minks (Restum et al. 1998). Although numerous chlorinated pesticides and other persistent toxic substances were present in the fish, the dietary treatments were expressed as targeted concentrations of total PCBs. Diets were formulated to provide 0, 0.25, 0.5, or 1.0 ppm PCBs by substituting carp from Lake Huron for ocean fish in the control diet. To determine whether the effects of exposure were permanent, half of the parental (P1) animals were switched from the treatment diets to the control diet after whelping the first of two F1 generations. Total exposure time for the P1 minks that were switched to the control diet after weaning was about 6 months, and the P1 minks that were continued on the treatment diets until termination of the study were exposed for approximately 16–18 months. Effects of gestational and lactational exposure on reproductive performance of the first F1 generation were examined by switching half of the F1 offspring to the control diet at weaning (offspring were exposed for about 3 months), and continuing the remaining offspring on their parental diet throughout their lifetime (continuous exposure for 12–15 months). The second F1 generation included kits born to the P1 dams that were exposed for 6 months followed by 10–12 months of consumption of control diet prior to whelping, as well as kits born to the P1 dams that were continuously exposed over an 18-month period. F2 generation minks consisted of kits born to the first F1 generation and exposed to PCBs either during gestation and lactation only, or from gestation throughout their lifetime. Effects included delayed onset of estrus, as determined by vulvar swelling and time of mating, in P1 and F1 females that were continuously exposed to the mid and high doses of PCBs. There were no significant differences in breeding performance (numbers of confirmed bred) and reproductive performance (number whelped/number mated) in the P1 and F1 females. Survivability of F1 and F2 offspring was markedly decreased in the mid- and high-dose groups. The reduced survivability of the F1 kits predominately occurred after birth during the lactation period. For example, the first F1 litter produced by the F0 generation showed a 70.5% survivability at birth (compared with 94.6% in controls), but by the end of lactation, 6 weeks after birth, the average survivability was 23% (compared with about 73% in controls). In several exposure groups, there were decreased percentages of mated females that gave birth, but the decreases were not statistically significant. The failure to demonstrate statistical significance may have been due to small sample sizes for several of these groups. For example, in a high-dose F1 group, 2/4 mated females gave birth (50%), compared with 11/14 (79%) in the F1 control group.

Single Congeners. A series of toxicity studies was performed in which groups of 10 male and 10 female Sprague-Dawley rats were exposed to diets containing four dose levels of various single congeners for 13 weeks (Chu et al. 1994, 1995, 1996a, 1996b, 1998b; Lecavalier et al. 1997). Histological examinations of the female reproductive organs and mammary glands showed mild changes in the ovaries in 7/10 rats exposed to PCB 126 at 8.7 µg/kg/day, but not #0.83 µg/kg/day (Chu et al. 1994). The

ovarian changes were characterized by loss of oogonia in the primary follicles with degeneration of the inner layer of the corona. No effects in reproductive tissues were found in females following exposure to PCB 28 at #3,956 µg/kg/day; PCB 77 at 892 µg/kg/day; PCB 105 at #3,960 µg/kg/day; PCB 118 at #170 µg/kg/day; PCB 128 at #4,125 mg/kg/day; or PCB 153 at #4,397 µg/kg/day. Measurements of serum leutinizing hormone and follicle-stimulating hormone concentrations, performed only in the female rats exposed to PCB 28 and PCB 77, showed no exposure-related changes (Desaulniers et al. 1997).

Promotion of surgically-induced endometriosis was studied in B6C3F1 mice that were treated with PCB 126 or PCB 153 by gavage every 3 weeks for a total of 5 doses (Johnson et al. 1997). Dose levels were 0, 100, 300, or 1,000 µg/kg/day for PCB 126 and 0, 3, or 30 mg/kg for PCB 153. No significant changes in the size, weight, or histology of endometriotic lesions were induced by either congener. There also were no significant effects on ovarian or uterine weights, although histological examination of the ovaries (uterus not examined) from a small number of animals (three per group) suggested possible induction of ovarian atrophy by PCB 126.

A reproduction study of PCB 169 was conducted in which offspring of exposed female Wistar rats were mated (Smits-van Prooije et al. 1993). The maternal rats were treated with a single 0, 0.2, 0.6, or 1.8 mg/kg dose of PCB 169 by gavage on day 1 of gestation. Mating of male and female offspring as young adults (age not specified) resulted in significantly reduced mating success (females mated) and pregnancy rate at 1.8 mg/kg. Mating of female offspring with unexposed males as 1-year-old adults caused nearly a completely reduced number of mated females and zero pregnancy rate at 1.8 mg/kg.

Female C57BL/6J mice were fed PCB 77 in estimated dietary doses of 0, 0.6, or 7 mg/kg/day for 2 weeks before mating with unexposed males and subsequently throughout gestation and lactation (Huang et al. 1998b). Female offspring were fed the same diets as the dams from weaning until 7 weeks of age, at which time they were mated with unexposed males. Fecundity (percentage of mated females that gave birth) and pup survival at ages 4 and 21 days were reduced in the F0 females at 7 mg/kg/day. There were no effects on fecundity or litter size in the F1 females, although all of their offspring died before 4 days of age at $0.6 mg/kg/day. Other effects included reduced *in vitro* fertilizing ability of the eggs and increased degenerated eggs in the F1 females at $0.6 mg/kg/day; these end points were not evaluated in F0 females.

Other Relevant Information. Results from *in vitro* studies with oocytes obtained from superovulated $B6D2F_1$ mice showed that and 3,3',4,4'-tetraCB and Aroclors 1221, 1254, and 1268 significantly reduced

fertilization rates and increased the incidence of degenerative ova and abnormal 2-cell embryos (Kholkute et al. 1994a). Of the four PCBs tested, Aroclor 1254 was the most effective.

3.2.5.3.2 Male Reproductive Effects

Commercial Mixtures. High oral doses of commercial PCBs induced testicular effects in weanling rats, but not adult rats or mice. Adult mice that were exposed to 130 mg/kg/day Aroclor 1254 in the diet for 14 days had no treatment-related changes in relative weights of the testes or preputial and vesicular accessory glands (Sanders et al. 1974). Similarly, no effects on testis weight, epididymis weight, or testicular histology or cytogenicity were found in adult rats that were treated with 50 mg/kg/day Aroclor 1254 by gavage for 7 days (Dikshith et al. 1975). Weanling F344 rats that were administered 25 mg/kg/day Aroclor 1254 by gavage for 15 weeks, however, had significant reductions in seminal vesicle and cauda epididymal weights, caudal epididymal sperm counts, and body weight gain (Gray et al. 1993). These effects were not observed at lower doses of 0.1–10 mg/kg/day, and there were no changes in testicular sperm count and motility, testicular weight, serum levels of testosterone, weight of the testicular interstitial fluid, testosterone concentration in the interstitial fluid, or total testosterone in the interstitial fluid compartment of the testis. None of these mouse and rat studies evaluated reproductive capability.

Fertility was markedly reduced in male offspring of Holtzman rats that were lactationally exposed to Aroclor 1254 (Sager 1983; Sager et al. 1987, 1991). The maternal rats were treated with 8, 16, 32, or 64 mg/kg doses by gavage on lactation days 1, 3, 5, 7, and 9, and male offspring were mated with untreated females 130–150 days postweaning (Sager 1983; Sager et al. 1987). Significant decreases in numbers of implants and embryos were observed at 8 mg/kg/day (21 and 29% lower than controls, respectively) and higher doses, and there was either a significant decrease or a decline in number and percent of normal fertilized eggs and eggs at the two- to four-cell blastocyte stages at $16 mg/kg/day. The reduction in male fertility appears to be due to impaired ability of sperm to fertilize eggs because sperm production, morphology, and motility were not affected and plasma FSH and testosterone concentrations were not reduced (Sager et al. 1987, 1991). Seminal vesicle and ventral prostate weights were decreased at $16 mg/kg/day.

In contrast to the effects of Aroclor 1254 summarized above, fertility was not impaired in male offspring of Sprague-Dawley rats that were administered 0 or 30 mg/kg/day doses of Aroclor 1221, 1242, or 1260 by gavage on days 12–20 of gestation (Gellert and Wilson 1979). There were no exposure-related

changes in the percentage of male offspring (F1) siring progeny when they were mated with unexposed females at approximately 6 months of age, or in the sex ratio of the F2 offspring from this mating. Measurements of absolute testes and ventral prostate weights in the F1 males (relative weights not determined) showed no changes except for increased testes weight in the Aroclor 1260 group.

Limited information is available on reproductive effects of commercial PCB mixtures in male minks and monkeys. Mating performance and testicular histology were normal in four male minks that were fed 0.1 mg/kg/day Aroclor 1254 in the diet for approximately 6 months (Wren et al. 1987b). Aulerich and Ringer (1977) noted that long-term dietary exposure to Aroclor 1254 did not exert any apparent adverse effects on spermatogenesis in minks. Matings between unexposed females and PCB-treated males reportedly resulted in acceptable reproduction, but no additional study information was provided.

One of four male Rhesus monkeys that were fed 0.1 mg/kg/day Aroclor 1248 for 17 months developed decreased libido and dermal and ocular signs of PCB toxicity after the first year of exposure (Allen and Norback 1976). A testicular biopsy on the affected animal showed marked hypoactivity of the seminiferous tubules characterized by an absence of mature spermatozoa and a predominance of Sertoli cells. The remaining three males remained healthy and sexually active. Evaluation of sperm morphology and viability and the ability to fertilize unexposed females, performed during the first year of exposure, showed no effects in any of the four males.

Contaminated Fish. There were no effects on breeding performance in male minks in the 2-generation reproduction study of Lake Huron fish summarized in Section 3.2.5.3.1 (Restum et al. 1998). No differences in the number of attempted or confirmed matings, or testicular volumes, were observed among the P1 and F1 generation males.

Single Congeners. Reproductive effects were evaluated in offspring of female Wistar rats that were treated with a single 0, 0.2, 0.6, or 1.8 mg/kg dose of PCB 169 by gavage on day 1 of gestation (Smits-van Prooije et al. 1993). Mating of exposed male and female offspring as young adults (age not specified) resulted in significantly reduced mating success (females mated) and pregnancy rate at 1.8 mg/kg. Mating of exposed male offspring with unexposed females as 1-year-old adults resulted in a zero pregnancy rate at 1.8 mg/kg.

Female C57BL/6J mice were fed PCB 77 in estimated dietary doses of 0, 0.6, or 7 mg/kg/day for 2 weeks before mating with unexposed males and subsequently throughout gestation and lactation (Huang et al.

1998a). Male F1 offspring were fed the same diets as the dams from weaning through 7 and 17 weeks of age, at which time, they were mated with unexposed females. Evaluation of reproductive ability of the F1 males showed no effects es as indicated by changes in fecundity (percentage of mated females that gave birth), litter size, sex ratio, or pup survival. Testes weights were increased in 7 mg/kg/day F1 males at 3 weeks, but not at 9 or 19 weeks of age. Additionally, although there were no effects on breeding, *in vitro* sperm-fertilizing ability was reduced in 7 mg/kg/day F1 males at 19 weeks, but not at 9 weeks of age.

A series of toxicity studies was performed in which groups of 10 male and 10 female Sprague-Dawley rats were exposed to diets containing four dose levels of various single congeners for 13 weeks (Chu et al. 1994, 1995, 1996a, 1996b, 1998; Lecavalier et al. 1997). Histological examinations showed no effects in male reproductive tissues following exposure to PCB 28 at #3,783 µg/kg/day; PCB 77 at #768 µg/kg/day; PCB 105 at #4,327 µg/kg/day; PCB 118 at #683 µg/kg/day; PCB 126 at #7.4 µg/kg/day; PCB 128 at #3,534 µg/kg/day; or PCB 153 at #4,210 µg/kg/day. Measurements of serum testosterone concentrations, performed only in the male rats exposed to PCB 28 and PCB 77, showed no exposure-related changes (Desaulniers et al. 1997).

Other Relevant Data. Daily sperm production was reduced and percentages of abnormal sperm were increased in adult male rats 1–8 weeks following administration of a single subcutaneous dose of 18 or 60 mg/kg of 3,3',4,4'-tetraCB (PCB 77) (Faqi et al. 1998). No effects on testis histology or serum testosterone concentration were observed, and reproductive capability was not evaluated.

Effects on the testis were evaluated in adult male rats that were neonatally exposed to either Aroclor 1242 (. 10, 40, or 80 mg/kg/day) or Aroclor 1254 (. 10 or 40 mg/kg/day) by daily subcutaneous injection from birth to PND 25 (Cooke et al. 1996). Examinations at 135 days of age showed significantly increased testis weight at $40 mg/kg/day Aroclor 1242 and $10 mg/kg/day Aroclor 1254, and increased daily sperm production at 10 mg/kg/day Aroclor 1242 and $10 mg/kg/day Aroclor 1254. Sertoli cell proliferation was also increased in exposed rats (only examined in 15-day-old pups treated with 40 mg/kg/day Aroclor 1242). Both Aroclor 1242 and 1254 also suppressed serum thyroxine (T_4) concentrations and T_4 replacement decreased or eliminated the testicular effects. As discussed in Section 3.2.2.8 (Endocrine Effects), other studies also indicate that hypothyroidism is involved in PCB-induced testicular effects in neonatal rats. Fertility tests showed that all Aroclor 1242-treated rats successfully impregnated unexposed females (Aroclor 1254 was not tested).

3.2.5.3.3 Evaluation of Animal Studies

Reproductive toxicity in female animals has been established in a number of oral studies with commercial PCB mixtures. Effects have been observed in various species, including rats (e.g., prolonged estrus, decreased sexual receptivity, and reduced implantation rate in adults and/or their offpsring exposed via gestation and lactation), mice (decreased conception), minks (partial or total reproductive inhibition), and monkeys (prolonged menstruation, decreased fertility) (Allen et al. 1974a; Arnold et al. 1990, 1993a, 1993b, 1995; Aulerich and Ringer 1977; Backlin and Bergman 1995; Backlin et al. 1997, 1998a, 1998b; Barsotti et al. 1976; Brezner et al. 1984; Jones et al. 1997; Kihlstrom et al. 1992; Sager and Girard 1994; Welsch 1985). Minks and monkeys are particularly sensitive, with effects occurring in these species at doses in the range of 0.1–1 mg/kg/day in intermediate-duration studies, and as low as 0.02 mg/kg/day in monkeys following chronic exposure.

In minks, repeated exposure to 0.4–1.8 mg/kg/day doses of Aroclor 1254 or Clophen A50 caused reproductive failure that has been associated with fetal death following embryo implantation (Aulerich and Ringer 1977; Backlin and Bergman 1995; Backlin et al. 1997, Kihlstrom et al. 1992). No indications of PCB-induced impaired ovulation or implantation have been observed in minks, although histopathological studies indicate that fetal death is mediated by changes in the placental vasculature which cause degenerative changes in the maternal and fetal vessels during gestation (Backlin and Bergman 1995; Backlin et al. 1997, 1998a, 1998b; Jones et al. 1997; Kihlstrom et al. 1992). As discussed in Section 3.5.2, multiple mechanisms are likely to be involved in PCB-induced reproductive impairment in minks. Although these studies provide important information on the mechanism and sensitivity of reproductive toxicity in female minks, it is unclear if this species is an appropriate surrogate for human toxicity. Impaired ability to conceive and decreased fetal survival are well-documented in female monkeys following repeated oral exposures to Aroclors 1254 and 1248 (Allen et al. 1974a; Arnold et al. 1990, 1993a, 1993b, 1995; Barsotti et al. 1976). For example, reduced conception rates, as well as increased incidences of abortions, resorptions, or stillbirths, were observed in groups of 16 female Rhesus monkeys that were fed encapsulated Aroclor 1254 at dose levels of 0.02–0.08 mg/kg/day for 37 months before breeding and subsequently throughout mating and gestation (Arnold et al. 1995). There were no clear effects on reproduction at 0.005 mg/kg/day, the lowest tested dose in this study and in any species. This dose is the LOAEL for immunological effects in the maternal monkeys and developmental toxicity in their offspring (see Sections 3.2.3 and 3.2.6). Mechanisms for the reproductive effects in monkeys have not been elucidated, although Arnold et al. (1995) found no evidence that they were associated with endometriosis.

There is some evidence suggesting that noncommercial mixtures of PCBs may have the potential to induce estrogenic and anti-estrogenic effects in the offspring of exposed animals. There were no significant effects on number of implantation sites or litter size in rats that were exposed to 4 mg/kg/day of a PCB congener mixture simulating the congener content of human milk from 50 days prior to mating until birth (Hany et al. 1999b). Evaluation of the offspring, however, showed significantly increased relative uterine weight in immature females (PND 21) and reduced testes weights and serum testosterone levels in adult males (PND 170). No significant exposure-related adverse effects on reproductive parameters (mating, fertility, viability, lactation indices, litter size) were found in a 2-generation study of rats fed contaminated fish from Lake Huron or Lake Ontario (Feeley et al. 1998). A 2-generation reproduction study of Lake Huron-fed minks similarly found no effects on breeding or reproductive performance, although onset of estrus was delayed in P1 and F1 females and survivability was decreased in F1 and F2 offspring (Restum et al. 1998). From the available information, it is not possible to determine whether the different results from the minks and rat studies are due to physiological or biochemical differences between minks and rats, qualitative or quantitative differences in chemical composition of the fish flesh, or some other cause. Additional information on the estrogenic and antiandrogenic effects of PCBs is discussed in Mechanisms of Toxicity (Section 3.5.2).

A limited amount of information is available on reproductive effects of PCBs in male animals. Short-term exposure to high oral doses of Aroclor 1254 induced no changes in the weight or histology of the testes or accessory glands in adult rats exposed to 50 mg/kg/day for 7 days or mice exposed to 130 mg/kg/day for 14 days (Dikshith et al. 1975; Sanders et al. 1974). Weanling F344 rats that were treated with 25 mg/kg/day Aroclor 1254 by gavage for 15 weeks, however, had significant reductions in seminal vesicle and cauda epididymal weights, caudal epididymal sperm counts, and body weight gain (Gray et al. 1993). These effects were not observed at lower doses of 0.1–10 mg/kg/day, and there were no changes in other testicular end points including sperm count and motility, testicular weight, and serum levels of testosterone. The results of the Gray et al. (1993) study may be related to the age of the rats at the start of dosing (day 31), which is after the development of Sertoli cells is complete, and therefore may have missed the vulnerable period in the postnatal development of the testes (see discussion in Section 3.2.2.8.3). None of these mouse and rat studies evaluated reproductive capability. Observations on small numbers of animals indicated that mating performance and testicular histology were normal in male minks that were fed 0.1–0.9 mg/kg/day doses of Aroclor 1254 for 4–6 months (Aulerich and Ringer 1977; Wren et al. 1987b). One of four monkeys that were fed 0.1 mg/kg/day Aroclor 1248 for 17 months developed clinical signs of toxicity, decreased libido, and marked hypoactivity of the seminiferous tubules, including an absence of mature spermatozoa, after the first year of exposure (Allen and Norback

1976). The remaining three males remained healthy and sexually active, and none of the animals had effects on sperm morphology and viability and the ability to fertilize unexposed females, although the latter end points were only evaluated during the first year of exposure.

In contrast to the limited evidence for reproductive effects in exposed male adult animals, fertility was markedly reduced in male offspring of rats that were lactationally exposed to $8 mg/kg/day Aroclor 1254 (Sager 1983; Sager et al. 1987, 1991). The reduction in male fertility appears to be due to impaired ability of sperm to fertilize eggs because sperm production, morphology, and motility were not affected and plasma FSH and testosterone concentrations were not reduced (Sager et al. 1987, 1991). Fertility was not impaired in the male offspring of rats that were administered 30 mg/kg/day of Aroclor 1221, 1242, or 1260 by gavage during gestation (Gellert and Wilson 1979), but this study did not include postnatal exposure. Results of oral and subcutaneous studies with single congeners have shown that gestational, lactational, or adult exposures can adversely affect morphology and production of sperm and fertility in male rats and mice (Faqi et al. 1998; Huang et al. 1998a; Smits-van Prooije et al. 1993), although congeneric structure-activity relationships are unclear.

3.2.6 Developmental Effects

This section describes effects of exposure to PCBs on anthropometric measures at birth in humans as well as on physical growth during infancy. For consistency, the discussion of animal data is restricted mostly to these same end points. Effects of perinatal exposure to PCBs on other end points in the offspring, including changes in the thyroid gland and thyroid hormones, neurobehavior, and the immune and reproductive systems, are discussed in the respective sections in Chapter 3.

3.2.6.1 Summary

Anthropometric measures have been evaluated in newborn from women (1) exposed to PCBs through consumption of Great Lakes and ocean fish contaminated with PCBs and other environmentally persistent chemicals, (2) from the general population with no known high exposure to PCBs, (3) occupationally exposed to commercial PCB mixtures, and (4) accidentally exposed to PCBs and structurally-related chemicals. Some studies found significant negative associations between anthropometric measures at birth (and at early ages) and exposure to PCBs, whereas others found significant positive associations, and yet a third group reported no significant associations. The wide range of results may reflect the different degree of controlling for confounders and/or the different exposure measures. Of the studies of

women who consumed contaminated fish from the Great Lakes, only the Michigan study reported an association between reduced birth weight, head circumference, and gestational age in newborns and with body weight at 4 years with prenatal exposure to PCBs (PCBs in cord blood). A study of Lake Ontario fisheaters, which used similar measures of exposure as the Michigan study, found no significant association between birth weight, head circumference, or gestational age and prenatal exposure to PCBs. In two additional studies of Lake Michigan women, fish consumption had a positive effect on birth weight. A study of Swedish wives of Baltic Sea fishermen found an increased risk of low birth weight with increasing maternal blood concentrations of the PCB congener PCB 153 used as surrogate of PCB exposure during the year of childbirth. In a study of the general population in the Netherlands, prenatal exposure to PCBs (PCBs in cord blood) was associated with reduced birth weight, but not with head circumference or height at 10 days of age. Prenatal exposure in formula-fed children was associated with reduced growth between birth and 3 months, but no such association was seen in breast-fed children, suggesting that any detrimental effect observed in newborns due to prenatal exposure to PCBs may have been counteracted by the benefits of breast feeding. No significant association was seen between growth during the ages of 3–7, 7–18, or 18–42 months and any measure of exposure to PCBs. A study of the general population in Finland found no significant association between birth weight and the concentration of PCBs in breast milk. No firm conclusions can be made regarding growth and development of children and environmental exposures to PCBs, although perinatal exposure to high concentrations of PCBs and structurally-related chemicals, as occurred in *Yusho* and *Yu-Cheng*, affects birth weight and growth during early life.

Studies have been conducted in animals exposed to commercial PCB mixtures, single PCB congeners, and a reconstituted PCB mixture with a composition of congeners similar to the pattern found in human breast milk. The results of these studies suggest that primates are much more sensitive to the effects of perinatal exposure to PCBs than rodents. It also appears that unless very high doses are used, PCBs are not teratogenic. In general, studies in rodents have used relatively high doses of PCBs. Data in rodents treated with commercial PCB mixtures showed that developmental toxicity can occur in the absence of overt signs of maternal toxicity. Limited data from a study in rats exposed during gestation showed that Aroclor 1254 was more potent than Aroclor 1260 in reducing survival of the pups to weaning. These two Aroclors differ primarily in that Aroclor 1254 lacks congeners with 7–9 chlorines. Reduced birth weight was reported in offspring from Rhesus monkeys treated before mating and during gestation with low doses of commercial PCB mixtures. These monkeys also showed characteristic signs of PCB intoxication such as hyperpigmentation. In all of the monkey studies, signs of PCB intoxication were also evident in the mothers.

3.2.6.2 Human Studies

3.2.6.2.1 Growth and Development

3.2.6.2.1.1 Contaminated Fish Consumption

The Michigan Cohort. Birth weight, length, and gestational age were evaluated in 313 newborn infants in the Michigan study (Fein et al. 1984a, 1984b). A detailed description of the study design is presented in Section 3.2.4 Neurological Effects. Briefly, of the 313 infants, 242 were born to mothers who had consumed moderate to large quantities of Lake Michigan fish sometime during their lives, and 71 were born to mothers who did not consume Lake Michigan fish. In the exposed group, mean fish consumption, estimated by recall and duration of consumption, was 6.7 kg/year and 15.9 years, respectively; this rate is equivalent to two or three salmon or lake trout/month (Fein et al. 1984a, 1984b). Consumption during pregnancy was 4.1 kg/year. The mean PCB level in maternal serum among those eating Lake Michigan fish was 6.1 ppb (SD=3.7), while the mean among those reporting no fish consumption was 4.1 ppb (SD=2.7). The mean PCB residues also were significantly higher in breast milk samples from the fisheaters as compared to the nonfisheaters, 865.6 ppb (fat basis) versus 622.2 ppb (Fein et al. 1984a). Data on approximately 37 potential confounders, including smoking during pregnancy, were considered in the study analyses (Fein et al. 1984a, 1984b).

Overall, lower birth weight, smaller head circumference, and shorter gestational age were positively correlated with consumption of fish and levels of total PCBs in cord serum; however, when the two populations were divided according to the cord serum levels, the great majority in the low-level group were fisheaters, suggesting that fish consumption rates were poor indicators of PCB exposure. Fish consumption only during pregnancy did not predict either birth size or gestational age (Fein et al. 1984b). Approximately 75% of the children were re-examined at age 4 (Jacobson et al. 1990a, 1990b). Levels of total PCBs in maternal milk or cord serum, or total duration of breast-feeding, were not related to height or head circumference at 4 years, but prenatal PCB exposure was associated with lower weight at age 4.

The Oswego Cohort. A study similar to the one conducted in Michigan was initiated in Oswego County (New York) based on babies born between 1991 and 1994 (Lonky et al. 1996) (see also Section 3.2.4.2.1.1, Neurological Effects). Pregnant women were recruited from the office of one obstetric practice and, following interviews, were divided into three groups based on their estimated fish consumption. The high fish consumption group was composed of women who reported having eaten

$40 PCB-equivalent pounds of Lake Ontario fish in their lifetime (n=152) (the same as the Michigan high fish consumption group). The low consumption group reported eating <40 PCB-equivalent pounds (n=243), and the no-fish-consumption group had never eaten Lake Ontario fish (n=164). The mean PCB-equivalent pounds consumed in the high fish group was 388.47 (SD=859.0), while the mean among those in the low-fish-consumption group was 10.14 (SD=17.8). The three groups did not differ with regard to demographic, health, and nutritional data, maternal substance use, infant birth characteristics. The high-fish consuming group had a significantly heavier pre-pregnancy weight than the nonfisheating group. In contrast to findings from the Michigan study, which had higher levels of exposure, birth weight, head circumference, and gestational age were unrelated to fish consumption. In subsequent studies, the investigators analyzed the association between specific groups of PCB congeners (according to degree of chlorination) and neurobehavioral outcomes in the newborn, but they provided no information regarding such analyses being done for birth weight, head circumference, and gestational age.

The Green Bay Wisconsin Study. This study was designed to evaluate the reproductive effects associated with maternal consumption of contaminated Great Lakes fish (Dar et al. 1992). All women between the ages of 18 and 35 with positive pregnancy tests at two Green Bay Wisconsin obstetrical clinics were invited to participate in this study. The recruitment occurred between January 1, 1987 and January 1, 1988. Participants were asked to complete a self-administered questionnaire at the first prenatal visit including questions on fish consumption, socioeconomic status, medical, reproductive, family, and occupational histories as well as a section on maternal behaviors. Of the 1341 eligible women, 1,115 agreed to participate for an overall participation rate of 82.9%. Nonparticipants (n=226) completed a brief questionnaire and were found to have lower education, lower income, and were more likely to be nonwhite. Exposure to PCBs was estimated from fish consumption scores determined from the questionnaire responses and corroborated by serum analyses. Fish consumption scores were calculated for each participant based on the amount and species of fish consumed in the preceding year. Levels of PCBs in the 18 species of fish that participants reported consuming were based on Wisconsin Department of Natural Resources surveys. Estimated PCB mean intake scores were used to establish the exposure variable categories. The low fisheating group (n=522) included women who consumed no locally caught fish, while the medium group (n=401) contained participants whose PCB scores were greater than 0 but less than the 90th percentile. The high exposure group was composed of women whose PCB intake scores were above the 90th percentile (n=104). Neither the actual fish consumption scores nor the means of the PCB scores for each exposure group were reported (Dar et al. 1992).

Due to the high cost of serum PCB analyses, maternal serum specimens were drawn from a random sample of participants (n=100) to assess the validity of the fish consumption variables. Correlation analysis between the fish consumption variables and serum PCB levels yielded a correlation coefficient of 0.666. The sum of the individual congener levels of PCBs in serum ranged from 0.6 to 5.0 ppb. The mean for each exposure group was not provided. The birth size outcomes evaluated in this study included birth weight, birth length, head circumference, ponderal index, and birth weight percentiles for gestational age (method not specified). These data were abstracted from hospital and clinic reports. Only data on birth weight were presented in this publication due to the similarity of results obtained from the other parameters. Multiple regression analyses were performed to assess the relationship between fish consumption and the outcomes of interest. Effect modification was assessed through the inclusion of interaction terms in the models. Factors known to influence birth outcomes were included as potential confounders (sex of child, birth order, smoking, caffeine and alcohol consumption during pregnancy, gestational age, pregnancy weight gain, usual maternal weight, and demographic variables).

Birth weight was found to increase with increasing PCB exposure, based on the fish consumption scores. Maternal weight gain modified the effect of fish consumption (PCB exposure) on birth weight; birth weight increased with fish consumption in women gaining <34 pounds during pregnancy while there was little difference in mean birth weights for the three fish consumption categories in women gaining more than 34 pounds during pregnancy (Dar et al. 1992). Smoking and caffeine consumption were negatively related to birth weight while male infants were found to be slightly heavier than female infants.

The Sheboygan Wisconsin Study. A study of Sheboygan, Wisconsin residents was conducted in 1980 and 1981 to assess the relationship between maternal serum and breast milk PCB levels and infant health, behavior, and development (Smith 1984). Routine testing for PCBs in 1978 along the Sheboygan River in Wisconsin revealed that game fish had PCB levels far in excess of the standard set for fish by the FDA (5 ppm at the time of the study, currently 2 ppm). To ascertain the potential health risks associated with the elevated PCB levels, a study of mother-infant pairs was undertaken in 1980 and 1981. A total of 73 of the mothers were included in the study. The participants were divided into three groups based on their screening survey responses (it is unclear if these groups were meant to be exposure variable categories or simply a description of the study population). Group 1 (n=23 pairs) included women who were breast feeding and ate Lake Michigan or Sheboygan River fish at least twice a month for $3 years. Group 2 (n=39 pairs) included women who were breast feeding and ate Lake Michigan or Sheboygan River fish not more than twice a year (and had not done this for more than 3 years). Group 3 (n=11 pairs)

included women who were not breast feeding and ate Lake Michigan or Sheboygan River fish at least twice a month for 3 or more years.

Maternal serum and a breast milk sample were also taken during each of the mother-infant evaluations. The first evaluation took place during the second month of postnatal life while the second occurred at 4 months of age. Prenatal maternal serum samples were not taken for any of the participants. The maternal survey instrument included questions on demographic variables, work history, medical history including reproductive history and the most recent pregnancy, smoking and alcohol consumption, and general diet. Information on fish consumption also was collected from this questionnaire and included data on species, amount, and frequency of fish meals. The data collected on the infants included a health history, dietary history, and growth and development assessment (Smith 1984). Statistical analyses included preliminary descriptive analyses using t-tests and chi-square tests. Multiple regression and logistic regression were used for the final models.

The mean level of PCBs in the first maternal serum was 5.76 ppb (range=1.29–14.9 ppb) while the mean for the second was 5.48 ppb (range=1.15–14.1 ppb). These levels indicated a low exposure level (Smith 1984). The means were very similar between the three groups of mother-child pairs. The mean breast milk PCB levels (fat basis) in the first sample of Sheboygan women was 1.13 ppm (range=0.29–4.02 ppm) while the mean for the second sample was 1.14 ppm (range=0.34–3.79 ppm).

Several variables were significant predictors of serum PCB levels (first sample) in linear regression modeling. These included the mother's education, a fish diet after birth, occupation, total bilirubin, cholesterol, and phosphorus (negatively associated). Regression analyses indicated that serum PCB level and cholesterol were significant predictors of the first breast milk sample PCB level. Regression analyses examining the relationship between birth weight and serum PCB levels (first sample) found that maternal serum PCB level was positively associated with birth weight after controlling for gestational age, smoking, and mother's weight. Fish consumption was not included as a variable in this analysis. In this investigation, the first breast milk sample was collected 2 months postnatal, and breast feeding would decrease serum concentrations. Therefore, a major confounder not adjusted for was breast feeding duration.

The Wives of Swedish Fishermen Cohort Study. A study of the wives of fishermen from two established cohorts was conducted to investigate whether east coast wives with a presumably higher intake of fatty fish (and PCBs) were more likely to have adverse reproductive outcomes than those living on the west

coast and elsewhere in Sweden (Rylander et al. 1995). Cohorts of fisherman from the east and west coasts of Sweden were established based on membership in fishermen organizations from 1930 forward. Through linkage to the national Swedish population register and to registers at the local parish offices, 1,568 women from the east coast and 4,027 from the west coast who were, or had been, married to these fishermen were identified. From 1973 to 1991, 757 women from the east coast cohort gave birth to 1,501 infants while 1,834 women from the west coast cohort gave birth to 3,553 infants.

Exposure to PCBs was represented by a variable called "east coast affiliation." The east coast of Sweden borders the Baltic Sea, a source of fatty fish (salmon and herring) thought to be contaminated with persistent organochlorine compounds. The west coast of Sweden is thought to have less contamination than the east. The study was designed as a retrospective cohort investigation comparing children born to east coast women (exposed) and west coast women (unexposed) who were, or had been, married to fishermen. The majority of the data in this study was collected from the Swedish Medical Birth Registry, which includes information on maternal demographics, smoking during pregnancy, prenatal care, delivery, and pediatric assessments of the newborns. The principal end points evaluated in this study were birth weight, birth length, and head circumference. Two cutpoints were used as an indicator of low birth weight, 2,500 and 3,000 g.

Smoking was less frequent among west coast women during early pregnancy than among east coast women (22.7 versus 38%). In addition, there was a higher proportion of short women (<165 cm) among the east coast cohort as compared to the west (48.6 versus 39.2%). Weight distributions during early pregnancy were similar for both cohorts.

In order to assess the dietary habits of both the east and west coast fishermen's wives, interviews were conducted with a 5% random sample (n=38) of east coast cohort members and 2% random sample (n=31) of west coast cohort members. Equal numbers of female residents from the general population were also interviewed; these "control" women were matched to the east and west coast wives by age and county of residence. Both the east and west coast women, who were married to fishermen, ate locally caught fish, both lean and fatty species, twice as often as their referents. The fishermen's wives also consumed about 3 times the amount of fatty fish species/month as their referents.

Among the fishermen's wives, west coast cohort members ate significantly more lean fish species than the east coast cohort members. There were no statistically significant differences between the east and west

coast fishermen's wives in the total number of fatty fish meals eaten/month or in the amount of fatty fish consumed on a monthly basis.

Comparison between the cohorts showed that birth weight and head circumference, but not length at birth, were slightly reduced ($p<0.001$) in the east coast group. The effect on head circumference was observed even when multiple births and infants with major malformations were excluded. Odds ratios were calculated to evaluate the effect of cohort affiliation on low birth weight using a stratified analysis to control for confounders. East coast affiliation was significantly associated with low birth weight (<3,000 g) even after adjustment for gender of the child, maternal age, parity, marital status, and smoking. Stratified analyses for head circumference and birth length were not presented in this report.

In a more recent publication from this group, the authors examined the association between the concentration of 2,2',4,4',5,5'-hexaCB (PCB 153) in maternal serum during the year of childbirth and birth weight of 57 east coast low birth weight cases and 135 controls matched on gender, parity, and calendar year of birth (Rylander et al. 1998b). In 1995, blood samples were collected from the wives and ex-wives of fisherman from the east cost who had given birth during the period of 1973–1991. PCB 153 in maternal blood was used as biomarker of exposure to PCBs and the concentration during the year of childbirth was estimated using kinetic models. The median concentration in serum (fresh weight) in 1995 was 1.0 ppb for the case mothers compared to 0.92 ppb for control mothers. Rylander et al. (1998b) found an increase in the risk of a low birth weight at maternal blood PCB 153 concentrations of 300 and 400 ng/g (ppb, lipid basis).

3.2.6.2.1.2 General Population Exposure

The North Carolina Breast Milk and Formula Project. The North Carolina Breast Milk and Formula Project (NCBMFP) is a cohort study designed to assess the relationship between exposure to prenatal and postnatal PCBs and DDE and growth and development in infants and children (Rogan et al. 1986a, 1986b). A detailed description of this cohort study in presented in Section 3.2.4.2.1.2 (Neurological Effects). Briefly, the participants were administered a questionnaire while in the hospital following delivery. Maternal serum, cord blood, and placenta samples were collected at birth as well as colostrum, breast milk, or formula. The first follow-up visit occurred at 6 weeks with subsequent evaluations at 3 and 6 months postpartum. Breast milk or formula was collected at each of these visits. PCB levels in milk at birth averaged around 1.8 ppm (fat basis). A total of 912 children were available with at least

partial neonatal information (Rogan et al. 1986a). The outcomes evaluated in the neonatal period included birth weight and head circumference.

The relationships of birth weight and head circumference to PCB levels were assessed by multiple regression. The covariates (potential confounders) included in the analyses were infant race, sex, mother's age, education, occupation, smoking, alcohol consumption, prior pregnancies, maternal weight, center enrolling the participant, and jaundice. The analysis of head circumference also included the birth weight variable (Rogan et al. 1986a). The multiple regression analyses found no associations between birth weight or head circumference and PCB level. The birth weight decrement was noted for smokers as was the male-female difference. Larger mothers also had significantly larger babies. Head circumference was associated with the infant's birth weight and sex, and the mother's education and occupation (Rogan et al. 1986a).

The Dutch Mother-Child Study. The Dutch Mother-Child Study was designed as a prospective study to assess the possible adverse effects of prenatal and postnatal PCB and dioxin exposure. Details of this study are presented in Section 3.2.4 (Neurological Effects). Briefly, 207 pairs (105 breast-fed and 102 formula-fed) were from Rotterdam, a highly industrialized area, while 211 pairs (104 breast-fed and 107 formula-fed) were from Groningen, a semi-urban area in northern Holland (Koopman-Esseboom et al. 1994b). The exposure variables used in this study were maternal serum and milk samples as well as cord blood specimens.

The effect of prenatal cord blood PCB exposure on birth size at 10 days of age was evaluated using a series of linear regression models (one for each outcome) in 207 mother-infant pairs from Rotterdam. Covariates included in the models were parity, gestational age, smoking, alcohol use during pregnancy, and a factor representing parental height. Gender, an important determinant of size, was not included among the covariables for some reason even though there were significantly more boys in the breast-fed (59%) than in the formula-fed group (46%). This may have resulted in confounded effect measures. A significant decrease in birth weight at 10 days of age was observed with cord blood PCB exposure; birth weight declined by a mean of 86 g at the 50th percentile of exposure relative to the 10th percentile exposure levels while a mean 165 g decrease was observed for those in the 90th percentile of exposure relative to the 10th percentile. Head circumference and height at 10 days of age were not significantly associated with cord blood PCB levels. Similar effects were observed when using maternal plasma PCB levels as the exposure variable with weight, height, and head circumference as outcomes at 10 days of age (Patandin et al. 1998).

The effect of prenatal PCB exposure on growth rate was assessed in the formula-fed group (n=102) using linear regression modeling. Covariates were identical to those described in the previous paragraph, with the addition of the relevant variable value at 10 days of age in each model (e.g., change in birth weight between 0 and 3 months included birth weight at 10 days of age). Gender was not included in these models. Cord blood PCB levels showed a significant inverse association with growth rate at 0–3 months of age for each index (i.e., birth weight, height, and head circumference). Similar findings were observed when maternal plasma PCB levels were used as the exposure. Prenatal PCB levels in formula-fed children were not significantly associated with growth rate for any of the indices from 3–7, 7–18, or 18–42 months (Patandin et al. 1998).

The associations between growth rates and prenatal and postnatal PCB/dioxin levels also were evaluated in 107 children from Rotterdam who were breast-fed. Both cord blood levels and postnatal breast milk levels were included in these models as were the covariates described above. PCB levels (prenatal or postnatal) were not associated with growth rates at 0–3 months of age. Postnatal PCB/dioxin levels were negatively associated with a change in height between 3 and 7 months ($p=0.04$), but not with weight or head circumference growth rates. Pre- and postnatal PCB levels were not associated with changes in growth rate between 7–18 and 18–42 months of age in the breast-fed children (Patandin et al. 1998).

Finnish General Population Study. This study was part of follow-up studies into levels of dioxins, dibenzofurans, and PCBs in human milk coordinated by WHO/EURO. The objectives of the study were to correlate the birth weight and sex of a child to dioxins/dibenzofurans and PCB concentrations of its mother's milk and to evaluate personal and environmental determinants that correlated with the levels of these chemicals in human milk in two areas in Finland, an urban area and a rural area (Vartiainen et al. 1998). One hundred sixty-seven random human milk samples were collected 4 weeks after delivery for 2 weeks. Information on each mother and child was gathered by a questionnaire that included questions on all relevant covariates.

The concentration of PCBs in breast milk from urban and rural mothers was approximately 500 and 400 ppb (fat basis), respectively. The average weight for all children was 3,630 g and the median was 3,625 g. The mean weight of the urban children was not significantly different from rural children, although dioxin international TEQs were significantly higher in milk from urban mothers. No correlation was found between the weight of children and total PCBs in all of the children, in boys, in girls, among all primiparae, or in primiparae girls or boys. The birth weight, especially of boys, slightly decreased

with increasing concentrations of TEQs, 2,3,4,7,8-penta CDF, 1,2,3,7,8-pentaCDD, and 2,3,7,8-TCDD, but when the analysis was restricted to primiparae, the correlation lost statistical significance.

3.2.6.2.1.3 Occupational Exposure

Upstate New York Capacitor Manufacturers Study. This study comprised women workers of two facilities of the same company located in adjacent communities in upstate New York that manufactured capacitors using PCBs with Aroclors 1254, 1242, and 1016 as their primary dielectric fluid (Taylor et al. 1984). Birth certificates for pregnancies between 1958 and 1975 were used to obtain information on birth weight, maternal age, parity, year of birth, race, sex, and date of the last menses. The high-exposure workers were directly exposed to Aroclors during the manufacturing process for at least 1 year prior to birth of the infant; the workers with low exposure were employed in areas where Aroclors were not used directly.

Fifty-one infants born to 39 women with high exposure to PCBs had lower mean birth weights and shorter mean gestational ages than those of 337 infants born to 280 women with low exposure to PCBs. After adjusting for gestational age, however, the difference in birth weight was markedly reduced, suggesting that the difference in weight may have resulted partially from a shortened gestation period. Furthermore, while the infants born to the high-exposure women were, on the average, lighter than matched community controls, those born to low-exposure women were heavier than matched community controls; thus, a dose-response relationship was not established. While relatively little detail was given regarding the statistical analysis of the results, Taylor et al. (1984) state that they had no information on tobacco use, underlying medical conditions, maternal height, and history of low birthweight, all factors known to influence birthweight. In a follow-up study of the same population in which most of these confounders were accounted for, a significant effect of high-homolog exposure was seen for birth weight and gestational age (Taylor et al. 1989). The difference in birth weight between the two groups was 60 g. However, when gestational age was accounted for in addition to the other variables related to birth weight, estimated serum PCB was no longer a significant predictor of birth weight. Taylor et al. (1989) concluded that the data suggested a significant relation between increased estimated PCB level and decreased birth weight and gestational age, and that the decrease in birth weight is partially related to shortened gestational age.

3.2.6.2.1.4 Accidental Exposure

Yusho and Yu-Cheng. Decreased birth weight was a commonly reported effect of *Yusho* and *Yu-Cheng* exposure (Funatsu et al. 1971; Lan et al. 1987; Rogan 1989; Taki et al. 1969; Yamaguchi et al. 1971). A survey of 128 children known to have been *in utero* during or after *Yu-Cheng* exposure found that mean birth weight was decreased by approximately 15% compared to a group of 115 unexposed controls (Rogan et al 1988). Exposed children also were shorter than controls; these children were a few months to 6 years old. Lan et al. (1987) documented the decreased birthweight of 49 *Yu-Cheng* children exposed transplacentally and born between 1979 and 1986, and showed that the deficit continued through the second child born after the outbreak, but was not detectable in the third. In a review of the *Yusho* poisoning incident, Masuda (1994) stated that most babies were small-for-date and their postnatal growth curves were similar in shape to the national standard curves, but lower for some of the babies. Relative to unexposed controls, height and weight gains of school children with *Yusho* significantly decreased after the poisoning, and the same tendencies were observed in some of the girls (Masuda 1994). These tendencies to reduced growth were later found to be reversed, as subsequent increaments tended to be close to the average value in the control group (Yoshimura and Ikeda 1978). It should be kept in mind that in both the *Yusho* and *Yu-Cheng* poisoning episodes, there was exposure to relatively high concentrations of CDF and PCQ impurities. Further information on *Yusho* and *Yu-Cheng* incidents can be found in ATSDR (1994).

3.2.6.2.2 Evaluation of Human Studies

Anthropometric measures have been evaluated in children from women (1) who consumed Great Lakes fish contaminated with PCBs and other chemicals (Dar et al. 1992; Fein et al. 1984b; Jacobson et al. 1990a, 1990b; Lonky et al. 1996; Smith 1984), (2) who consumed Baltic Sea fish contaminated with organochlorines (Rylander et al. 1995, 1998b), (3) from the general U.S. (Rogan et al. 1986a, 1986b), Dutch (Patandin et al. 1998), and Finnish populations (Vartiainen et al. 1998), (4) who were occupationally exposed to commercial PCB mixtures (Taylor et al. 1984, 1989), and (5) who were accidentally exposed to PCBs and other structurally related chemicals in the *Yusho* and *Yu-Cheng* poisoning incidents (Funatsu et al. 1971; Lan et al. 1987; Rogan 1989; Yamaguchi et al. 1971). A comparison of PCB levels in blood and breast milk in some of these studies is included in Appendix A.

The results have been varied, with some studies finding significant inverse associations between exposure to PCBs and anthropometric measures at birth (and at early ages), some studies reporting significant

positive associations, and yet a third group reporting no significant associations. The wide range of results may reflect the different degree of controlling for confounders and/or the different exposure measures, levels, and substances. Also, in some cases, the difference in serum PCB levels between case studies and controls may have been too small to allow detection of differences between the variables measured. Of the studies of women who consumed contaminated fish from the Great Lakes, the Michigan study (Fein et al. 1984b; Jacobson et al. 1990a, 1990b) reported an association between reduced birth weight, head circumference, and gestational age in newborns and with body weight at 4 years with prenatal exposure to PCBs (PCBs in placental cord blood).

A study of wives of Swedish fishermen found that newborn infants born to mothers from the east coast (Baltic Sea) gave birth to infants with significantly lower birth weight and head circumference compared to infants born to mothers from the west coast (Rylander et al. 1995). East coast mothers were reported to consume more contaminated fatty fish (and PCBs) than women from the west coast where fish contamination was much less. In a subsequent study of east coast/Baltic Sea mothers (Rylander et al. 1998b), their low birth weight infants (1,500–2,750 g weight) were compared with control infants from this same cohort (3,250–4,500 g weight). Blood samples from the mothers were analyzed for PCB congener 153, which was used as a surrogate of total PCB exposure during the year of childbirth. The mothers of the low birth weight infants were reported to have a higher median PCB blood level (1,000 pg/g) compared to the control mothers (920 pg/g). These researchers also found an increased risk of low birth weight with increasing maternal blood concentrations of PCB 153 (at 300 or 400 ng/g, lipid basis).

In the Dutch general population cohort, prenatal exposure to PCBs (PCBs in cord blood) was associated with a reduced birth weight, but not with head circumference or height at 10 days of age (Patandin et al. 1998). Prenatal exposure (as measured by cord and maternal blood PCB levels) in formula-fed children was associated with reduced growth (weight, length, and head circumference) between birth and 3 months. No such association was seen in breast-fed children, suggesting to the investigators that any detrimental effect observed in newborns due to prenatal exposure to PCBs may have been counteracted by the benefits of breast feeding. Additionally, there were no significant associations between growth during the ages of 3–7, 7–18, or 18–42 months and any measure of exposure to PCBs. In an occupational study, Taylor et al. (1989) studied high PCB exposed females employed in capacitor manufacturing facilities and compared them with female workers in low PCB exposure jobs. A significant association was observed between the increased estimated PCB exposure level and decreased birth weight and gestational age. In addition, effects on birth weight and growth during early life have been demonstrated

following perinatal exposure to high concentrations of PCBs and structurally-related chemicals during the *Yusho* and *Yu-Cheng* poisoning incidents.

Consumption of PCB-contaminated fish had a positive effect on birth weight in two studies of Lake Michigan women (Dar et al. 1992; Smith 1984). This finding could be related to the beneficial effects of certain fatty acids in fish (Olsen et al. 1990). In one of these studies (Smith 1984), the concentration of PCBs in breast milk was higher than in breast milk from women from the Michigan cohort (1.13 vs 0.87 ppm) in the Jacobson study discussed above. In the other study (Dar et al. 1992), fish consumption levels were less than in the Jacobson study.

In the Oswego cohort of Lake Ontario fish consumers (Lonky et al. 1996) there was no significant association between prenatal exposure to PCBs, assessed by the same fish consumption measures as in the higher exposure Michigan study, and birth weight, head circumference, or gestational age. In addition, a study of the general population in Finland found no significant association between birth weight and the concentration of PCBs in breast milk (Vartiainen et al. 1998). In this study, the mean concentration of PCBs in milk (0.4–0.5 ppm) was slightly lower than in the Dutch general population study (0.62 ppm) (Koopman-Esseboom et al. 1994b; Patandin et al. 1998).

For those studies with effects, there is consistency in the outcome of lower birth weight for infants exposed *in utero* to maternal body burdens of PCBs. This association remains constant regardless of the method by which PCB exposure is measured (e.g., estimate by fish consumption or actual body burdens in maternal blood). Jacobson et al. (1990b) have demonstrated that, even at the age of 4 years, the children most highly exposed to PCBs weighed less on the average than those with the least exposure. This tendency can also be seen in the Dutch study (Patandin et al. 1998), which reported a significant association between lower infant growth rate in 0–3 month olds and mothers' body burden as demonstrated by cord and maternal PCB levels. The consistency with which this finding has been demonstrated strengthens the position that PCBs (and related substances) are developmental toxicants. In addition, birth weight is a sound indicator of newborn development and health.

3.2.6.3 Animal Studies

Developmental effects discussed in this section are restricted mainly to effects on fetal development, birth weight and weight gain in early life, and teratogenicity. Information regarding effects on the thyroid, immune system, and reproduction in offspring following perinatal exposure to PCBs is presented in Sections 3.2.3, 3.2.4, and 3.2.5, respectively.

The highest NOAEL values and all reliable LOAEL values for developmental effects for each study are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.6.3.1 Birth Weight and Early Development

Oral Exposure

Commercial PCB Mixtures. Doses of #100 mg/kg/day Aroclor 1254 administered on Gd 6–15 by gavage did not affect maternal weight gain nor induce developmental toxicity in Wistar rats, as evidenced by number of litters, litter size and weight, and number of resorption sites (Villeneuve et al. 1971). Morse et al. (1996b) also reported that treatment of Wistar rats on Gd 10–16 with up to 25 mg Aroclor 1254/kg/day by gavage did not affect maternal body weight, fetal body weight, number of live fetuses, late gestational death, number of resorptions, number of live pups born, sex ratio, and postnatal death. However, a study with Aroclor 1254 in Holzman rats reported a significant reduction in fetal weight at 5 mg/kg/day and reduced fetal survival at 15 mg/kg/day after dosing also on Gd 6–15; both effects were dose-related (Spencer 1982). A dose of 2.5 mg/kg/day was without effect. Maternal body weight loss occurred at 30 mg/kg/day. Decreased survival of pups to weaning was found in Sherman rats administered nine doses of 100 mg Aroclor 1254/kg by gavage on Gd 7–15 (Linder et al. 1974), but that treatment did not affect the number of litters or the litter size at birth. Doses of #50 mg/kg/day did not affect survival at weaning. No effect on the number of litters, litter size, survival to weaning, or body weight at weaning were observed in the offspring of rats treated with doses of 100 mg/kg/day Aroclor 1260 on Gd 7–15 (Linder et al. 1974). Offspring from Long-Evans rats exposed to 4 mg Aroclor 1254/kg/day via the diet starting 50 days prior to mating until birth had significantly reduced weight at birth, and on PND 7, 14, and 21 (Hany et al. 1999b). This was observed in the absence of any overt sign of maternal toxicity. There were no significant treatment-related effects on number of pups/litter, number of implantation sites, or sex ratio.

Litter size was significantly reduced in Osborne-Mendel rats fed a diet that provided approximately 25 mg Aroclor 1254/kg/day during gestation and lactation (Collins and Capen 1980c). Body weight of pups at 21 days was significantly reduced with dietary PCB levels of 2.5 and 25 mg/kg/day, but was not affected at birth or at 7 and 14 days. Doses of approximately 13.5 mg Aroclor 1254/kg/day administered during gestation and lactation induced high early mortality in the pups (Overmann et al. 1987). Doses of approximately 1.3 mg/kg/day significantly decreased pups' weight on PND 21 and 14, and doses of 0.13 mg/kg/day decreased pups' weight on PND 14 (Overmann et al. 1987). Doses of 1.3 mg/kg/day had no significant effect on maternal weigh or food consumption. High early mortality was also observed in pups from Long-Evans rats treated with 4 or 8 mg Aroclor 1254/kg/day from Gd 6 through PND 21 (Goldey et al. 1995). Wistar rats that were treated with 10 mg/kg/day Aroclor 1254 by gavage during gestation had delayed parturition and decreased litter size (Brezner et al. 1984). This dose level resulted in no weight gain in the dams. Offspring of these rats that were exposed throughout lactation experienced decreased pre- and postweaning survival, premature vaginal opening, and delayed first estrus, but no effects on sexual differentiation, estrus cycle, mating, or pregnancy.

Offspring of mice exposed to doses up to 12.5 mg Aroclor 1254/kg/day on Gd 6–18 did not show adverse developmental effects, as judged by number of litters, number of dead and reabsorbed fetuses, fetal weight, incidence of gross malformations, and skeletal development (Welsch 1985). A single, but much higher dose (244 mg/kg) of Aroclor 1254 given on Gd 9 to pregnant mice significantly increased the percentage of fetuses with hydronephrosis, did not induce cleft palate, and did not affect the number of resorptions and number of dead and live fetuses (Haake et al. 1987). Maternal weight gain was not influenced by PCB treatment (Haake et al. 1987).

Doses up to 10 mg Aroclor 1254/kg/day administered to rabbits on Gd 1–28 did not induce developmental toxicity, as monitored by total number of fetuses, number of viable fetuses or resorption sites, fetal weight, fetal liver weight, and placental weight (Villeneuve et al. 1971). Doses of 12.5 mg/kg/day or higher, however, significantly increased the number of dead fetuses, resorption sites, and fetuses aborted. Aroclor 1248 did not affect litter size, appearance, or postnatal mortality in New Zealand rabbits administered doses of #91 mg/kg/day in the diet for 11 weeks, but a dose of 28 mg/kg/day induced focal liver necrosis in the offspring (Thomas and Hinsdill 1980).

Administration of 2.2 mg of Clophen A50/kg/day by gavage to pregnant guinea pigs during Gd 16–60 caused high incidence of fetal mortality, but did not cause maternal lethality or any other overt sign of maternal toxicity (Brunstrom et al. 1982). Also, administration of 2.5 mg/kg/day Clophen A50 (50%

chlorine by weight) to guinea pigs on Gd 18–60 significantly increased the frequency of dead fetuses and stillbirth (27.4 versus 9.4% in controls), decreased litter size, and decreased maternal weight gain during gestation and birth weight (Lundkvist 1990). First vaginal opening was abnormal in the surviving offspring, occurring at an older age and with a shorter duration, but there was no significant effect on age at first ovulation.

A dose of 0.1 mg/kg/day Aroclor 1254 administered in a juice-oil emulsion to two Cynomolgus monkeys starting at Gd 60 resulted in delivery of dead, term infants after approximately 105 days of dosing (Truelove et al. 1982). The infant of one monkey that was similarly treated with 0.4 mg/kg/day had reduced birth weight and weight gain, and later died of bronchopneumonia at 139 days of age. The only sign of maternal toxicity was loss of fingernails. Resorption and/or abortion occurred in monkeys that were bred 3 months following dietary treatment with 0.8 mg/kg/day Aroclor 1248 for 2 months (Allen et al. 1974a). Chronic developmental data are limited to studies in monkeys. In these studies, the exposure period ranged from 12 to 37 months. Pregnant Rhesus monkeys that were fed a diet that provided approximately 0.007 or 0.03 mg/kg/day Aroclor 1016 for a total of 12 months (before mating and during gestation) experienced uncomplicated pregnancies, carried their infants to term, and delivered viable offspring (Barsotti and Van Miller 1984). Information regarding maternal body weight and age was not provided. Head circumference and crown-to-rump length were not affected by treatment with Aroclor 1016, but mean birth weight in the high-dose group was significantly lower than in controls. Both groups of neonates showed hyperpigmentation. At weaning, body weight in the high-dose group was still lower than in controls, but the difference was not statistically significant. Dose-related early abortions were reported in female monkeys fed a diet that provided 0.1 or 0.2 mg/kg/day Aroclor 1248 for 15 months (five of eight in the low-dose group and four of six in the high-dose group); this period included breeding, gestation, and lactation (Allen and Barsotti 1976). Mean birth weight in both groups was significantly lower than in controls and remained low for the next 12 weeks. Skeletal development was not affected by PCB treatment. At 2 months of age, the infants had signs of PCB intoxication such as facial acne, swollen eyelids, loss of eyelashes, and hyperpigmentation of the skin, and three of the six infants died of PCB intoxication between days 44 and 329. Gross and microscopic examination of the major organs revealed a rudimentary thymus, extremely small spleen lymph nodes, hypocellularity of the bone marrow, and degenerative changes in the liver. Maternal toxicity, evidenced as facial acne, swollen eyelids, and lack of facial hair, was observed at weaning. One year after receiving a control diet, the same females from the Allen and Barsotti (1976) study were bred again (Allen et al. 1980). Mean birth weights of the infants of the former high-dose mothers were significantly lower than those of controls, and signs of PCB intoxication (hyperpigmentation about the hairline) developed during suckling. Early

infant mortality was also observed (two of four in the former low-dose group and two of seven in the former high-dose group). Histological examination of infant tissues showed hypocellularity of the thymus and of lymph nodes in the spleen and hyperplastic gastritis.

Arnold et al. (1995, 1997) treated female monkeys with Aroclor 1254 for 37 months (0.005, 0.02, 0.04, or 0.08 mg/kg/day) after which time they were mated with untreated males; dosing continued through mating and gestation. Treatment ceased when the infants were 7 weeks old. The young monkeys were sacrificed at the age of 122 weeks. Statistical analysis of the results showed a significant increasing dose-related trend in fetal mortality incidence rates (combined fetal and postpartum deaths). However, when only the treated groups were compared, there was no evidence of such a trend. Results of the Fisher's exact test showed a significant increase rate for only the highest dose group with the 0.02 mg/kg/day dose approaching significance. Mean birth weight was not significantly affected by maternal treatment with Aroclor 1254. The major clinical findings in the offspring from treated females were the presence of inflammation and/or enlargement of the tarsal (Meibomian) gland, nail lesions, and gum recession.

Single Congeners. No effect on maternal body weight or on gestational length, litter size, percent live births, birth weight, or pup weight at weaning was observed following administration of 0.001 mg/kg/day PCB 126 or 8 mg/kg/day PCB 77 to pregnant Sprague-Dawley rats on Gd 10–16 (Seo et al. 1995). Administration of 0.001 mg/kg/day of PCB 126 to Long-Evans rats beginning 5 weeks before and continuing through gestation and lactation did not result in any significant effect on neonatal mortality, birth weight, litter size, or on weight gain monitored up to PND 60 (Rice 1999a)

Pregnant C57BL/6J mice given up to 21 mg PCB 77/kg/day by gavage on 5 consecutive days beginning on days 1, 6, or 11 of pregnancy showed no adverse effect on maternal body weight or on pup weight, crown-rump length, litter size, sex ratio, day of eye opening, or upper incisor eruption (Rodriguez et al. 1997). Administration of a single gavage dose of approximately 0.8 or 1 mg PCB 126/kg to pregnant C57B46 mice on Gd 10 significantly increased the percentage of fetuses with cleft palate (Zhao et al. 1997b). However, no fetuses with cleft palate were seen after administration of up to 271 mg PCB 153. Combined administration of PCB 126 and PCB 153 significantly reduced the incidence of cleft palate compared to that produced by PCB 126 alone.

Defined Experimental Mixtures. Offspring from Long-Evans rats exposed via the diet to 4 mg/kg of a reconstituted PCB mixture composed according to the congener pattern in human breast milk starting 50 days prior to mating until birth had significantly reduced weight at birth, and PND 7, 14, and 21 (Hany

et al. 1999b). This was observed in the absence of any overt signs of maternal toxicity. There were no significant treatment-related effects on number of pups/litter, number of implantation sites, or sex ratio. Arnold et al. (1999) administered a PCB mixture of congeneric composition similar to that found in Canadian breast milk to Rhesus and Cynomolgus monkeys during the first 20 weeks of life; each infant received 0.0075 mg PCBs/kg/day. There was no statistically significant difference between the control and treated groups for body weight gains throughout the study.

3.2.6.3.2 Evaluation of Animal Studies

Studies in animals suggest that primates are much more sensitive to the effects of perinatal exposure to PCBs than rodents. It also appears that unless very high doses are used, PCBs are not teratogenic in animals. Hydronephrosis was reported in mice treated with a single dose of 244 mg Aroclor 1254/kg on Gd 9 (Haake et al. 1987) and increased incidence of fetuses with cleft palate was reported by Zhao et al. (1997b) following treatment of mice with a single dose of 0.8 mg of PCB 126/kg, a dioxin-like congener. Treatment with up to 271 mg/kg of the di-*ortho*-substituted congener PCB 153 did not induce cleft palate (Zhao et al. 1997b). Susceptibility to both hydronephrosis and cleft palate formation by dioxin-like congeners is a trait that segregates with the Ah locus (Hassoun et al. 1984) and Zhao's findings are consistent with this fact.

Data in rats treated with commercial PCB mixtures showed that developmental toxicity can occur in the absence of overt signs of maternal toxicity as evidenced by reduced fetal weight and viability in a study by Spencer (1982) and reduced birth weight and postnatal growth in a study by Hany et al. (1999). It should be noted that Villaneuve et al. (1971) and Morse et al. (1996b) reported adverse developmental effects at Aroclor 1254 doses much higher than those used by Spencer (1982) in similarly designed experiments. It is possible that the Holzman strain of rats, which Spencer (1982) used, is more susceptible than the Wistar rat, which the other two studies used. Hany et al. (1999b), in addition to testing Aroclor 1254, also treated rats with a reconstituted PCB mixture of congeneric composition similar to the pattern found in human breast milk and both mixtures induced similar reductions in birth weight and in weight gain during lactation.

Linder et al. (1974) administered Aroclor 1254 or Aroclor 1260 at the same dose levels on Gd 7–15 to Sherman rats and found that Aroclor 1254 significantly reduced survival to weaning, whereas Aroclor 1260 did not. These two Aroclors differ primarily in that Aroclor 1254 lacks congeners with 7–9 chlorines (Albro et al. 1981), but further information is needed before speculating as to which PCB

congeners might or might not be responsible for neonatal lethality. Studies in rats which included *in utero* and lactational exposure to Aroclors suggest that transfer of PCBs via milk may be considerable, as decreased body weight was seen in the pups after weeks of nursing even though body weight at birth was not significantly different than unexposed rats (Collins and Capen 1980c; Goldey et al. 1995; Overmann et al. 1987). It should be kept in mind that, in general, studies in rats used fairly high doses of Aroclors to the dams such that it is reasonable to assume that breast milk had the potential to accumulate high concentrations of PCBs.

As previously mentioned, monkeys seem to be much more sensitive to developmental effects of PCBs than rodents. Reduced birth weight was reported in offspring from Rhesus monkeys treated before mating and during gestation with 0.03 mg Aroclor 1016/kg/day (Barsotti and Van Miller 1984). These monkeys also showed characteristic signs of PCB intoxication such as hyperpigmentation. Reduced mean birth weight also was reported in monkeys exposed to Aroclor 1248 (Allen and Barsotti 1976; Allen et al. 1980). Doses even smaller of Aroclor 1254 (0.005 mg/kg/day), while not significantly affecting birth weight or growth, produced clear signs of PCB intoxication manifested as skin, nail, and gum lesions (Arnold et al. 1995, 1997). In all of these studies in monkeys, maternal toxicity was also evident.

3.2.7 Genotoxic Effects

3.2.7.1 Summary

The genotoxicity of PCBs has been tested in *in vivo* and *in vitro* studies with generally negative results. End points that have been examined in these studies include gene mutations in bacteria and Chinese hamster V79 cells, chromosomal aberrations in human lymphocytes and rat and mouse bone marrow cells and spermatogonia, micronuclei in mouse bone marrow cells, and dominant lethal mutations in rat sperm cells.

3.2.7.2 *In Vivo* Studies

Available information on *in vivo* genotoxic effects of PCBs in humans is limited by confounding exposures that involved mixtures of chemicals. Chromosomal aberrations and sister chromatid exchanges in peripheral lymphocytes were increased in 32 workers involved in the manufacturing of DELOR 103 and DELOR 106 (Czechoslovakia-made PCBs with three and six chlorine atoms in the biphenyl ring, respectively) for 2–25 years (Kalina et al. 1991). These increases over control values

achieved statistical significance in workers exposed for >10 years. Although control and exposed groups were matched regarding smoking and alcohol drinking habits, the exposed workers were also exposed to benzene and formaldehyde. Another occupational study found a moderately increased incidence of chromatid exchanges in peripheral lymphocytes from a group of 12 workers who were exposed to PCBs following a fire in an electric station (Melino et al. 1992). The authors also observed that the number of chromosome breaks per cell was often higher in the exposed subjects than in unexposed controls. Additionally, lymphocytes from the exposed workers appeared to be more fragile than those from unexposed individuals. However, exposure to toxic chlorinated dioxins and/or furans generated during the fire may have occurred.

Six of 16 workers engaged in cleaning oil from old transformers showed abnormal banding pattern for *fes* oncogene-related proteins; all of the 6 were smokers (Brandt-Rauf and Niman 1988). Since none of the nonsmoking workers (six) showed this pattern, the role of PCBs, if any, in the induction of genetic abnormalities described in this study cannot be ascertained. A control unexposed group was not included in this study.

PCBs gave generally negative results in *in vivo* assays in animals (see Table 3-4). Several studies investigated genotoxic effects in rats following acute oral exposure to PCBs. Single doses #5,000 mg/kg of Aroclor 1242 administered by gavage did not induce chromosome abnormalities in bone marrow cells or spermatogonial cells of rats (Green et al. 1975a). In the same study, doses #750 mg/kg/day Aroclor 1254 administered for a 5-day period did not increase the incidence of chromosomal abnormalities in rat bone marrow cells. Dominant lethal mutations were not induced in male Osborne-Mendel rats following gavage treatment with a single dose of 625–2,500 mg/kg Aroclor 1242, or with five daily doses of 125 or 250 mg/kg Aroclor 1242 or 75–300 mg/kg Aroclor 1254 (Green et al. 1975b). Rats treated with a single dose of 1,295 mg/kg Aroclor 1254 showed evidence of DNA damage in hepatocytes 4–12 hours after treatment (Robbiano and Pino 1981). However, this damage was no longer detectable 48 hours after treatment due to DNA repair. Whysner et al. (1998) administered Aroclor 1260 as a single dose of 50 mg/kg or as a concentration of 200 ppm in the diet for 14 days. Neither the single dose nor the exposure in the diet produced detectable DNA adducts in the liver.

Two studies that examined genotoxic effects of intermediate-duration oral exposure to PCBs were identified. Rats administered 0.25–25 mg/kg/day Aroclor 1254 in their diets for #35 days had no evidence of chromosomal damage in bone marrow and spermatogonial cells (Garthoff et al. 1977). Dietary exposure to 1.25 or 5 mg/kg/day Aroclor 1254 for 70 days did not induce dominant lethal

Table 3-4. Genotoxicity of Polychlorinated Biphenyls *In Vivo*

Species (test system)	End point	Results	Reference	Polychlorinated biphenyl mixture
Mammalian cells:				
Rat spermatogonia	Chromosomal abnormalities	–	Dikshith et al. 1975	Aroclor 1254
Rat spermatogonia	Chromosomal abnormalities	–	Dikshith et al. 1975	Aroclor 1254
Rat hepatocytes	DNA fragmentation	+	Robbiano and Pino 1981	Aroclor 1254
Rat spermatogonia	Chromosomal abnormalities	–	Green et al. 1975a	Aroclor 1242
Rat bone marrow cells	Chromosomal abnormalities	–	Green et al. 1975a	Aroclor 1242
Rat bone marrow cells	Chromosomal abnormalities	–	Green et al. 1975a	Aroclor 1242
Rat sperm cells	Dominant lethal mutation	–	Green et al. 1975b	Aroclor 1242
Rat sperm cells	Dominant lethal mutation	–	Green et al. 1975b	Aroclor 1254
Mouse bone marrow cells	Micronuclei	–	Bruce and Heddle 1979	Aroclor 1254
Mouse sperm cells	Chromosomal abnormalities	–	Bruce and Heddle 1979	Aroclor 1254
Nonmammalian cells:				
Chicken embryos	Chromosomal abnormalities	–	Blazak and Marcus 1975	Aroclor 1242
Ring dove	Chromosomal abnormalities	+	Peakall et al. 1972	Aroclor 1254
Drosophila melanogaster	Chromosomal abnormalities	–	Nilsson and Ramel 1974	Clophen 30
D. melanogaster	Chromosomal abnormalities	–	Nilsson and Ramel 1974	Clophen 50

DNA = deoxyribonucleic acid; – = negative result; + = positive result

mutations in male rats (Green et al. 1975b). Lack of dominant lethality was indicated by no consistent changes in numbers of implantations and dead implantations per pregnant untreated female. The 70-day duration of the feeding study covered the spermatogenic cycle of the rat.

PCBs did not induce chromosomal aberrations when tested in *Drosophila melanogaster* (Nilsson and Ramel 1974) and chicken embryos (Blazak and Marcum 1975), although slight effects were reported in ring dove embryos (Peakall et al. 1972).

3.2.7.3 *In Vitro* Studies

PCBs are generally nongenotoxic in *in vitro* assay systems (see Table 3-5). Aroclor 1254 was not mutagenic in the bacteria *S. typhimurium* with or without exogenous metabolic activation (Bruce and Heddle 1979; Heddle and Bruce 1977; Schoeny et al. 1979). Gene mutations also were not induced by Aroclor 1242 or Clophen A60 in Chinese hamster V79 cells (Hattula 1985). Varying results were found in two assays for Aroclor 1254-induced chromosomal damage in cultured human lymphocytes, but the different findings may be the consequence of a higher test concentration in the positive study (Hoopingarner et al. 1972; Sargent et al. 1989). Aroclor 1254 induced DNA damage in rat liver cells *in vitro* as indicated by an increase in unscheduled DNA synthesis (Althaus et al. 1982), but it was not reported whether the genotoxic doses were also cytotoxic.

Chromosome breakage and micronuclei were not induced in human lymphocytes in whole blood or isolated cultures following *in vitro* exposure to the single congener 3,3'4,4'-hexaCB (PCB 77) (Belpaeme et al. 1996). In another study, PCB 77, but not Aroclor 1254, induced DNA adducts in the Hep G2 human cell line and in primary fetal rat and quail hepatocytes (Dubois et al. 1995).

3.2.7.4 Evaluation of Genotoxicity Studies

The generally negative results of *in vitro* and *in vivo* genotoxicity studies indicate that commercial PCB mixtures are not potent genotoxicants. Although PCBs have been found to be generally inactive as mutagens in *S. typhimurium* strains and in several other tests of genotoxicity that may be predictive of tumor initiation activity, *in vitro* studies with rat microsomes have indicated that metabolism of lower chlorinated congeners can lead to covalently modified macromolecules including proteins and DNA (Hayes 1987; Robertson and Gupta 2000; Silberhorn et al. 1990). Therefore, although the available data

Table 3-5. Genotoxicity of Polychlorinated Biphenyls *In Vitro*

Species (test system)	End point	Results: With activation	Results: Without activation	Reference	Polychlorinated biphenyl mixture
Prokaryotic organisms:					
Salmonella typhimurium (plate incorporation)	Gene mutation	–	–	Schoeny et al. 1979	Aroclor 1254
S. typhimurium (plate incorporation)	Gene mutation	–	–	Heddle and Bruce 1977	Aroclor 1254
S. typhimurium (plate incorporation)	Gene mutation	–	–	Bruce and Heddle 1979	Aroclor 1254
Eukaryotic organisms:					
Chinese hamster V79 cells (tissue culture)	Gene mutation	No data	–	Hattula 1985	Aroclor 1242
Chinese hamster V79 cells (tissue culture)	Gene mutation	No data	–	Hattula 1985	Clophen A60
Human lymphocytes (tissue culture)	Chromosomal abnormalities	No data	–	Hoopingarner et al. 1972	Aroclor 1254
Rat hepatocytes (tissue culture)	DNA repair synthesis	No data	+	Althaus et al. 1982	Aroclor 1254
Human lymphocytes (tissue culture)	Chromosomal damage	No data	+	Sargent et al. 1989	Aroclor 1254

DNA = deoxyribonucleic acid; – = negative result; + = positive result

indicate that PCBs are not potent genotoxicants, there is some experimental support for the possible involvement of genotoxic mechanisms in the development of PCB-induced cancer.

3.2.8 Cancer

3.2.8.1 Summary

The carcinogenicity of PCBs in humans has been investigated in retrospective cohort mortality studies that investigated cancer in exposed workers, and in case-control studies of environmental exposure that examined associations between serum or adipose tissue levels of PCBs and occurrence of cancer. Some of the mortality studies suggest that occupational exposures to PCBs were associated with cancer at several sites, particularly the liver, biliary tract, intestines, and skin (melanoma). A report of liver cancer in *Yusho* victims appears to support the occupational hepatocarcinogenicity data. There is no clear association between occupational exposures to PCBs and cancer in other tissues, including the brain, hematopoietic, and lymphatic systems. Case-control studies of the general population are inconclusive with respect to associations between environmental exposures to PCBs and risk of breast cancer or non-Hodgkin's lymphoma, although there are preliminary indications that particular subgroups of women may be at increased risk for breast cancer. Overall, the human studies provide some evidence that PCBs are carcinogenic. In contrast to the studies in humans, there is conclusive evidence that commercial PCB mixtures are carcinogenic in animals based on induction of tumors in the liver and thyroid.

3.2.8.2 Human Studies

Most of the information on the carcinogenicity of PCBs in humans is available from cohort mortality studies of workers exposed during the manufacture and use of capacitors and case-control studies of breast cancer in women exposed to background levels in the environment.

3.2.8.2.1 Liver, Biliary Tract, and Gall Bladder

Occupational Exposure. A small excess risk of liver-related cancer was found in studies of workers from two capacitor manufacturing plants in New York and Massachusetts (Brown 1987b; Brown and Jones 1981). The workers had completed at least 3 months of employment between 1940 and 1976 in areas of the plants considered to represent the potential for the highest exposure to PCBs. Aroclor 1254 was used at first, but usage was later changed to Aroclor 1242, and finally to Aroclor 1016. Historical exposure

data were not available, but personal time-weighted average concentrations of Aroclor 1016 in 1977 ranged from 0.024 to 0.393 mg/m^3 at Plant 1 and 0.170 to 1.26 mg/m^3 at Plant 2. These data were collected shortly after changes in work practices and engineering controls were effected, and the use of PCBs was reduced to 25% of the 1976 level. The workers were also exposed to additional chemicals, including trichloroethylene, toluene, and methyl isobutyl ketone. The first study (Brown and Jones 1981) included 2,567 total subjects comprised of 968 workers (583 males, 385 females) in Plant 1 and 1,599 workers (675 males and 924 females) in Plant 2. Of these workers, 25.5 and 8.2% in Plant 1 and 19.4 and 8.9% in Plant 2 were exposed for 3–10 years and $10 years, respectively. The second study (Brown 1987b) was conducted after 7 additional years of observation on 2,588 total subjects, comprised of 981 workers (593 males, 388 females) in Plant 1 and 1,607 workers (677 males and 930 females) in Plant 2. Expected numbers of deaths were based on U.S. white male and white female cause-specific mortality rates. A slight increase in mortality due to cancer of the liver, biliary tract, or gall bladder (3 observed/1.07 expected, SMR=280, 95% CI 58–820) that was not statistically significant ($p>0.05$) was found in the first study (Brown and Jones 1981). The follow-up study (Brown 1987b) identified two additional cases of liver/biliary tract/gall bladder cancer, which made the excess statistically significant (5 observed/1.9 expected, SMR=263, CI not reported, $p<0.05$). Four of the five cancer cases occurred in women who worked in Plant 2 (4 observed/0.9 expected, SMR=444, CI not reported, $p<0.05$). Although the four cases in women occurred in the plant with the higher exposure range, there was no clear increase in the risk of liver/biliary tract/gall bladder cancer with increasing latency (time from start of exposure to end of observation) or length of employment; however, the confidence in this analysis is low due to the small numbers of deaths. Reclassification of the data showed that only two of the five deaths were from liver cancer and the remaining three were in the biliary tract (two cases) or gall bladder (one case) (Brown 1987b). Additionally, one of the two liver cancers was not a primary carcinoma as it metastasized from another site (unknown). If the metastatic liver cancer is not included in the analysis, the SMR for liver and biliary tract cancer in the whole cohort loses statistical significance (SMR=210, p$0.05) (Nicholson and Landrigan 1994). Other findings included a slight increase in rectal cancer in the first study, but not in the follow-up (see Section 3.2.8.2.3). Limitations of these studies include small number of deaths, relatively short periods of observation, and possible misclassification of the cause of death because it is not clear in every case if death was due to primary cancer of the liver, biliary tract, or gall bladder.

Liver cancer was not statistically significantly increased in a retrospective cohort mortality study of 7,075 workers from two capacitor manufacturing/repairing plants in New York (Kimbrough et al. 1999a). An unspecified number of the male workers in this study were included in the cohort studied by Brown (1987b) and Brown and Jones (1981) summarized above. The Kimbrough et al. (1999a) cohort was

comprised of hourly workers (2,984 male, 2,544 female) and salaried workers (1,078 male, 469 female) employed for at least 90 days between January 1, 1946 and June 15, 1977, and followed to death or January 31, 1993, whichever came first. Follow-up was essentially complete (98.7%), and the mean age at end of employment ranged from 31 to 35 years in the four subgroups, mean follow-up time was 31 years, mean age of all cohort members alive at the end of follow-up was 57 years, and mean age at death was 62 years. PCB exposures were predominantly to Aroclor 1254 from 1946 to 1954, Aroclor 1242 from 1954 to 1971, and Aroclor 1016 from 1971 to 1977. Exposures were qualitatively classified as high, low, or undefinable based on types and locations of jobs and some area measurements. No personal exposure monitoring was performed, although previously reported data on 290 self-selected workers from one of the plants had serum PCB levels in ranges of 6–2,530 and 1–546 ppb (ng/mL) for lower and higher chlorinated homologs, respectively (Wolff et al. 1982a). Workers with high exposure jobs had direct PCB contact (dermal and/or inhalation), workers with low exposure jobs primarily had inhalation exposure to background levels of PCBs in the plant, and workers with undefinable exposures had exposures that varied depending on where tasks were performed. Exposure-specific analysis was limited to workers with the greatest potential for exposure, (i.e., hourly workers who ever worked in a high-exposure job, worked for at least 6 months in a high-exposure job, or worked for at least 1 year in a high-exposure job). Workers who exclusively worked in high-exposure jobs could not be analyzed as a separate group due to small numbers (112 males, 12 females). SMRs were calculated for hourly and salaried workers by gender, length of employment (6 or 12 months), and latency categories (<20 or $20 years), using age-, sex-, race-, and time-specific U.S. general population rates for comparison. No statistically significant elevations in mortality from cancer of the liver and biliary passages were found in any of the groups, including in the most highly exposed workers, and SMRs for liver/biliary cancer did not statistically significantly increase with length of cumulative employment and latency. SMRs for cancer of the liver and biliary passages were <100 in the male hourly workers (2 observed/2.5 expected, SMR=80, 95% CI 10–289), female hourly workers (2 observed/2.2 expected, SMR=89, CI 11–321), and male salaried workers (1 observed/1.2 expected, SMR=79, CI 2–439), and could not be calculated in the female salaried workers due to no observed cases (0.3 expected). Other findings in this study included a suggestive increase in mortality from intestinal cancer as discussed in Section 3.2.9.2.2. A healthy worker effect was demonstrated by SMRs that were less than expected for mortality from all causes and from all cancers.

Interpretation of the Kimbrough et al. (1999a) findings is complicated by a few study limitations and biases, including some exposure misclassifications related to use of length of employment alone as a surrogate of exposure, potentially insufficient dosage differences between exposed and comparison

groups, a degree of selection bias due to the healthy worker effect that may have resulted in an underestimate of SMRs, concern for low statistical power due to small numbers of deaths from site-specific cancers in some of the groups (e.g., female hourly workers with high exposure and $20 years latency), relatively young age at follow-up, and use of the general population for comparison rather than an internal control group or a group of workers from another company. These issues are discussed by Bove et al. (1999), Frumkin and Orris (1999), and Kimbrough et al. (1999b). Some of the limitations are typical of occupational cohort mortality studies, and strengths of the study include its size (the largest cohort of PCB workers ever studied) and essentially complete follow-up of long duration. Unresolved are the puzzling Kimbrough et al. (1999a) findings of significantly lower than expected mortality from all cancers among males and the lower number of observed cases of liver and biliary tract cancers among females compared to the smaller cohort studied by Brown et al. (1997b), a subset of the same study population. These unresolved findings suggest that ascertainment of cancer mortality was not complete in this study. Overall, the study limitations are sufficient to cast doubt on the negative findings for liver and biliary tract cancer and other site-specific cancers. Increases in mortality from intestinal cancer, rectal cancer, and melanoma are summarized in Sections 3.2.8.2.2, 3.2.8.2.3, and 3.2.8.2.4, respectively.

Mortality from liver and bile duct cancer was increased (2 observed cases/0.78 expected, SIR=256, 95% CI 31–926) in a small group of workers at a Swedish capacitor manufacturing facility (Gustavsson and Hogstedt 1997; Gustavsson et al. 1986). The subjects were exposed to PCBs of 42% chlorine content for an average of 6.5 years between 1965 and 1978. Airborne PCB levels measured on one occasion in 1973 were 0.1 mg/m^3, and dermal exposure was common. The first study of these workers included 142 males and had a median latency time of 13 years (Gustavsson et al. 1986). The second study added 9 years of follow-up and included 242 males (Gustavsson and Hogstedt 1997). Although only two cases were observed in the category for liver and bile duct cancers, both cases were relatively rare bile duct types (a cholangiocarcinoma of the primary bile duct in one high-exposure worker employed for 3 years, and an adenocarcinoma of the Papilla Vaterii in one low-exposure worker employed for 9 years).

Mortality from cancers of the digestive system, which included the liver and biliary tract, was increased in a study of 544 male and 1,556 female workers involved in the manufacture of PCB-impregnated capacitors in a plant in Italy (Bertazzi et al. 1987). The workers were employed for a minimum of 1 week between 1946 and 1978 and were examined during 1946–1982. PCB mixtures containing 54% chlorine (Aroclor 1254 and Pyralene 1476) were used until 1964; these were progressively replaced by mixtures containing 42% chlorine (Pyralene 3010 and 3011) until 1970, when only Pyralene 3010 and 3011 were used. The maximum quantities of PCBs were used in 1967–1968, and the use of PCBs was abandoned

completely since 1980. Area samples taken in 1954 and 1977 showed air PCB concentrations ranging from 5.2 to 6.8 mg/m^3 (Aroclor 1254) and 0.048 to 0.275 mg/m^3 (Pyralene 3010), respectively. Concentrations of total PCBs on workers' hands in 1977 and 1982 ranged from 0.3 to 9.2 and 0.09 to 1.5 µg/cm^2, respectively. Deaths from digestive system cancers were statistically significantly increased in males when compared with national rates (6 observed/1.7 expected, SMR=346, 95% CI 141–721) and local rates (6 observed/2.2 expected, SMR=274, 95% CI 112–572). The digestive system category was not defined, but is not specific for the stomach and intestines as the six cases included cancers of the liver (one case), biliary tract (one case), and pancreas (two cases), as well as stomach (two cases). Follow-up evaluation of the cohort by Tironi et al. (1996) after an additional 9 years of latency found that mortality from digestive system cancers was still increased in comparison to local rates (10 observed/5.1 expected, SMR=195, CI 94–359), although the excess was not as high as found previously. Other findings included increased mortality from hematological neoplasms as discussed in Section 3.2.8.2.6. Limitations of the Bertazzi et al. (1978) and Tironi et al. (1996) studies include questionable grouping of digestive system cancers; small number of cases; short minimum exposure period; lack of pattern or trend when data were analyzed by duration of exposure, latency, and year of first exposure; and some cancer deaths in males with low potential for direct PCB exposure.

Mortality from cancer of the liver, biliary passages, and gall bladder was not increased in the Sinks et al. (1992) study of capacitor manufacturing workers or Loomis et al. (1997) study of electric utility workers summarized in Section 3.2.8.2.4.

Contaminated Fish Consumption. Mortality from liver cancer was not increased in the Svensson et al. (1995a) study of Swedish east coast (Baltic Sea) and west coast fisherman summarized in Section 3.2.8.2.2.

Yusho and Yu-Cheng Exposures. A retrospective study of 887 male and 874 female patients that were observed for an average of 11 years following registration as *Yusho* victims found statistically significantly ($p<0.01$) increased mortality from liver cancer in the males compared to national death rates (9 observed/1.61 expected, SMR=559, 95% CI not reported) (Kuratsune et al. 1987). Elevated mortality from liver cancer was also seen in the females, but the increase was not statistically significant (2 observed/0.66 expected, SMR=304, $p>0.05$). Comparisons based on local death rates also showed a statistically significantly increased mortality from liver cancer in the males (9 observed/2.34 expected, SMR=385, $p<0.01$) but not in females (2 observed/0.79 expected, SMR=253, $p>0.05$), as well as in males when early liver cancer cases (those occurring <9 years after poisoning) were excluded

(4 observed/1.04 expected, SMR=385, p<0.05). However, because the geographic distribution of liver cancer deaths was markedly uneven (there was no significant increase in one of two locations), the cancer could not be conclusively associated with *Yusho* exposure.

A retrospective mortality study of 1940 *Yu-Cheng* cases summarized in Section 3.2.8.2.6 found no statistically significantly increased mortality from cancer of the liver and intrahepatic bile ducts (Hsieh et al. 1996).

3.2.8.2.2 Gastrointestinal Tract

Occupational Exposure. Mortality from cancer of the stomach or intestines was not statistically significantly increased in the Kimbrough et al. (1999a) study of capacitor workers summarized in Section 3.2.8.2.1, although the rate for intestinal cancer (large and small intestine) was elevated and approached statistical significance (20 observed/12.7 expected, SMR=157, 95% CI 96–242) in the hourly female subgroup of workers. Most of these cancers occurred in women with $20 years of latency and the increase in this subgroup was statistically significant (SMR=189, 95% CI not reported, p<0.05). There was no increasing trend with length of employment and the SMR was 100 for women employed for $10 years with a latency period of $20 years (Kimbrough et al. 1999b). Comparison with the regional population resulted in a SMR which is still elevated (SMR=120, 95% CI 74–186) and similar to the SMR of 157 based on the national rates (Kimbrough et al. 1999b). Due to the small number of cases, healthy worker effect bias, and exposure misclassification bias, it is remarkable that an elevation of intestinal cancer was found among hourly women workers. However, this finding must be viewed as suggestive given the limitations of the study.

Deaths from cancers of the digestive system were statistically significantly increased in the Bertazzi et al. (1987) study of capacitor workers summarized in Section 3.2.8.2.1. This category was not specific for the stomach and intestines as it included other parts of the digestive system, including the liver and biliary tract. Of six observed deaths from digestive system cancers, one was due to hepatocellular carcinoma and another from a cancer of the biliary tract.

Mortality from cancer of the stomach or intestine was not statistically significantly increased in other studies of capacitor manufacturing workers (Brown 1987b; Brown and Jones 1981) summarized in Section 3.2.8.2.1, or in the Loomis et al. (1997) study of electric utility workers summarized in Section 3.2.8.2.4. Mortality from cancer of the digestive organs (not otherwise specified) was not

increased in the Sinks et al. (1992) study of capacitor manufacturing workers summarized in Section 3.2.8.2.4.

Contaminated Fish Consumption. Cancer incidences were studied in cohorts of fisherman from the Swedish east coast (on the Baltic Sea) (2,896 subjects) and Swedish west coast (8,477 subjects) (Svensson et al. 1995a). Both cohorts ate almost twice as much fish as the general regional populations, although intake of fatty fish was higher in the east coast fisherman (Svensson et al. 1995b). Plasma levels of PCB congeners, particularly non-*ortho* PCBs, were also higher in the east coast fisherman compared west coast fisherman and referents from both coasts; the sum of non-*ortho*, mono-*ortho*, di-*ortho*, and other congeners expressed as the TEQ was about 2 times higher in the east coast fisherman than in those from the west coast. The incidence of stomach cancer was increased in the east coast fisherman when compared with both the regional general population (Incidence Rate Ratio [IRR]=1.6, 95% CI 1.0–2.4) and the west coast fisherman (IRR=2.2, CI 1.3–3.5). Stomach cancer was not increased in the west coast fisherman when compared to the regional general population. Although the east and west coast fisherman ate almost twice as much fish as controls, and intake of fatty fish and PCBs was higher in the east coast fisherman compared to the west coast fisherman and referents from both coasts, the east coast fisherman also consumed smoked fish (a risk factor for stomach cancer) twice as often as the west coast fisherman (Svensson et al. 1995a).

Yusho and Yu-Cheng Exposures. The retrospective study *Yusho* victims summarized in Section 3.2.8.2.1 found no statistically significant ($p<0.05$) increased mortality from cancer of the stomach or esophagus (Kuratsune et al. 1987). The retrospective study of *Yu-Cheng* victims summarized in Section 3.2.8.2.6 found no statistically significantly increased mortality from cancer of the stomach or small intestine (Hsieh et al. 1996).

3.2.8.2.3 Rectum

Occupational Exposure. An elevation in rectal cancer was found in the first of two studies of two capacitor manufacturing plants in New York and Massachusetts (Brown 1987b; Brown and Jones 1981). Background information on these studies is provided in Section 3.2.8.2. Brown and Jones (1981) found that rectal cancer mortality was statistically significantly ($p<0.05$) increased in 1,309 females (3 observed/ 0.5 expected, SMR=600, 95% CI not reported) from Plant 2, but not among 675 males from Plant 2 (0 observed/0.20 expected), 924 males from Plant 1 (1 observed/0.31 expected, SMR=323, 95% CI not reported), 385 females from Plant 1 (0 observed/0.18 expected), or 2,567 total males and females from

both plants (4 observed/1.19 expected, SMR=336, 95% CI 92–860). Follow-up evaluation (Brown 1987b) of 2,588 total workers from both plants after a further 7 years of observation found no additional deaths from rectal cancer (4 observed/1.9 expected, SMR=211), and the excess in the females from Plant 2 was no longer statistically significant because of the small numbers of observed and expected cases (3 observed/0.8 expected, SMR=375, $p>0.05$).

There was a non-statistically significant ($p>0.05$) increase in rectal cancer mortality (4 observed/ 2.3 expected, SMR=169, 95% CI 46–434) in the female hourly worker subgroup of the capacitor manufacturing workers studied by Kimbrough et al. (1999a) (Section 3.2.8.2.1). Mortality from rectal cancer was not increased in the Sinks et al. (1992) study of capacitor manufacturing workers or Loomis et al. (1997) study of electric utility workers summarized in Section 3.2.8.2.4.

Contaminated Fish Consumption. Mortality from cancer of the rectum or colon was not statistically significantly increased in the Svensson et al. (1995a) study of Swedish east coast (Baltic Sea) and west coast fisherman summarized in Section 3.2.8.2.2.

Yusho and Yu-Cheng Exposures. The retrospective study *Yusho* victims summarized in Section 3.2.8.2.1 found no statistically significantly increased mortality from cancer of the rectum, sigmoid colon, and anus (Kuratsune et al. 1987).

3.2.8.2.4 Skin

Occupational Exposure. Mortality analysis of 3,588 workers (2,742 male, 846 female) employed at an Indiana capacitor manufacturing facility when PCBs were used (1957–1977) provided evidence of exposure-related malignant melanoma (NIOSH 1991; Sinks et al. 1992). The mean latency was 19.2 years (range, 0.04–32.5 years), mean duration of employment was 4.1 years (range, 1 day–20.2 years), and mean age at hire was 27 years (range, 16.8–62.6 years). Aroclor 1242 was used until 1970, and Aroclor 1016 was used subsequently. Area monitoring for PCBs in 1977 showed mean concentrations ranging from 0.016 to 0.076 mg/m^3. The workers were also exposed to various solvents (toluene, xylene, methyl ethyl ketone, trichloroethylene, and 1,1,1-trichloroethane) and unspecified metals from brazing and soldering operations. Mortality from all causes and all cancers was lower than expected, indicating a healthy worker effect. More deaths were observed than expected for malignant melanoma (8 observed/2 expected, SMR=4.1, 95% CI 1.8–8.0, $p<0.01$). The excess mortality from melanoma affected both men and women. All eight melanoma deaths occurred $5 years after first

employment, and three occurred in individuals who had worked for >10 years. One of the eight cases of malignant melanoma was diagnosed 2 months before starting employment and should not have been included in the analysis; the excess mortality remained when this case was excluded from analysis (SMR=3.5, 95% CI 1.4–7.3). Two other melanoma cases possibly should have been excluded from analysis due to low risk from short-term exposure of <6 months; however, PCBs are bioaccumulative and exposure may have been high. A ninth worker died with malignant melanoma listed as a contributory cause of death; this person had worked at the plant for 1 month and died 20 years after exposure. There was no clear relationship between malignant melanoma and latency or duration of employment. Analysis performed to determine if a dose-response relationship existed between average estimated cumulative PCB exposure (duration of employment multiplied by a primarily qualitative exposure intensity rating) and mortality showed no statistically significant differences in estimated exposures between the workers that died from malignant melanoma and other workers at the same plant. Other findings in this study included a non-statistically significant increase in brain cancer as discussed in Section 3.2.8.2.5. Limitations of this study include possible insensitivity of mortality as an index of risk for malignant melanoma; inability to evaluate risk of cancers with long latency periods (<10% of the person-years at risk were accumulated with $20 years of latency); insufficient monitoring data, which precluded detailed exposure weighting; and exposure intensity ratings, which may have resulted in exposure misclassification and obscured a dose-response relationship. Screening of the affected workers for malignant melanoma was recommended based on the conclusion that the workers were at excess risk (NIOSH 1990).

There were non-statistically significant ($p>0.05$) increases in mortality from skin melanomas in the hourly male workers (5 observed/3.8 expected, SMR=130, 95% CI 42–303), hourly female workers (3 observed/2.0 expected, SMR=144, 95% CI 30–421), and salaried male workers (4 observed/1.9 expected, SMR=210, 95% CI 57–538 in the Kimbrough et al. (1999a) study of capacitor manufacturing workers summarized in Section 3.2.8.2.1.

Mortality from malignant melanoma was not statistically significantly different than expected in the Gustavsson and Hogstedt (1997) study of capacitor manufacturing workers summarized in Section 3.2.8.2.1.

Preliminary data, reported in letters to the editor of the journal, indicated that the incidence of malignant melanoma was increased in a small group New Jersey petrochemical refinery workers who were involved in processes that used Aroclor 1254 (Bahn et al. 1976, 1977; Lawrence 1977; NIOSH 1977). Two cases

of malignant melanoma were observed in 31 men believed to have been heavily exposed to PCBs; when compared to 0.04 expected cases based on national rates, the increase was statistically significant (p=0.001). An additional malignant melanoma was observed in another group of 41 workers believed to have had less exposure. Aroclor 1254 had been used over a 9-year period ending in the late 1950s, but PCB exposure was not quantified and concurrent exposure to other potential and known carcinogens was not evaluated. Other limitations of this study include the small number of cases and cohort size, and the use of expected cancer rates based on U.S. population data rather than on local New Jersey rates.

Loomis et al. (1997) analyzed cancer mortality among 138,905 men employed as electric utility workers for at least 6 months between 1950 and 1986 at five power plants. No increases in total-cancer mortality were found when data were analyzed by total cohort, duration of employment, job category, or estimated cumulative exposure to PCBs in insulating fluids. Mortality from malignant neoplasms of the skin was not increased in the total cohort (116 observed/111.9 expected, SMR=1.04, 95% CI 0.86–1.24), although relative risk of malignant melanoma by duration of employment appeared to increase in the job category with the greatest potential for dermal exposure to PCBs (i.e., in mechanics, but not in electricians, lineman and cable splicers, or laborers and material handlers). The mortality RR for malignant melanoma in mechanics employed for >0–5 years and >5–10 years were 2.57 (95% CI 1.06–6.20, based on eight deaths) and 3.16 (95% CI 0.92–10.85, based on three deaths), respectively; analysis for >10 years duration was precluded by small number of deaths. Analysis of mortality by cumulative exposure was only performed for the total cohort (all job categories combined). Mortality from malignant melanoma in the total cohort increased with increasing cumulative exposure; the RRs relative to unexposed men were 1.23 (95% CI 0.56–2.52), 1.71 (95% CI 0.68–4.28), and 1.93 (95% CI 0.52–7.14) for men with <2000, >2000–10,000, and >10,000 hours of cumulative exposure, respectively, without consideration of latency. A latency interval of 20 years yielded RRs of 1.29 (95% CI 0.76–2.18), 2.56 (95% CI 1.09–5.97), and 4.81 (95% CI 1.49–15.50) for the same cumulative exposure levels, although the RR for the highest exposure category is based on only one death. Although mortality from melanoma was highest among workers in the job category with the greatest potential for dermal exposures, this study is limited by small numbers of subjects in the higher exposure and longer latency groups, as well as possible incomplete control of confounding due to exposure to sunlight.

Of 55 transformer workers who were exposed to Askarel PCBs (0.00001–0.012 mg/m^3) for a mean duration of 3.75 years, 2 gave a history of removal of a melanoma (type not reported) (Emmett et al. 1988a). No melanomas were reported by 56 age-matched nonexposed subjects, and the difference between the groups was not statistically significant.

Contaminated Fish Consumption. Cancer incidences were studied in the Svensson et al. (1995a) study of Swedish east coast (Baltic Sea) and west coast fisherman summarized in Section 3.2.8.2.2. When compared to the regional general populations, incidences of lip cancer were significantly increased in both the east and west coast fisherman (SIR=2.6, 95% CI 1.1–5.4 and SIR=1.9, CI 1.3–2.8, respectively), and incidences of squamous cell skin cancer were increased in the east and west coast fisherman (SIR=2.3, CI 1.5–3.5 and SIR=1.1, CI=0.9–1.4, respectively). The incidence of skin cancer in the east coast fisherman was also higher than in the west coast fisherman (SIR=1.9, CI 1.2–3.1). Mortality from skin cancer was increased in the fisherman from the west coast (SMR=3.05, CI=0.99–7.13), but not east coast (SMR=0, CI=0.00–15.4). Mortality from melanoma was not increased in either the east coast fisherman (SMR=0, CI=0.00–1.73) or west coast fisherman (SMR=0.67, CI=0.25–1.46). The investigators noted that exposure to ultraviolet (UV) radiation in sunlight is too small to explain the observed difference in skin cancer sunlight and that UV light is not a risk factor for lip cancer.

General Population Exposures. An increased annual occurrence of ocular melanoma was discerned in Ohio residents during 1967–1977 (1.09 cases/100,000 persons/year versus 0.6 in other reports), with no statistically significant difference in the number of cases reported from year to year (Davidorf and Knupp 1979). No relationship between PCB exposure and the occurrence of ocular melanoma was suggested by a crude state-wide geographic comparison, which showed that distribution of the cancer was similar in counties with and without presumed elevated exposures, as indicated by high PCB levels in fish or presence of industries that might use PCBs.

3.2.8.2.5 Brain and Central Nervous System

Occupational Exposure. Suggestive increases in mortality from brain cancer were reported in the Sinks et al. (1992) study of capacitor manufacturing workers and Loomis et al. (1997) study of electric utility workers summarized in Section 3.2.8.2.4. Sinks et al. (1992) found a non-statistically significant increase in brain cancer mortality in 3,588 male and female workers based on five cases in both sexes compared to 2.8 expected (SMR=1.8, 95% CI 0.6–4.2, $p>0.05$). There was no clear relationship between brain cancer and latency or duration of employment, although there was an indication that brain cancer deaths were more common among those with a longer duration of employment (three deaths occurred after $10 years). Additional analysis was performed to determine if a dose-response relationship existed between average estimated cumulative PCB exposure and mortality from brain cancer. This analysis showed no statistically significant differences in estimated exposures between the workers that died from

brain cancer and other workers at the same plant, although the estimated exposure of the brain cancer fatalities was approximately twice as high as that of the other workers.

Loomis et al. (1997) found that mortality from brain cancer was increased among mechanics who were dermally exposed to PCBs in capacitor fluids for 0–10 years (RR=1.84, 95% CI 0.90–3.78). There were no deaths from brain cancer in mechanics employed for >10 years. Mortality from brain cancer was also increased in the total cohort of electric utility workers with cumulative exposures of <2,000 hours (RR=1.61, 95% CI 0.86–3.01) and 2,000–10,000 hours (RR=1.79, 95% CI 0.81–3.95) and no latency period. The RRs of 1.6–1.8 rose to about 2.0 when a latency period of 5 years was used, but latencies of 10 and 20 years diminished or eliminated the effect. There were no deaths from brain cancer in workers in the highest cumulative exposure category (>10,000 hours). The lack of either strong or consistent associations of brain cancer with exposure, as well as the tendency for brain cancer mortality rates to decline with longer employment and greater exposure, weakens support for a causal relation.

Mortality from cancer of the brain and nervous system was not statistically significantly different than expected in Kimbrough et al. (1999a) and Gustavsson and Hogstedt (1997) retrospective studies of capacitor manufacturing workers summarized in Section 3.2.8.2.1.

3.2.8.2.6 Hematological

Occupational Exposure. Hematological cancers were increased in the Bertazzi et al. (1987) study of 544 male and 1,556 female Italian capacitor manufacturing workers summarized in Section 3.2.8.2.2. Mortality from hematological neoplasms was statistically significantly ($p<0.05$) higher than expected in the females based on local rates (4 observed/1.1 expected, SMR=377, 95% CI 115–877). All four of the hematologic neoplasms in females were associated with lymphatic tissue (three deaths from Hodgkin's disease and one death from lymphosarcoma). Mortality from hematological neoplasms was also increased in females compared to national rates (4 observed/1.5 expected, SMR=266, CI not reported), and males compared to both national rates (3 observed/0.8 expected, SMR=375, CI not reported) and local rates (3 observed/1.1 expected, SMR=263, CI not reported), but these increases were not statistically significant. Follow-up evaluation of the cohort after an additional 9 years of latency found an additional death from a hematologic neoplasm (lymphatic leukemia) in the females (no change in males), although the increase in the women was no longer statistically significant based on local rates (5 observed/3.5 expected, SMR=141, 95% CI 46–330) (Tironi et al. 1996).

Mortality from cancers of the lymphatic and hematopoietic tissues was not increased in other studies of capacitor manufacturing workers summarized in Sections 3.2.8.2.1 and 3.2.8.2.4 (Brown 1987b; Brown and Jones 1981; Gustavsson and Hogstedt 1997; Gustavsson et al. 1986; Kimbrough et al. 1999a; Sinks et al. 1992), or in the Loomis et al. (1997) study of electric utility workers summarized in Section 3.2.8.2.4.

Contaminated fish Consumption. There was no increased mortality from Hodgkin's lymphoma or non-Hodgkin's lymphoma in the Svensson et al. (1995a) study of Swedish east coast (Baltic Sea) and west coast fisherman summarized in Section 3.2.8.2.2. Mortality from multiple myeloma was increased in the fisherman from the east coast (SMR=3.08, 95% CI=1.24–6.35) and west coast (SMR=2.08, CI=0.76–4.53), as was mortality from leukemia was increased in the east coast fisherman (SMR=1.38, CI=0.45–3.22).

General Population Exposures. Two studies reported an association between risk of non-Hodgkin's lymphoma and exposure to PCBs (Hardell et al. 1996; Rothman et al. 1997). Adipose tissue concentrations of total PCBs and 34 non-coplanar congeners were compared in 27 Swedish hospital patients with non-Hodgkin's lymphoma (NHL) (B-cell type) and 17 surgical controls without malignancy (Hardell et al. 1996). Analysis of three coplanar congeners (PCB 77, 126, and 169) was performed in 20 of the cases and all 17 controls. The mean total PCB concentration, calculated as the sum of the non-coplanar congeners, was about 33% higher (p=0.06) in the cases than controls. Mean levels of 11 individual non-coplanar congeners were statistically significantly ($p<0.05$) increased compared to controls; the difference was most significant (p#0.01) for PCB 156 and PCB 208. Mean concentrations of the three coplanar congeners were not statistically significantly different in the cases and controls. An increased risk of NHL (OR=2.7, 95% CI 0.8–9.4]) was calculated for cases with total PCB concentrations higher than the median concentration (1,300 ng/g lipid) of the total group.

An association between serum PCBs and increased risk of NHL was found in a nested prospective case-control study of Maryland residents (Rothman et al. 1997). Serum levels of total PCBs were compared in 74 cases of NHL and 147 matched controls identified in a cohort established in 1974. The mean time to diagnosis after enrollment into the cohort was 12.1 years. The mean PCB concentration was statistically significantly higher in the cases than controls (10% increase, p=0.0014). Conditional logistic regression analysis showed that the risk of NHL increased significantly with increasing PCB serum concentrations (p for trend=0.0008); the ORs in the two highest concentration quartiles were 2.8 (95% CI 1.1–7.6) and 4.5 (95% CI 1.7–12.0). Additional analysis indicated that the effect of PCBs on risk of NHL was increased among participants who were seropositive for Epstein-Barr virus early antigen.

Yusho and Yu-Cheng Exposures. A retrospective cohort mortality study followed, 1940 *Yu-Cheng* cases (929 males, 1,011 females, >95% of all registered cases) for 12 years following exposure in 1979 (Hsieh et al. 1996). The average age of the subjects was 27 years at the beginning of the study. Mortality from Hodgkin's disease was increased in comparison to Taiwan national or local rates in the males (SMR=61.17, 95% CI 1.55–340.72 or SMR=86.45, 95% CI 2.19–481.52, respectively). There was no statistically significantly increased mortality from leukemia. The retrospective study *Yusho* victims summarized in Section 3.2.8.2.1 found no statistically significantly increased mortality from leukemia (Kuratsune et al. 1987).

3.2.8.2.7 Breast

Occupational Exposure. Mortality from breast cancer was not increased in the Brown and Jones (1981), Brown (1987b), and Kimbrough et al. (1999a) studies of capacitor manufacturing workers summarized in Section 3.2.8.2.1.

General Population Exposures. Eight case-control studies compared breast tissue concentrations of PCBs in women with breast cancer and women with benign breast disease or who died in accidents. Four of these studies found higher average levels of total PCBs or individual congeners in breast fat among the cases than in controls (Dewailly et al. 1994; Falck et al. 1992; Guttes et al. 1998; Wasserman et al. 1976). Wasserman et al. (1976) reported that the mean concentration of total PCBs in malignant breast tissue of nine Brazilian women collected after diagnosis (date not reported) was about 3 times higher ($p<0.01$) than that found in adjacent breast glandular or adipose tissue from the same women, or in normal breast tissue from five controls. Total PCB levels in breast fat were 40% higher ($p<0.02$) in 20 Connecticut patients with breast cancer compared to 20 age-matched controls who had benign breast disease (Falck et al. 1992); adipose samples were obtained near the time of diagnosis in 1987.

Dewailly et al. (1994) measured breast adipose levels of total PCBs and 10 individual congeners in Canadian women with benign breast disease (n=17), breast cancer with estrogen receptor (ER)-positive breast cancer cells (n=9), and breast cancer with ER-negative cells (n=9). Analysis near the time of diagnosis during 1991–1992 showed no statistically significant group differences in total PCBs. Mean congener concentrations in ER-negative cases were generally lower than those in control subjects, although the difference was statistically significant ($p=0.02$) only for PCB 118. ER-positive case patients, however, showed congener levels that were generally higher than controls, with the difference reaching statistical significance ($p=0.05$) for PCB 99. Guttes et al. (1998) measured concentrations of total PCBs

and 12 congeners in breast fat samples from 45 German women with breast cancer and 20 controls with benign breast disease; samples were obtained at the time of diagnosis in 1993–1994. Geometric mean levels of total PCBs were not statistically significantly different in the cases and controls after statistical adjustment for age differences. Mean concentrations of PCB 126 and PCB 153 were 25% (p=0.04) and 24% (p=0.08) higher, respectively, in the cases compared to controls.

In contrast to the findings summarized above, the four other case-control breast tissue studies found no increased levels of total PCBs or individual congeners in patients with breast cancer (Aronson et al. 2000; Liljegren et al. 1998; Mussalo-Rauhamaa et al. 1990; Unger et al. 1984). There was no statistically significant difference in mean PCB levels in breast fat from 14 newly diagnosed Danish breast cancer patients and 21 noncancer patients, or in samples taken at autopsy from groups of 18 deceased women with breast cancer and 35 deceased women without breast cancer (Unger et al. 1984). Mussalo-Rauhamaa et al. (1990) similarly found no statistically significant difference in mean concentrations of PCBs in breast fat of 44 breast cancer cases from Finland and 33 controls without cancer.

Breast adipose tissue concentrations of PCB congeners were assessed in a case-control study of 43 Swedish women with invasive breast cancer and 35 controls with benign breast disease (Liljegren et al. 1998). Total or individual levels of 36 non-coplanar congeners, or individual levels of coplanar congeners 3,3',4,4'-tetraCB (PCB 77), 3,3',4,4',5-pentaCB (PCB 126), and 3,3',4,4',5,5'-hexaCB (PCB 169), did not statistically significantly differ between cases and controls in the entire group or in subgroups of pre- and postmenopausal women. Analysis of coplanar congeners was limited to PCB 77, PCB 126, and PCB 169 in 19 cases and 19 controls. Logistic regression analysis was used to estimate risk associated with exposure to elevated tissue levels of total non-coplanar congeners, and each of the three coplanar congeners in all women, as well as subgroups who were postmenopausal, had ER-positive tumors, or were postmenopausal with ER-positive tumors. Based on age- and parity-adjusted data, increased risks of breast cancer were associated with PCB 77 (tissue concentration >4.5 ng/g lipid) in postmenopausal women (OR=5.8, 95% CI 0.8–42), women with ER-positive tumors (OR=5.0, CI 0.8–28) and postmenopausal women with ER-positive tumors (OR= 33, CI 1.8–588), and PCB 126 (>145 ng/g lipid) in women with ER-positive tumors (OR=5.1, CI 0.8–30). An OR for PCB 126 in postmenopausal ER-positive women was not calculated due to insufficient data.

Aronson et al. (2000) investigated the association between risk of breast cancer and breast adipose tissue concentrations of total PCBs or 14 individual congeners. Analyses were performed on biopsy tissue from 217 Canadian women diagnosed with breast cancer and 213 matched controls with benign breast lesions.

Mean breast fat concentrations of total PCBs and individual congeners were not statistically significantly higher in the cancer cases than in controls. Multiple logistic regression was used to calculate ORs for increasing tissue concentrations of total PCBs and individual congeners. When the whole group was adjusted for age, menopausal status, and other confounders, risk was increased for 2,3,3',4,4'-pentaCB (PCB 105) and 2,3,4,4',5-pentaCB (PCB 118). The ORs for these congeners increased linearly with tissue concentrations (p values for trend #0.01), reaching 3.17 (95% CI 1.51–6.68) for PCB 105 and 2.31 (CI 1.11–4.78) for PCB 118 in the highest concentration categories ($13 and $50 µg/kg, respectively). Risks for breast cancer were even higher among premenopausal women for PCB 105 (OR=3.91, CI 1.73–8.86) and PCB 118 (OR=2.85, CI 1.24–6.52). Risks among postmenopausal women were elevated for PCB 170 and PCB 180, but these were not clearly tissue concentration-related.

Four case-control studies used serum PCB concentrations as the marker of exposure with measurements performed after the diagnosis of breast cancer (Moysich et al. 1998, 1999b; Wolff et al. 1993; Zheng et al. 2000). Studies by Moysich et al. (1998, 1999b) were based on 154 cases of postmenopausal breast cancer and 192 matched postmenopausal controls from western New York. Based on serum congener analysis performed within 3 months of diagnosis in 1986–1991, exposure was characterized as total PCBs, total number of detected peaks, and three congener groups (less chlorinated, moderately chlorinated, and more highly chlorinated PCBs). No statistically significant differences in mean PCB concentrations were found between cases and controls in the total sample, or in subgroups of who breast-fed (85 cases, 106 controls) or never lactated (46 cases, 61 controls), using any of the measures of exposure (Moysich et al. 1998). Additionally, analyses using unconditional logistic regression showed that higher serum levels of total PCBs, moderately chlorinated congeners, more highly chlorinated congeners, or greater number of detected peaks, were not associated with increased risk of breast cancer. There was an indication of a small increase in risk for women with detectable levels of less chlorinated congeners (OR=1.66, 95% CI 1.07–2.88), and in parous women who had never lactated having higher serum levels of total PCBs (OR=2.87, CI 1.01–7.29), moderately chlorinated congeners (OR=3.57, CI 1.10–8.60), and detected congener peaks (OR=3.31, CI 1.04–11.3). Further study of the 154 cases and 192 controls investigated the association between cytochrome P-4501A1 genotype and breast cancer risk in the postmenopausal women (Moysich et al. 1999b). An increased risk of breast cancer was associated with elevated serum PCB concentrations ($3.73 ng/g of serum) among women with *CYP1A1* polymorphism, as indicated by an OR of 2.96 (95% CI 1.18–7.45) in women carrying at least one *CYP1A1* valine for isoleucine substitution allele (*Ile:Val* or *Val:Val)*, compared with women with lower serum PCB levels and who were homozygous for the isoleucine allele (*Ile:Ile*). No effect of *CYP1A1* was found among women with lower serum levels of PCBs or women homozygous for the isoleucine allele.

Zheng et al. (2000) investigated the relationship between serum PCBs and breast cancer risk in a case-control study of 475 Connecticut patients with confirmed cancer and 502 age-matched controls with benign breast diseases. Blood samples were collected at the time of recruitment in 1995–1997 and analyzed for 9 PCB congeners. There were no statistically significant differences in mean serum level of total PCBs between the cases and controls in all subjects or subgroups of cases based on stage of cancer at time of diagnosis, type of cancer treatment, type of cancer or benign breast disease, or ER status. Logistic regression analysis showed no statistically significant association between breast cancer risk (OR) and serum total PCB levels for all subjects or subgroups based on parity, lactation, or menopausal status. Additionally, mean serum levels for each of three structure-activity congener groups (potentially estrogenic, potentially antiestrogenic and immunotoxic (dioxin-like), and phenobarbital-type cytochrome P-450 (*CYP1A* and *CYP2B*) enzyme inducers) were comparable between the cases and controls, and there was no statistically significant increasing trend for risk of breast cancer with increasing serum levels of these congener groups.

Wolff et al. (1993) found that mean concentrations of total PCBs were 19% higher in the sera of 58 New York City women with breast cancer than in 171 matched cancer-free controls, but the difference only approached statistical significance (p=0.058). The blood samples were collected between 1985 and 1991 and analyzed within 6 months of cancer diagnosis. Conditional logistic regression analysis showed that the relative risk of breast cancer increased less than 2-fold for a change in serum PCB levels from 3.9 ng/mL (10^{th} percentile) to 10.6 ng/mL (90^{th} percentile; OR=1.70, 95% CI 0.79–3.68), and that there was no statistically significant positive trend (p=0.16) with increasing concentrations of PCBs.

Wolff et al. (2000) found a more conclusive lack of association between serum total PCBs and breast cancer in a prospective investigation nested within the same cohort from which the women in their 1993 study were selected. Cases (n=148) and individually matched controls (n=295) were identified among women whose blood had been collected at least 6 months before diagnosis in October 1994. In addition, among 84 cases and 196 controls, two or more consecutive annual blood samples were available to estimate elimination half-lives of total PCBs. Cases and controls had similar mean serum levels of PCBs, and this difference remained statistically nonsignificant when ER status of the tumors was considered. Additionally, PCB half-lives did not differ between cases and controls. Conditional logistic regression analysis showed no positive association between serum PCB levels and risk of breast cancer; although ORs were elevated in the three upper concentration quartiles relative to the lowest one, none of the ORs were statistically significant and there was no evidence of a trend (p=0.23). The risk of breast cancer was not influenced by menstrual status, lactation history, or PCB half-life.

Five other prospective studies similarly found no association between serum levels of PCBs and breast cancer incidence using blood samples collected prior to diagnosis (Dorgan et al. 1999; Helzlsouer et al. 1999; Høyer et al. 1998; Hunter et al. 1997; Krieger et al. 1994). Total plasma PCBs were determined in blood samples collected during 1989–1990 in a prospective study of the health of nurses in the United States (Hunter et al. 1997). Mean plasma levels of PCBs did not statistically significantly differ between 230 women who developed breast cancer before June 1992 and pair-matched controls who did not subsequently develop breast cancer. There was no association between plasma levels of PCBs and established or suspected risk factors for breast cancer (e.g., menopausal status, age, age at menarche, age at birth of first child, number of children, and history of lactation), nor any increase in relative risk of breast cancer with increasing concentrations of PCBs. It should be noted that the short follow-up period (not more than 3 years) could have contributed to the negative findings.

A prospective nested case-control study of blood samples collected in 1964–1971 compared serum levels of total PCBs in 150 California women (50 white, 50 black, 50 Asian) who were later diagnosed with breast cancer with levels in matched controls who did not develop breast cancer in the interval at least 6 months after the blood was drawn through the end of 1990 (Krieger et al. 1994). Serum samples were collected an average of 14 years prior to cancer diagnosis. Mean concentrations of PCBs were not statistically significantly different in cases or controls in the total group or racial/ethnic subgroups, regardless of menopausal and estrogen-receptor status. Conditional logistic regression analyses showed no statistically significant trends for increased breast cancer risk with increasing serum PCB levels or by year of diagnosis or length of follow-up.

A case-control study of 240 breast cancer patients and 477 matched controls (two controls for each case) was nested within a prospective study conducted in Denmark (Høyer et al. 1998). Blood samples were collected in 1976 and breast cancer was diagnosed during the following 17 years. Conditional logistic regression analysis indicated no increased risk of breast cancer with increasing serum levels of total PCBs. Exclusion of women who developed breast cancer within 5 years of serum sampling did not alter the results. The average amount of time between the collection of the serum sample and the diagnosis of breast cancer is unclear.

Serum PCBs were measured in blood samples collected in 1974 or 1989 in a breast cancer case-control study nested within a prospective cohort study of Washington County, Maryland residents (Helzlsouer et al. 1999). A group of 346 women who were diagnosed with breast cancer by June, 1994 (i.e., after a follow-up period of up to 20 years) were matched to 346 cancer-free controls by age, race, menopausal

status, and date of blood donation. Mean and median concentrations of total PCBs in 1974 or 1989 were not statistically significantly different in women who subsequently developed breast cancer than in controls. Logistic regression analysis showed that the risk of breast cancer did not statistically significantly increase with increasing serum levels of total PCBs measured in 1974 or 1989. Additionally, there was no statistically significantly increased risk of breast cancer associated with increasing concentrations of 26 individual congeners, or three groupings of congeners based on structure-activity considerations (potentially estrogenic, potentially antiestrogenic and immunotoxic (dioxin-like), and phenobarbital-type cytochrome P-450 (*CYP1A* and *CYP2B*) enzyme inducers). Length of follow-up, menopausal and estrogen-receptor status, lactation and birth history, and putative high-risk genotypes for detoxification enzymes such as glutathione transferase (*GSTM1*, *GSTT1*, *GSTP1*, or *COMT*) did not contribute to an increased risk of breast cancer.

The association between breast cancer and PCB exposure was evaluated in 105 cases and 208 matched controls in a prospective nested case-control study using the Columbia, Missouri breast cancer serum bank (Dorgan et al. 1999). Breast cancer was diagnosed up to 9.5 years after blood samples were obtained between 1977 and 1987. Exposure was estimated using lipid-adjusted serum levels of total PCBs and 27 individual congeners. The percent of participants with total PCBs or individual congeners above the assay detection limit was not statistically significantly higher in cases compared to controls. Conditional logistic regression analysis of the entire group showed no indication of increased relative risk of breast cancer among women with elevated serum levels of total PCBs or individual congeners. A positive association between serum PCB 138 concentration and breast cancer was suggested (p=0.07) when blood was collected close to the time of diagnosis (#2.7 years, 53 cases, 104 controls), based on RRs of 1.7 (95% CI 0.7–4.2) and 1.9 (CI 0.8–4.8) in the middle and highest concentration tertiles, respectively.

3.2.8.2.8 Other Sites

Occupational Exposure. Pancreatic cancer was increased in 1,939 males employed between 1947 and 1975 at a transformer manufacturing plant in Canada (Yassi et al. 1994). Only a very small number of transformers contained PCBs, and there was considerably more exposure to mineral oil refined predominantly from naphthenic base crudes. Therefore, unlike the typical studies of capacitor manufacturing workers who were mainly exposed to PCBs, the transformer plant workers in the Yassi et al. (1994) study were predominantly exposed to mineral oil not containing PCBs. Mortality from pancreatic cancer was statistically significantly increased. Based on 11 observed deaths, SMRs for

pancreatic cancer ranged from 2.92 (95% CI 1.17–6.01) to 12.9 (95% CI 2.59–37.7), depending on cohort definition and acceptability criteria used. The authors also reported that those who entered the cohort prior to 1960 had a higher mortality risk than those who entered later. Additionally, SMRs for pancreatic cancers were higher in the departments in which transformers were assembled than in departments in which the exposures were thought to be lower. The role of PCBs is unclear due to the exposure to other transformer chemicals and study limitations such as the fact that no medical history of the workers was provided. Wong (1995) raises serious concerns about the Yassi et al. (1994) pancreatic cancer findings. For instance, in the group with the highest SMR, three cancers were reported. One of these had worked for <1 year and had died within 1 year of leaving the plant. Another case that was "possibly" linked to employment at the plant had worked at the plant for 1 year. Thus, two of the three cases in the group with the highest SMR had neither sufficient duration of exposure nor latency for their cancers to be considered occupationally related to PCB exposure at the plant.

Kidney adenocarcinoma was found in three male public utility workers (aged 34–56) exposed to unspecified PCBs while servicing and repairing transformers for 5–14 years (Shalat et al. 1989). The workers were employed by the same company during the same period, but the total exposed population was not reported. Although kidney cancer is relatively rare in young men (range, 1.3–29.7 cases/100,000 in the age group of the subjects), an association between PCB exposure and kidney cancer cannot be demonstrated due to limitations of the study, particularly exposure to other chemicals including unspecified organic solvents and herbicides.

Mortality from pancreatic, kidney, or urinary cancer was not statistically significantly increased in other studies of capacitor manufacturing workers summarized in Sections 3.2.8.2.1 and 3.2.8.2.4 (Brown 1987b; Kimbrough et al. 1999a; Sinks et al. 1992), or in the Loomis et al. (1997) mortality study of electric utility workers summarized in Section 3.2.8.2.4.

Contaminated Fish Consumption. Mortality from pancreatic cancer was not increased in the Svensson et al. (1995a) study of Swedish east coast (Baltic Sea) and west coast fishermen summarized in Section 3.2.8.2.2.

Yusho and Yu-Cheng Exposures. The retrospective study of *Yusho* victims summarized in Section 3.2.8.2.1 found no statistically significant ($p<0.05$) increased mortality from cancer of the pancreas (Kuratsune et al. 1987.).

3.2.8.2.9 Evaluation of Human Studies

The carcinogenicity of PCBs in humans has been investigated in retrospective occupational studies that investigated cancer mortality in exposed workers, and in case-control studies of environmental exposure that examined associations between serum or adipose tissue levels of PCBs and occurrence of cancer. As discussed below and summarized in Appendix A, some of these studies provide meaningful evidence that PCBs are carcinogenic in humans.

Occupational mortality data indicate that exposures to PCBs during capacitor manufacturing and repairing were associated with cancer of the liver, biliary tract and/or gall bladder, intestinal cancer, and skin melanoma. A slight but statistically significant increase in cancer of the liver, biliary tract, and gall bladder category was found by Brown (1987b) based on a small number of deaths (five cases) in a cohort of 2,588 workers (SMR=263, $p<0.05$). Of the five deaths, four occurred in women from one plant, and two deaths were from liver, two from biliary tract, and one from gall bladder cancer. One of the two liver cancers was not a primary carcinoma as it metastasized from another site, and the SMR loses statistical significance if the metastatic liver cancer is not included in the analysis. No analysis was performed to assess risk from biliary cancer alone. Mortality from cancer of the liver/biliary tract/gall bladder was not statistically significantly increased in any of the other occupational studies of PCB workers (Bertazzi et al. 1987; Gustavsson and Hogstedt 1997; Gustavsson et al. 1986; Kimbrough et al. 1999a; Loomis et al. 1997; Sinks et al. 1992), although Bertazzi et al. (1987) did observe one death from biliary tract cancer and another from a primary liver cancer in six cases classified as digestive system cancers, and there were two deaths from relatively rare types of bile duct cancers in the small cohort of 242 workers evaluated by Gustavsson and Hogstedt (1997). Because no individual study indicated a statistically significantly increased risk of primary liver/biliary tract/gall bladder cancer, Nicholson and Landrigan (1994) combined the results from the various studies available at the time by summing observed and expected cases. Based on a total of 8 observed and 2.8 expected cases from studies of capacitor manufacturing workers from three cohorts (Bertazzi et al. 1987; Brown 1987b; Brown and Jones 1981; Gustavsson et al. 1986), statistically significant increases were found for liver/biliary tract/gall bladder (SMR=285, $p=0.008$) and for biliary tract/gall bladder separately ($p<0.05$, SMR not reported). Although the Nicholson and Landrigan (1994) analysis is based on combined results from cohorts having different durations and levels of exposure, latencies, and follow-up, and did not include data from the most recent studies (Gustavsson and Hogstedt 1997; Kimbrough et al. 1999a), it provides an indication that PCBs are associated with cancer of the liver, biliary tract, and/or gall bladder in humans. The finding for biliary cancer is particularly meaningful considering its relatively rare nature and the fact that data on liver and

biliary cancers were not reported separately in most studies. Additionally, support for the hepatocarcinogenicity of PCBs in humans is provided by data indicating that mortality from liver cancer was increased following *Yusho* exposure (Kuratsune et al. 1987).

Data suggestive of PCB-related intestinal cancer were reported in the Kimbrough et al. (1999a) mortality study of capacitor workers. Mortality from cancer of the intestines (large and small) was not statistically significantly increased in any of the groups of male or female workers in this study; however, the mortality rate for intestinal cancer in the hourly female subgroup was elevated and approached statistical significance (SMR=157, 95% CI 96–242). Additionally, most of the intestinal cancer cases (18 of 20) in this subgroup occurred in women with $20 years of latency, who had a rate that was significantly elevated (SMR=189, $p<0.05$). There was no trend for increased risk with cumulative exposure; however, there is low precision in this analysis due to a particularly small number of deaths in each exposure duration category. There was no indication of increased mortality from stomach cancer in this study, or from cancer of the intestines or stomach in other studies of PCB workers. Bertazzi et al. (1978) did report a statistically significantly increased mortality from digestive system cancers in male workers (SMR=274, $p<0.05$), but this classification included cancers of the liver, biliary tract, and pancreas as well as stomach, and no deaths from intestinal cancer were reported. Additionally, follow-up evaluation of the same cohort after an additional 9 years of latency showed that mortality from digestive system cancer was no longer statistically significantly increased. The incidence of stomach cancer was significantly elevated in Swedish fisherman that had high intake of PCBs in fish (Svensson et al. 1995a), but the effect cannot be definitely attributed to PCBs because consumption included smoked fish and PCBs were not the only contaminants in the fish.

Mortality from malignant melanoma was statistically significantly increased in one study of capacitor workers (Sinks et al. 1992). The excess mortality affected both men and women (SMR=350, $p<0.01$). Because the number of deaths was relatively small and a dose-response relationship or increase with latency could not be established, the results of this study are not conclusive. Two other studies support the skin cancer finding of Sinks et al. (1992). Bahn et al. (1976, 1977) observed two cases of malignant melanoma in 31 refinery workers believed to have been heavily exposed to PCBs (Bahn et al. 1976, 1977). Although the increase was statistically significant (p#0.001), it cannot definitely be attributed to PCBs because the workers were exposed to other chemicals in the refinery. Mortality from malignant melanoma appeared to increase with cumulative exposure and latency among electric utility power plant mechanics who were dermally exposed to PCBs in capacitor fluids (Loomis et al. 1997). Although SMRs were elevated at 2.57 (95% CI 1.06–6.20) and 3.16 (95% CI 0.92–10.85) in mechanics employed for

>0–5 years and >5–10 years, respectively, the association between malignant melanoma and exposure is unclear due to a small number of deaths, which precluded assessment of employment durations longer than 10 years and analysis by cumulative exposure and latency. Considering the limitations of the Bahn et al. (1976, 1977) and Loomis et al. (1997) studies, the apparent relationships to PCB exposure are more uncertain than in the Sinks et al. (1992) study, although the data from the three studies collectively suggest an association.

Other findings in the Sinks et al. (1992) study of capacitor manufacturing workers and Loomis et al. (1997) study of electric utility workers included slightly increased mortality from brain cancer in some subgroups. Because confidence intervals on risk ratios were broad with lower 95% limits <1.0, and there was no clear relationship between brain cancer and exposure duration, level, or latency, the association between PCBs and brain cancer is uncertain.

There is also no clear association between PCBs and hematopoietic or lymphatic cancers. Investigations of a cohort of Italian capacitor workers found statistically significantly increased mortality from hematological neoplasms in females in the first study (SMR=377, CI 115–877, $p<0.05$) (Bertazzi et al. 1987), but not upon follow-up after an additional 9 years of latency (SMR=141, CI 46–330) (Tironi et al. 1996). All of the cases of hematological neoplasms (five observed) were associated with lymphatic tissues, including three deaths from Hodgkin's disease (Hodgkin's lymphoma). Although mortality from lymphatic or hematopoietic cancers was not increased in any of the other studies of capacitor manufacturing workers, the cases of Hodgkin's disease observed by Bertazzi et al. (1987) may be consistent with the significantly increased mortality from Hodgkin's disease found in *Yu-Cheng* victims (Hsieh et al. 1996). Two background environmental exposure studies found that mean concentrations of PCBs in adipose tissue (Hardell et al. 1996) and serum (Rothman et al. 1997) were statistically significantly higher in patients with NHL than in controls without NHL. Although these studies also showed that the risk of NHL increased significantly with increasing levels of PCBs in adipose and serum, additional information is needed to conclude that the NHL is specifically due to PCBs. The absence of consistent evidence for lymphatic cancers might result from the rarity of these cancers, and not from a lack of an association between PCBs and the cancers.

Associations between exposure to PCBs and breast cancer were investigated in a few of the occupational retrospective cohort mortality studies and in a number of case-control studies of women with background environmental exposures. The occupational studies found no indications of increased mortality from breast cancer in female capacitor manufacturing workers who were mainly exposed to PCBs by the

inhalation and/or dermal routes (Brown 1987b; Brown and Jones 1981; Kimbrough et al. 1999a). The environmental exposure studies assessed relationships between breast cancer and levels of PCBs in breast fat or blood in the general population. These studies were generally conducted to investigate a hypothetical role of organochlorine compounds, including PCBs, in the development of breast cancer. In environmental case-control studies that compared breast tissue PCB concentrations in women with and without breast cancer, some reported higher levels of total PCBs and/or congeners in breast fat among cases than in controls (Dewailly et al. 1994; Falck et al. 1992; Guttes et al. 1998; Wasserman et al. 1976), whereas others found no elevated breast adipose PCB levels in breast cancer cases (Aronson et al. 2000; Liljegren et al. 1998; Mussalo-Rauhamaa et al. 1990; Unger et al. 1984). Risk analyses, performed in two of the tissue studies, suggest increased risks of breast cancer associated with increased tissue levels of some congeners in subgroups of women that were postmenopausal or had estrogen receptor-positive tumors (Aronson et al. 2000; Liljegren et al. 1998). Other case-control studies used serum PCB concentrations as the marker of exposure with blood samples taken after the diagnosis of breast cancer (Moysich et al. 1998, 1999b; Wolff et al. 1993; Zheng et al. 2000), or prospectively collected prior to diagnosis (Dorgan et al. 1999; Helzlsouer et al. 1999; Høyer et al. 1998; Hunter et al. 1997; Krieger et al. 1994; Wolff et al. 2000). None of the serum studies found significantly different mean blood levels of PCBs in breast cancer cases and controls. Additionally, logistic regression analyses showed no statistically significant associations between breast cancer risk and serum PCBs in most of the serum studies. The negative findings in the serum studies were generally not influenced by menopausal or estrogen receptor status, birth or breast-feeding history, types of congeners, and/or other contributing or confounding factors. Increased risks were associated with serum PCBs in postmenopausal women who were parous and had never breast-fed or in postmenopausal women with a putative high-risk *CYP1A1* variant genotype (Moysich et al. 1998, 1999b), but these findings are only suggestive due to small number of subjects and variance with another study (Zheng et al. 2000).

As discussed above, associations between PCBs and breast cancer have been reported in only a few of the many case-control studies. Inconsistencies in the results could be related to methodological differences in the studies. For example, most of the breast tissue studies are limited by small numbers of subjects and/or inadequate control for known breast cancer risk factors, not all of the blood studies adjusted for serum lipids or factors such as menstrual status, parity, and duration of lactation, and only some studies used congener-based exposure assessment or considered timing of exposure assessment relative to the etiology of cancer. Many of the better designed studies have been prospective, using blood samples obtained a number of years prior to the diagnosis of cancer (Dorgan et al. 1999; Helzlsouer et al. 1999; Høyer et al. 1998; Hunter et al. 1997; Krieger et al. 1994; Wolff et al. 2000), but none of the prospective studies found

a relationship between serum PCBs and breast cancer. Interpretations, however, are hampered by differences in target analytes since some studies have found associations with congeners that were not considered in larger studies due to expense of analysis. Although the overall epidemiologic evidence for an association between breast cancer and PCBs is inconclusive, there are meaningful preliminary indications that specific subgroups may be at risk.

The human studies examining the cancer causing effect of PCBs often have methodological limitations. However, the evidence, taken in totality, indicates a potential cancer causing effect for PCBs. EPA determined that the human data are inadequate, but suggestive, of carcinogenicity (IRIS 2000), and IARC (1987) concluded that the evidence for carcinogenicity to humans is limited.

3.2.8.3 Animal Studies

Reliable cancer effect levels (CELs) are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.8.3.1 Inhalation Exposure

No studies were located regarding carcinogenicity of PCBs in animals following inhalation exposure.

3.2.8.3.2 Oral Exposure

Chronic Oral Bioassays. A number of oral cancer studies of commercial PCB mixtures have been performed in animals. As summarized below, these studies demonstrate the hepatocarcinogenicity of PCBs as well indicate that the thyroid is a site of tumorigenesis.

Hepatocellular carcinomas developed in female Sherman rats fed an estimated dose of 5 mg/kg/day Aroclor 1260 (purity not reported) for .21 months (Kimbrough et al. 1975). Almost all treated rats (170 of 184) exhibited a few to multiple tan nodules on the surface of the liver and more upon sectioning. Only one control rat had gross abnormalities of the liver. Hepatocellular carcinomas were diagnosed in 14.1% (26 of 184) of the treated rats and 0.6% (1 of 173) of the controls. Neoplastic nodules were found in 84.7% (144 of 170) of the treated rats with surface nodules and in none of the controls (0 of 173). The total reported incidence of neoplastic liver lesions was 92.4% (170 of 184) in treated rats and 0.6% (1 of 173) in controls. Incidences of neoplastic lesions were not increased in tissues other than liver (all major tissues and organs were examined).

Liver tumors also developed in Sprague-Dawley rats fed an estimated average dose of 4.2 mg/kg/day Aroclor 1260 (purity not reported) for 24 months (Norback and Weltman 1985). In treated rats that survived 18 months or longer, 95.7% (45 of 47) of the females and 15.2% (7 of 46) of the males had hepatocellular carcinomas or neoplastic nodules, indicating a sex-related effect. Of the 47 treated females, 43 had trabecular carcinomas and/or adenocarcinomas, and another 2 had neoplastic nodules only. Of the 46 treated males, 2 had trabecular carcinomas and another 5 had neoplastic nodules. Incidences of hepatocellular neoplasms in control rats were 0% (0 of 32) in males and 2% (1 of 49) in females; the 1 female had a single neoplastic nodule. Hepatocellular lesions progressed as follows: centrilobular cell hypertrophy at 1 month; foci of altered cells at 3 months and areas at 6 months; neoplastic nodules at 12 months; trabecular carcinoma at 15 months; and adenocarcinoma at 24 months. The authors observed that, while the tumors met morphologic criteria for malignancy, they were relatively nonaggressive because they did not metastasize to distant organs or invade blood vessels. Mortality was not affected, probably because of the late appearance and slow growth of the tumors. Preneoplastic lesions in the biliary tract (simple cholangiomas, also referred to as bile duct hyperplasia) occurred at a higher incidence in treated males and females (14 and 21%, respectively) than in control males and females (2 and 2%, respectively).

Liver neoplastic nodules and hepatocellular carcinomas developed in 50% (63 of 126) and 48.4% (61 of 126) of male Wistar rats, respectively, that were fed Clophen A60 (60% chlorine by weight) at an estimated dosage of 5 mg/kg/day for up to 832 days (Schaeffer et al. 1984). The incidences of these lesions were significantly ($p<0.05$) higher than control values of 3.8% (5 of 131) and 0.8% (1 of 131), respectively. Combined incidences of neoplastic nodules and hepatocellular carcinomas were 98.4% (124 of 126) and 4.5% (6 of 131) in the treated and control groups, respectively. Time-dependent progression from altered foci to neoplastic nodules to hepatocellular carcinoma was observed. The Clophen A60 mixture was reported to be free of CDFs, but it is not certain whether these contaminants were absent from the mixture because no information was provided on detection limit or analytical technique, nor is it known whether and how the mixture may have been treated to remove CDFs.

In a study conducted by NCI (1978), male and female Fischer 344 rats were fed Aroclor 1254 (purity not determined) in estimated doses of 1.25, 2.5, or 5.0 mg/kg/day for 104–105 weeks. Low incidences of hepatocellular carcinomas and unspecified adenomas occurred in the middle- and high-dose groups, but in none of the control or low-dose groups, which contained 24 rats each. The incidences of combined tumors were 4.2% (1 of 24) and 12.5% (3 of 24) in the middle- and high-dose males, respectively, and 4.5% (1 of 22) and 8.3% (2 of 24) in the middle- and high-dose females, respectively. Analysis of these

results revealed no statistically significant difference between treated groups and matched controls. Re-examination and reclassification of the NCI (1978) liver data by Ward (1985) found that total tumor incidence (hepatocellular adenomas and carcinomas) was significantly increased ($p<0.05$) in the high-dose males. Tumor incidences for males in the control, low-, middle-, and high-dose groups were 0% (0 of 24), 4.2% (1 of 24), 8.3% (2 of 24), and 29.2% (7 of 24), respectively, and the response showed a significant ($p<0.01$) dose-related trend.

There were no nonhepatic tumors clearly related to Aroclor 1254 treatment in the NCI (1978) study, although adenocarcinomas in the stomach, jejunum, or cecum of two treated males (mid-dose group) and two treated females (low- and mid-dose groups), and a stomach carcinoma in one treated male (high-dose group) were found. Although their incidences were not statistically significant, the low historical incidences of these lesions suggested that they were treatment-related. Morgan et al. (1981) re-examined the NCI (1978) gastrointestinal data and found increased incidences of stomach metaplasia that were dose-related and stomach adenocarcinomas in six treated rats. When compared with incidences of stomach adenocarcinomas in historical controls (1 of 3,548), the total incidence (6 of 144) was statistically significant. This comparison may not be appropriate, however, because the Aroclor 1254-treated animals were specially examined. The investigators commented that the stomach adenocarcinoma and intestinal metaplasia appeared to be related and might have the same initiating mechanism. They concluded that Aroclor 1254 led to induction of intestinal metaplasia and probably to induction of adenocarcinoma in the glandular stomachs of Fischer 344 rats. No correlation between rats having stomach and liver lesions was found. Ward (1985), who also re-examined the NCI (1978) gastrointestinal data, noted that the metaplastic lesions were similar to those seen in monkeys, but differed in being focal and singular, while monkey lesions were diffuse. The appearance of the few metaplastic lesions in the stomachs of controls differed from those in treated rats, which resembled precancerous lesions induced by gastric carcinogens. A significant dose-related trend in combined incidences of lymphomas and leukemias in male rats also was found by NCI (1978), but incidences in each dose group were not statistically significantly different from matched controls.

In another study of Aroclor 1254 (purity not reported), no neoplastic nodules or hepatocellular carcinomas developed in small groups of Sherman rats (10 per sex) treated with estimated dietary doses as high as 72.4 mg/kg/day for 8 months (Kimbrough et al. 1972). Increased incidences of adenofibrosis of the liver were observed, but this lesion was not considered precancerous by the investigators. Sensitivity of this study is limited by the small number of animals, and the short duration may be insufficient to express possible carcinogenicity and to draw any negative conclusions.

The carcinogenicity of a lower chlorinated PCB mixture was evaluated in male Wistar rats that were fed Clophen A30 (42% chlorine by weight) in estimated dosages of 5 mg/kg/day for up to 832 days (Schaeffer et al. 1984). Liver neoplastic nodules and hepatocellular carcinomas were diagnosed in 29.2% (38 of 130) and 3.1% (4 of 130), respectively, in the treated rats compared to 4% (2 of 53) and 2% (1 of 53), respectively, in controls. The increased incidence of neoplastic nodules is statistically significant ($p<0.05$), but this pathology classification could have included nonneoplastic hyperplasia as well as benign adenomas. Combined incidences of neoplastic nodules and hepatocellular carcinomas were 7.7% (10 of 130) and 4.5% (6 of 131) in the treated and control groups, respectively.

A panel of pathologists re-evaluated seven of the PCB cancer studies in rats for the purpose of minimizing differences among studies that may have been due to the diagnostic criteria used or individual variability among pathologists (Moore et al. 1994). Also, under a new diagnostic criteria and nomenclature, lesions that had been previously diagnosed as neoplastic nodules were now classified as either hepatocellular hyperplasia or hepatocellular adenoma. A study was defined as "a protocol that examined the pathological effects associated with the chronic dietary exposure to a PCB mixture in one sex of rat." The studies re-examined were: Kimbrough et al. (1975) (Aroclor 1260), Norback and Weltman (1985) (Aroclor 1260), NCI (1978) (Aroclor 1254), and Schaeffer et al. (1984) (Clophen A30). In general, the results showed consistency in diagnoses between the original reports and the re-evaluation. One key difference was a change in some diagnoses from neoplastic nodule to focus of cellular alteration, which downgraded the finding to a nonneoplastic lesion. The results led the authors to conclude that PCBs with a 60% chlorine content consistently induce a high yield of liver tumors in rats, which supported the original findings. In addition, the reassessed results now showed that the studies in which rats were fed mixtures with 54 or 42% chlorination showed no statistically significant increases in liver tumors, and that there was no clear sensitivity differences in tumor response between males and females.

A more recent carcinogenicity study provides comparative data on the four most widely used commercial Aroclor mixtures (1016, 1242, 1254, and 1260) in rats (General Electric Co. 1997a, 1997b; Mayes et al. 1998). This is a comprehensive investigation designed to clarify carcinogenic differences in the mixtures by allowing direct comparisons of Aroclors using current tumor diagnostic criteria, and thereby address some of the limitations in previous studies and problems associated with inter-study comparisons. Groups of 50 male and 50 female Sprague-Dawley rats were fed Aroclor 1016, 1242, 1254, or 1260 in the diet for 24 months at three dose levels per compound (two for Aroclor 1242) in ranges of 2.0–11.2, 2.0–5.7, 1.0–6.1, or 1.0–5.8 mg/kg/day, respectively. One control group of 100 males and 100 females was used for the entire study (i.e., for all Aroclors). The base feed contained <0.15 ppm of PCBs

(estimated dose <0.01 mg/kg/day). The Aroclor 1016, 1242, 1254, and 1260 test mixtures contained PCDD concentrations of 0.6, 0, 20, and 0 ppb, respectively, and PCDF levels of 0.035, 2.9, 23, and 4.9 ppm, respectively. The Aroclor 1254 was treated for PCDF removal because the level was considered higher than the acceptable range. The cleanup procedure removed >99% of the PCDFs, and additionally reduced the concentration of congener 3,3',4,4',5-pentaCB (PCB 126) by approximately 35%, which was still about 2 times greater than that of "ordinary" Aroclor 1254. It was subsequently found that the tested lot of Aroclor 1254 had been made by a modified procedure that was used only in the final years of manufacture, and accounted for <1% of the total Aroclor 1254 production for the years 1958–1977 (Frame 1999).

Comprehensive histological examinations were performed on all rats in the high-dose and control groups at the end of the study (24 months), as well on animals in all groups that died prior to 24 months (Mayes et al. 1998). Evaluations of all remaining animals included the liver, brain, mammary gland, thyroid (males only), and gross lesions. Statistically significantly increased tumor incidences were found in the liver and thyroid, while significant decreases occurred in the mammary gland. The response in the liver was both Aroclor- and sex-dependent (much greater in females than males), consisted primarily of benign hepatocellular adenomas and, for females, increased with dose and followed the general incidence pattern of Aroclor 1254 > Aroclor 1260 . Aroclor 1242 > Aroclor 1016. For females exposed to Aroclor 1254, percentages with liver tumors were statistically significantly (p#0.05 or p#0.01) increased as follows: hepatocellular adenomas in all dose groups at 1.4, 2.9, and 6.1 mg/kg/day (36, 52, and 54%, respectively, vs. 1% in controls), hepatocellular carcinomas in the middle- and high-dose groups (8 and 12%, respectively, vs. 0%), and hepatocholangiomas in the middle-dose group (12 vs. 4%). For females exposed to Aroclor 1260, hepatocellular adenomas were significantly increased in all dose groups at 1.4, 2.8, and 5.8 mg/kg/day (18, 20, and 42%, respectively, vs. 1% in controls), and hepatocellular carcinomas and hepatocholangiomas were increased at the high dose (10 and 6%, respectively, vs. 0% in controls). For females exposed to Aroclor 1242, hepatocellular adenomas were increased in both dose groups at 2.8 and 5.7 mg/kg/day (20 and 24%, respectively, vs. 1% in controls). For females exposed to Aroclor 1016, hepatocellular adenomas were increased in the middle- and high-dose groups at 5.4 and 11.2 mg/kg/day (10 and 10%, respectively, vs. 0% in controls). In males, liver tumor responses were nonsignificant (p>0.05) in all groups except for increased hepatocellular adenomas at the highest dose (4.1 mg/kg/day) of Aroclor 1260 (14 vs. 4% in controls). The liver neoplasms did not adversely affect survival rates in any of the Aroclor-exposed groups.

Thyroid follicular cell adenomas were significantly (p#0.05 or p#0.01) increased in male rats in a non-dose-related and non-Aroclor-related manner (Mayes et al. 1998). Increased percentages of males with follicular cell adenomas were induced by Aroclor 1242 in both dose groups at 2.0 and 4.0 mg/kg/day (10 and 10%, respectively, vs. 1% in controls), Aroclor 1254 in all dose groups at 1.0, 2.0, and 4.3 mg/kg/day (12, 8, and 10%, respectively, vs. 1%), and Aroclor 1260 in the low and middle-dose groups at 1.0 and 2.0 mg/kg/day (12 and 8%, respectively, vs. 1%). The morphologic appearance of the thyroid tumors were reported to be characteristic of those that developed as a secondary response to chronic overstimulation by thyroid-stimulating hormone. The incidence of spontaneous mammary gland tumors (fibroadenoma, adenoma, and/or adenocarcinoma) in females was statistically significantly decreased by Aroclor 1254 at the high dose of 6.1 mg/kg/day (27 vs. 45% in controls), and Aroclor 1260 in the low and middle-dose groups of 1.4 and 2.8 mg/kg/day (35 and 36%, respectively, vs. 45% in controls). Additionally, statistically significant negative trends (p#0.05) for total mammary tumors occurred for Aroclors 1242, 1254, and 1260.

Oral carcinogenicity evaluations of commercial PCB mixtures in mice are limited to two less-than-lifetime studies that did not examine tissues other than liver (Ito et al. 1973; Kimbrough and Linder 1974). Incidences of benign hepatomas were statistically significantly increased in male Balb/cJ mice fed an estimated dose of 49.8 mg/kg/day Aroclor 1254 (purity not reported) for 11 months, but not in mice similarly treated for 6 months followed by a 5-month recovery period (Kimbrough and Linder 1974). The hepatoma incidences were 0% in two control groups (0 of 34 and 0 of 24), 45.5% (10 of 22) in the 11-month exposure group, and 4.2% (1 of 24) in the 6-month exposure group. No malignant tumors were observed, but the investigators noted that the tested mouse strain only rarely develops hepatomas spontaneously and considered the hepatomas to be potentially malignant. Additionally, adenofibrosis occurred in all of the 22 mice treated for 11 months.

Liver nodular hyperplasia and hepatocellular carcinomas were found in 58.3% (7 of 12) and 41.7% (5 of 12) of dd strain mice, respectively, that were fed an estimated dose of 65 mg/kg/day Kanechlor 500 (52–54% chlorine by weight, purity not reported) for 32 weeks (Ito et al. 1973). Neither of these incidences was significantly increased compared to control values of 0% (0 of 6), but the statistical power of this study is low due to the small number of animals, relatively short treatment duration, and no posttreatment observation period. Proliferative liver lesions were not observed in mice fed lower doses (32.5 or 13 mg/kg/day) of Kanechlor 500, or in mice similarly exposed to the lower chlorinated mixtures Kanechlor 400 (48% chlorine by weight) or Kanechlor 300 (40–42% chlorine by weight) at estimated

dietary doses of #65 mg/kg/day for 32 weeks. Limitations of this study include small numbers of animals, relatively short treatment period, and no observation period following treatment.

The EPA has derived cancer potency estimates for oral exposure to PCBs (Cogliano 1998; EPA 1996c, 2000a). Based on rat liver tumor incidence data for Aroclors 1260, 1254, 1242, and 1016 from the Mayes et al. (1998)/General Electric Co. (1997a, 1997b) study and Aroclor 1260 from the Norback and Weltman (1985) study, a range of upper-bound slope factors were calculated to represent the potency of representative classes of environmental PCB mixtures. A three-category approach is used that considers how environmental processes (partitioning, chemical transformation, and bioaccumulation) affect each exposure pathway or situation by altering the composition and cancer potential of the original PCB mixtures. The highest slope factor (2.0 per [mg/kg]/day) is for the high risk and persistent category, which is used for pathways in which environmental processes are likely to increase risk, such as food chain exposure, sediment or soil ingestion, dust or aerosol inhalation, and exposure to dioxin-like, tumor-promoting, or persistent congeners. Due to the potential for higher sensitivity in early life, the highest slope factor is also used for all early-life exposures. An intermediate slope factor (0.4 per [mg/kg]/day) is used for the low risk and persistence category, which is appropriate for exposure pathways in which environmental processes tend to decrease risk, such as drinking water ingestion of water soluble congeners, inhalation of evaporated congeners, and dermal exposure (because PCBs are incompletely absorbed through the skin). The lowest slope factor (0.07 per [mg/kg]/day) applies to the lowest risk and persistence category, and is used when congener or homologue analyses of an environmental mixture verify that congeners with more than four chlorines comprise <0.5% of total PCBs, as well as the absence of dioxin-like, tumor-promoting, and persistent congeners. For the upper slope factor of 2 per (mg/kg)/day, doses corresponding to risk levels ranging from 10^{-4} to 10^{-7} are $5x10^{-5}$ to $5x10^{-8}$ mg/kg/day, respectively, as indicated in Figure 3-2.

Tumor Promotion Studies. It is well documented that orally administered commercial PCB mixtures can promote tumors in the liver (hepatocellular carcinomas, adenomas, and neoplastic nodules) and lung (alveologenic adenomas) of rats and mice following initiation with carcinogens such as N,N'-dimethylnitrosamine (DMNA), N,N'-diethylnitrosamine, N-ethyl-N'-hydroxyethylnitrosoamine, hexachlorocyclohexanes, 2-acetylaminofluorene, and 3'-methyl-4-dimethylaminoazobenzene (Anderson et al. 1986, 1991, 1994; Beebe et al. 1993; Buchmann et al. 1991; Hirose et al. 1981; Ito et al. 1973; Kimura et al. 1976; Nishizumi 1976; Preston et al. 1981; Silberhorn et al. 1990; Tatematsu et al. 1979). These studies typically administered the tumor initiator with a proliferative stimulus (e.g., hepatotoxic

dose, partial hepatectomy, or neonatal administration) and showed effects using higher chlorinated PCB mixtures (>50% chlorine by weight, particularly Aroclors 1254 and Kanechlor 500) as the promoter.

The promoting activity of PCBs is also indicated by short-term assays in which orally administered commercial mixtures or single congeners promoted development of putative preneoplastic lesions in rat liver following induction by various initiators (Anderson et al. 1991; Buchmann et al. 1991; Deml and Oesterle 1982, 1987; Deml et al. 1983; Hemming et al. 1993; Laib et al. 1991; Oesterle and Deml 1983, 1984; Pereira et al. 1982; Preston et al. 1985; Rose et al. 1985; Sargent et al. 1991; Silberhorn et al. 1990). Enzyme-altered hepatic foci, identified by alterations in adenosine triphosphatase (ATPase), GGT, or placental glutathione S-transferase (PGST) activity, were used as markers of promoting activity. The commercial PCBs showing promotion in these studies were usually the higher-chlorinated mixtures Aroclor 1254 or Clophen A50. The congener studies have shown promoting activity with non-*ortho* PCBs such as PCB 77 and PCB 126; mono-*ortho*-substituted PCBs such as PCB 105 and PCB 114, and di-*ortho* PCBs such as PCBs 47, 49, and 153. Although structurally diverse congeners show promoting activity, the co-planar PCBs appear to be most effective. Additional information on tumor promotion by PCBs is discussed in Section 3.5.2 (Mechanisms of Toxicity).

3.2.8.3.3 Dermal Exposure

Dermal carcinogenicity studies of PCBs consist of skin tumor initiation and promotion assays. A single dose of 0.1 mg of Aroclor 1254 (purity not reported) showed no conclusive initiator activity when applied to the shaved skin of female CD-1 mice followed by promotion with the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA) twice weekly for 32 weeks (DiGiovanni et al. 1977). The finding is inconclusive due to low skin papilloma incidence and lack of control mice treated with TPA alone. Aroclor 1254 (purity not reported) was not a skin tumor promoter when applied to the shaved skin of female CD-1 mice (0.1 mg/mouse, twice weekly for 30 weeks) that were initiated with dimethyl-benzanthracene (DMBA) (Berry et al. 1978, 1979), or to female HRS/J hairless mice (1 mg/mouse, twice weekly for 20 weeks) that were initiated with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) (Poland et al. 1983). These results must be interpreted with caution since only one dose level of Aroclor 1254 was tested, and the doses may have been too low, as indicated by slight, although not statistically significant, promotion in the Poland et al. (1983) study. The initiation and promotion studies tested sufficient numbers of animals and, except as noted above, included positive and negative control groups. Pretreatment with a single topical dose of 0.1 mg of Aroclor 1254 inhibited skin tumor initiation by DMBA in female CD-1 mice by as much as 45% (Berry et al. 1979).

3.2.8.3.4 Evaluation of Animal Studies

The carcinogenicity of several commercial PCB mixtures has been evaluated in a number of chronic oral bioassays in rats. The most comprehensive and adequately performed study compared Aroclors 1016, 1242, 1254, and 1260 and found that all four mixtures induced liver tumors when fed to female Sprague-Dawley rats (General Electric Co. 1997a, 1997b; Mayes et al. 1998). Aroclor 1260 also induced liver tumors in male rats. The liver response was both Aroclor- and sex-dependent (much greater in females than males), consisted primarily of benign hepatocellular adenomas and, in females, increased with dose in the general potency pattern of Aroclor 1254 > Aroclor 1260 . Aroclor 1242 > Aroclor 1016. Previous lifetime dietary exposure studies found that commercial mixtures with 60% chlorine content (Aroclor 1260 and Clophen A60) induced liver tumors in three strains of rats (Kimbrough et al. 1975; Moore et al. 1994; Norback and Weltman 1985; Schaeffer et al. 1984), with indications of a sex-dependent response (stronger in females) in one of these studies (Norback and Weltman 1985). Many of the rat liver tumors were benign, although sequential morphologic analyses demonstrated the eventual progression of the benign liver lesions to malignant carcinomas (Norback and Weltman 1985). Lifetime carcinogenicity tests of commercial PCB mixtures containing <60% chlorine were performed in only a few studies prior to the Mayes et al. (1998) bioassay. Liver tumors were reportedly induced by Aroclor 1254 in Fischer 344 rats (NCI 1978; Ward 1985) and a 42% chlorine mixture (Clophen A30) in Wistar rats (Schaeffer et al. 1984), but re-evaluation of these studies using current diagnostic criteria showed no statistically significant increases in tumor incidences or clear sensitivity differences in tumor responses between males and females. The chronic rat studies provide a limited amount of evidence for neoplastic or preneoplastic changes in tissues other than the liver. Incidences of preneoplastic lesions in the biliary tract were increased in both sexes by exposure to Aroclor 1260 (Norback and Weltman 1985), although the response was greater in females. There was a suggestive indication of Aroclor 1254-induced precancerous intestinal metaplasia and adenocarcinomas in the stomach of rats in one study (Morgan et al. 1981; NCI 1978; Ward 1985). The preneoplastic lesions in the biliary tract and stomach have not been reported in other studies, particularly Mayes et al. (1998). Statistically significant increases in thyroid gland follicular cell adenomas were induced by Aroclors 1242, 1254, and 1260 in males, but not females (Mayes et al. 1998).

The oral carcinogenicity of commercial PCB mixtures has also been tested in mice, but these studies are limited by intermediate-duration exposures, lack of postexposure observation, and histological examinations that were limited to the liver. These studies generally indicate that less-than-lifetime dietary

exposure to commercial mixtures with 42–60% chlorine induced precancerous liver lesions (Ito et al. 1973; Kimbrough and Linder 1974; Kimbrough et al. 1972).

It is well documented that oral exposure to commercial PCBs and single congeners can promote preneoplastic lesions and tumors in the liver and lung of rats and mice following initiation with other carcinogens (Anderson et al. 1986, 1991, 1994; Beebe et al. 1993; Buchmann et al. 1991; Deml and Oesterle 1982, 1987; Deml et al. 1983; Hemming et al. 1993; Hirose et al. 1981; Ito et al. 1973; Kimura et al. 1976; Laib et al. 1991; Nishizumi 1976; Oesterle and Deml 1983, 1984; Pereira et al. 1982; Preston et al. 1981, 1985; Rose et al. 1985; Sargent et al. 1991; Silberhorn et al. 1990; Tatematsu et al. 1979). The commercial PCBs showing promotion in these studies were usually the higher-chlorinated mixtures such as Aroclor 1254. Congeners showing promoting activity are structurally diverse, although the co-planar PCBs appear to be most effective.

Several studies in which relatively low doses (0.1 mg/mouse) of Aroclor 1254 were applied to the skin of mice showed no conclusive initiation or promotion activity (Berry et al. 1978, 1979; DiGiovanni et al. 1977; Poland et al. 1983). Studies corroborating these findings on skin tumor initiation, promotion, and inhibition or evaluating the carcinogenicity of PCBs applied to the skin without initiators or promoters have not been performed.

Before the comprehensive four-Aroclor comparative carcinogenicity study was conducted by Mayes et al. (1998), only commercial PCBs mixtures with 60% chlorine had been adequately tested, and there was controversy about whether mixtures with lower chlorine content were carcinogenic. The liver and thyroid tumor results of the Mayes et al. (1998) rat study, in addition to confirming the carcinogenicity of higher chlorinated PCBs, provide compelling evidence that all commercial PCB mixtures can cause cancer in animals. The sufficiency of the evidence of carcinogenicity of PCBs in animals is recognized by both EPA (Cogliano 1998; IRIS 2000) and IARC (1987).

3.3 HEALTH EFFECTS IN WILDLIFE POTENTIALLY RELEVANT TO HUMAN HEALTH

3.3.1 Overview

The EPA's final rule strictly limiting the manufacture, processing, distribution, and use of PCBs under Section 6(e) of the Toxic Substances Control Act was promulgated in 1979 (EPA 1979a). A technical support document for the EPA rule was comprised primarily of a draft environmental impact statement that outlined the significance of the release of PCBs into the environment from both human and wildlife health perspectives (EPA 1978c). Health effects in wildlife that were cited in the support document included the following: mortality in piscivorous birds; reproductive impairment in monkeys, minks, ring doves, and American kestrels; immunotoxicity in monkeys and birds; and endocrine and neurobehavioral effects in birds. A variety of other health effects have since been evaluated in wildlife, some of which may be relevant to human health. Environmental monitoring studies have shown that PCBs are highly persistent in the environment (see Section 6.3, Environmental Fate), and therefore continue to present a potential health hazard to humans.

Wildlife may be regarded as sentinels for human health. Wildlife sentinel species data may be used for several purposes related to exposure and hazard assessment (NRC 1991; van der Schalie et al. 1999), including the following: (1) provide additional weight of evidence in a human health risk assessment; (2) act as an early warning for potential effects in humans (e.g., by identifying new locations of potential concern for human health, or identifying new end points of potential human concern not previously observed in experimental animal studies); (3) suggest potential cause-and-effect relationships for further study; (4) investigate the bioavailability of contaminants from environmental media; and (5) monitor contamination in the food web, such as during the course of remedial actions. Reviews of public health considerations regarding toxic substances (including PCBs) in the Great Lakes region have incorporated effects in wildlife in a weight-of-evidence analysis of the potential for detrimental effects in humans in the region, particularly in human populations that rely heavily on Great Lakes fish for their dietary protein (Johnson et al. 1998b, 1999).

The purpose of this section is to provide a qualitative synopsis of health effects in wildlife to address the potential concern that effects observed in wildlife that are attributable to PCB exposure may also occur in humans, and to highlight information in the wildlife database that contributes to the weight of evidence supporting the critical effects that form the basis for the chronic- and intermediate-duration oral MRLs.

A hazard identification table (Table 3-6) is provided to quickly scan the wildlife database for taxa or categories of toxicological end points that are of particular interest. Since Table 3-6 is intended for hazard identification, data concerning effects from parenteral exposures were included, as well as oral, inhalation, and dermal routes that are more directly relevant to human environmental exposures. Table 3-6 distinguishes between correlational evidence from field observations and experimental evidence. Correlational evidence from field studies inherently has multiple sources of uncertainty, many of which are controlled in experimental studies. Observations that indicate a positive relation between environmental PCB exposure (sometimes represented by PCB concentration in tissue) and an adverse health effect in free-ranging wildlife are represented in Table 3-6 as correlational field observations. Effects that were observed in experimental studies under controlled or closely monitored exposure conditions were included in the table as experimental observations. However, no entry was made in Table 3-6 for responses that were reported in an experimental study to be equivocal, ambiguous, or not statistically significant.

Several reviews of the PCB ecotoxicological literature (e.g., DOI 1986, 1996; Hansen 1987b; Safe 1994; WHO 1993) provided much of the information included in this section. Information contained in the reviews was supplemented by individual studies that were not otherwise represented, such as more recent studies. The number of sources identified for an individual Table 3-6 entry does not necessarily reflect the number of studies showing that effect for the following reasons: (1) several reviews may have reported the same results from a single study, or (2) a single source document may report effects in multiple studies, but the source is represented only once for a given entry. Information included in Table 3-6 was limited to effects in fish and birds, and in mammals that had not been bred to reduce genetic variability (e.g., Table 3-6 includes data on monkeys and minks, but excludes data on laboratory-bred strains of rats, mice, rabbits, etc.). No data concerning effects in fungi, invertebrates, microbes, or terrestrial or aquatic plants were included. Among the classes of organisms represented in Table 3-6, mustelids (primarily minks and ferrets), galliform birds (primarily domestic fowl and quail), and freshwater fish were the most frequently studied (see Table 3-6). End points that received the most attention in the wildlife toxicology literature on PCBs were mortality, reproductive, developmental, and endocrine effects, and enzyme induction. Additional categories of end points that were relatively frequently addressed were immunological, neurological/behavioral, and hepatic effects. Most of the toxicological information represented in Table 3-6 was derived from experimental studies, but the focus of some experiments was influenced by observations in earlier field studies of correlations between levels of environmental PCBs and the occurrence and/or severity of toxicological effects in wildlife.

Table 3-6. PCB Hazard Identification in Wildlife

Adverse biological effect	Wild mammals				Birds			Reptiles	Amphibians		Fish	
	Primate	Mustelid	Cetacean, pinniped	Other	Piscivore	Galliform	Other	Turtle	Frog	Toad	Freshwater	Marine
Mortality	O_{E1}[5]	O_{E1}[4,5,26,32] O_{E3}[4,25,28,32] O_{E4}[4]		O_{E3}[4]	O_{E3}[4]	O_{E1}[5] O_{E3}[4,7,32]	O_{E3}[4,32]		O_{E1}[27] O_{E3}[32]	O_{E1}[9] O_{E3}[9,32]	O_{E1}[5,28] O_{E2}[5] O_{E3}[4,32]	O_{E3}[4]
Systemic effects												
Respiratory		O_{E4}[13]				O_{E3}[32]						O_{E3}[32]
Cardiovascular		O_{E3}[32] O_{E4}[24]	O_{C4}[2]			O_{E3}[32]						
Gastrointestinal	O_{E1}[5] O_{E3}[4]	O_{E1}[5]	O_{C4}[2]			O_{E3}[28]						
Hematological		O_{E4}[13]									O_{E3}[4,32]	
Musculo-skeletal						O_{E3}[32]					O_{E3}[4,32]	
Hepatic	O_{E3}[4,28]	O_{E1}[5] O_{E3}[19,28] O_{E4}[13,24] O_{E5}[4,5]			O_{E3}[4]	O_{E1}[5] O_{E2}[5] O_{E3}[10,28,32] O_{E5}[4]	O_{E1}[5] O_{E3}[4]				O_{E3}[4,32]	O_{E3}[32]
Renal		O_{E4}[24]	O_{C4}[2]			O_{E3}[28]					O_{E3}[32]	
Endocrine	O_{E3}[28]	O_{E3}[32] O_{E4}[19,24,30] O_{E5}[5]	O_{E3}[10] O_{E4}[3,32] O_{C4}[2,23]		O_{E3}[28,32] O_{C4}[3]	O_{E3}[32]	O_{E1}[4,5,7,32] O_{E3}[28,32]				O_{E3}[4,10]	O_{E3}[3,32]
Dermal/ ocular	O_{E1}[5] O_{E3}[4,5,28]	O_{E3}[32]	O_{C4}[2]								O_{E3}[32]	
Body weight	O_{E1}[5] O_{E3}[5,28]	O_{E1}[5,32] O_{E3}[5,28,32]				O_{E2}[5]	O_{E1}[5]			O_{E3}[10]	O_{E3}[4,32]	
Metabolic		O_{E5}[5]					O_{E1}[4,32]		O_{E3}[9]		O_{E3}[4,32]	

Table 3-6. PCB Hazard Identification in Wildlife *(continued)*

Adverse biological effect	Wild mammals				Birds			Reptiles	Amphibians		Fish	
	Primate	Mustelid	Cetacean, pinniped	Other	Piscivore	Galliform	Other	Turtle	Frog	Toad	Freshwater	Marine
Enzyme induction		O_{E1}[5] O_{E3}[28] O_{E4}[29] O_{E5}[5]				O_{E1}[5,13,28,32] O_{E2}[5,32] O_{E3}[5,10,28,32]	O_{E1}[5] O_{E2}[5] O_{E3}[4]				O_{E1}[5] O_{E3}[5]	O_{C4}[10]
Blood chemistry	O_{E1}[5]	O_{E5}[5]				O_{E3}[28]	O_{E1}[4]				O_{E3}[4,32]	
Immunological/ lymphoreticular	O_{E1}[5] O_{E3}[28]	O_{E4}[13,24]	O_{E4}[16] O_{C4}[3,10,20]		O_{C4}[31]	O_{E1}[5] O_{E3}[32]	O_{E1}[5] O_{E3}[32]				O_{E3}[18,32]	O_{E3}[10]
Neurological/ behavioral	O_{E2}[7] O_{E3}[5,7,10,16,28]	O_{E2}[10] O_{E4}[13,24]		O_{E3}[4]		O_{E3}[7,32]	O_{E3}[4,10,32]				O_{E2}[5] O_{E3}[4]	O_{E3}[32]
Reproductive	O_{E3}[4,5,10,28]	O_{E1}[4] O_{E3}[1,4,5,10,25,27,32] O_{E4}[4,12,17,24,26,32] O_{E5}[32] O_{C4}[7,11]	O_{C4}[2,3,10,32] O_{E4}[3,32]	O_{E3}[10,32]	O_{C4}[3,21]	O_{E3}[4,28,32]	O_{E1}[5] O_{E3}[4,10,28]	O_{C4}[3]			O_{E3}[10,32] O_{C4}[4]	O_{E3}[28,32] O_{C4}[4]
Developmental	O_{E3}[4,5,7,10]	O_{E3}[6,25,32,34] O_{E4}[12,24]		O_{E3}[10]	O_{E1}[5] O_{C1}[7] O_{C4}[10,32]	O_{E1}[4,5,10] O_{E3}[10,32]		O_{E4}[3]	O_{E1}[27] O_{E3}[9]	O_{E1}[9] O_{E3}[9]	O_{E3}[32] O_{C4}[32]	
Egg shell	Not relevant for wild mammals				O_{C4}[4]	O_{E3}[32]	O_{E3}[5] O_{C4}[4]		Not relevant for amphibians or fish			
Genotoxic							O_{E3}[4]					
Cancer	No cancer data											

Entry:

Effect Code
O = effect was observed, as reported in the source document.
Blank = effect was either not evaluated, or evaluated but not observed (including equivocal, ambiguous, or not statistically significant responses)

Subscript:

Observation Type
E = experimental observation
C = correlational field observation

PCB Exposure
1 = dioxin-like PCB congener (AhR binder; planar; chlorine para-substituted and non- or mono-*ortho*-substituted)
2 = non-dioxin-like PCB congener (poorly binds to AhR; non-planar; chlorine di-, tri-, or quatro-*ortho*-substituted)
3 = commercial PCB mixture (e.g., Aroclor 1016)
4 = "weathered" (i.e., environmentally degraded and/or metabolized) PCB mixture, usually in combination with other chemicals (e.g., PCBs in wild-caught fish)
5 = unspecified PCB

Superscript:

Source Documents (including reviews of PCB toxicity in wildlife and experimental studies not otherwise represented)

1 = Backlin et al. 1998b
2 = Bergman and Olsson 1985
3 = Crisp et al. 1998
4 = DOI 1986
5 = DOI 1996
6 = EPA 1980f
7 = Giesy and Kannon 1998
8 = Geisy et al. 1994
9 = Gutleb et al. 1999
10 = Hansen 1987b
11 = Harding et al. 1999
12 = Heaton et al. 1995a
13 = Heaton et al. 1995b
14 = Hornung et al. 1998
15 = Jarman et al. 1996
16 = Johnson et al. 1998
17 = Johnson et al. 1999
18 = Jones et al. 1979
19 = Käkelä et al. 1999
20 = Kannan et al. 1993
21 = Murk et al. 1996
22 = Nisbet et al. 1996
23 = Olsson et al. 1994
24 = Restum et al. 1998
25 = Ringer et al. 1991
26 = Risebrough 1999
27 = Rosenshield et al. 1999
28 = Safe 1994
29 = Shipp et al. 1998a
30 = Shipp et al. 1998b
31 = van der Schalie et al. 1999
32 = WHO 1993
33 = Wren et al. 1987a
34 = Wren et al. 1987b

There is some question as to the relevance of experimental studies in wildlife using single congeners or well-described commercial mixtures to situations involving environmental exposures of free-ranging wildlife to weathered PCBs (i.e., PCB mixtures in environmental media, such as the water column or animal tissues, that have undergone selective environmental degradation, bioaccumulation, and/or metabolism of component PCB congeners). Reviews (Giesy et al. 1994; WHO 1993) identified the following difficulties in extrapolating from toxicity observed in wildlife experimental studies to effects expected in wildlife in the environment: (1) most of the experimental studies in fish and wildlife tested the effects of commercial mixtures of PCBs, so the identity of the particular components (or interactive sets of components) that caused the effects is not generally known; (2) tests were generally conducted in environmentally unrealistic conditions; (3) because of differences between congeners in environmental fate, bioaccumulative potential, and species-specific degree of metabolism, weathered mixtures of PCBs in various environmental compartments (e.g., the water column and animal tissues) frequently bear little resemblance to the original commercial mixture that was released into the environment; (4) PCB exposure in the environment invariably involves co-exposure to other pollutants that may interact to produce effects that were not observed under experimental conditions. For example, among mink studies, weathered total PCBs in fish were found to be more potent than commercial PCB mixtures, possibly because the weathering process selectively removed the less toxic congeners or possibly because of interactions with other contaminants (Giesy and Kannan 1998; Giesy et al. 1994).

3.3.2 Health Effects in Wildlife

Biological responses in wildlife to exposures to individual PCB congeners and commercial PCB mixtures varied widely, possibly reflecting not only variability in susceptibility among species, but also differences in mechanism of action or selective metabolism of individual congeners (DOI 1986, 1996; WHO 1993). More highly chlorinated congeners tend to bioaccumulate most readily, and PCBs tend to biomagnify in the food chain, reaching relatively high, toxic concentrations at higher trophic levels, such as in piscivorous birds (e.g., gulls, terns, and cormorants) and mammals (e.g., minks, otters, seals, and sea lions) (EPA 1978c; WHO 1993). It is generally accepted that dioxin-like PCB congeners (i.e., those that can assume a planar configuration and exhibit high affinity for the Ah receptor) are more potent toxicants than other congeners (i.e., those with multiple chlorine substitution in ring positions 2 and 6) (DOI 1996; Giesy and Kannon 1998; Giesy et al. 1994). Of interest is the observation that the patterns of toxicities in seals may be changing with apparent decreasing global burdens; certain pathological changes are now more closely associated with methyl sulfonyl metabolites of DDE and PCBs than with parent coplanar PCBs (Olsson et al. 1994). Reproductive effects in birds and piscivorous mammals appear to be Ah

receptor-mediated, since planar, dioxin-like PCB congeners are more effective in inducing these effects than non-dioxin-like congeners (Giesy and Kannan 1998). In a comprehensive review of the literature concerning the ecotoxicology of planar PCBs, DOI (1996) concluded that the chinook salmon, domestic chicken, mink, and Rhesus macaque were among the most sensitive species to effects from planar PCB exposure.

In aquatic organisms including fish, PCB toxicity was enhanced by flow-through experimental conditions as compared to static exposure conditions, and commercial Aroclor mixtures with moderate chlorine content were generally more toxic than commercial mixtures with low or high percentage chlorination (WHO 1993). Fish in early life stages were more vulnerable than adults to PCB toxicity (WHO 1993). In birds, acute toxicity in experiments was generally positively related to degree of chlorination of the commercial mixture (WHO 1993). Avian reproduction was impaired primarily due to decreased egg hatchability and increased embryotoxicity (WHO 1993). Available evidence indicates that PCBs do not directly affect egg shell thickness in birds, but may indirectly affect egg shells by decreasing food consumption and thereby reducing body weight (WHO 1993). PCBs are ubiquitous and continuously circulating in the global environment, and appear to be gradually redistributing toward the marine environment (WHO 1993). For this reason, and because marine mammals are near the top of the food chain, piscivorous marine mammals are regarded as potentially the most sensitive wildlife receptors to PCB exposure (DOI 1996; WHO 1993). Field studies suggested, and subsequent experimental studies confirmed, that accumulated PCBs impair pinniped (e.g., seals and sea lions) reproduction by preventing implantation of the embryo; whether this effect is caused by endocrine disruption remains unresolved (WHO 1993). The endocrine disruptive potential of PCBs and other persistent and bioaccumulative pollutants has been critically reviewed in the literature (e.g., Crisp et al. 1998; DeRosa et al. 1998; Risebrough 1999); the wildlife toxicology database summarized in Table 3-6 indicates that PCBs have induced endocrine-related effects in a variety of taxa.

Of particular interest are PCB-induced effects in wildlife that contribute to the weight of evidence supporting the oral MRL derivations, including neurological/behavioral, immunological, and dermal effects. The intermediate-duration MRL is based on neurodevelopmental alterations in infant Rhesus monkeys that were postnatally fed a constituted mixture of PCB congeners analogous to those found in human breast milk. The chronic-duration MRL is based on immunological and dermal/ocular effects in Rhesus monkeys resulting from long-term oral exposure to Aroclor 1254 (see Section 2.3, Relevance to Public Health and Appendix A for further details concerning MRL rationale and derivations). Effects that

occurred in monkeys at doses proximate to the LOAELs used to derive the MRLs included decreased conception and fetal mortality.

Effects in wildlife that are potentially related to neurological impairment included alterations in central nervous system neurotransmitter levels, retarded learning, increased activity, and behavioral changes. Significantly reduced dopamine levels were observed in certain regions of the brain in adult pigtailed macaques provided Aroclor 1016 or Aroclor 1260 orally at 0.8, 1.6, or 3.2 mg/kg/day for 20 weeks; reduced dopamine levels persisted after termination of exposure (Geisy and Kannon 1998; Safe 1994). Offspring of Rhesus monkeys provided Aroclor 1248 in the diet at 0.5–2.5 mg/kg before and during gestation showed hyperactivity and other behavioral deficits (Geisy and Kannon 1998; Hansen 1987). "Long-term neurobehavioral changes" were seen in monkeys provided an unspecified PCB mixture (DOI 1996). Two-and-a-half and 5-year-old monkeys exhibited retarded learning and inefficient response behavior following a 20-week oral exposure to a PCB mixture immediately after birth (Johnson et al. 1998). Similarly, retarded learning, increased locomotor activity, impaired discrimination reversal learning, and increased hyperactivity were observed in monkeys provided Aroclor 1248 for an unspecified duration (Safe 1994). Brain catecholamine levels were altered in minks exposed (by an unreported route and duration) to PCB 136 (Hansen 1987). Minks provided diets of carp containing weathered PCBs for up to 182 days showed listlessness and nervousness, as well as anorexia, hindlimb paralysis, and sporadic seizures prior to death (Heaton et al. 1995b). Brain weight was significantly reduced in F1 adult female minks exposed to weathered PCBs (in carp) *in utero*, during lactation, and through the diet until 1.5–14 months postpartum (Restum et al. 1998). Significantly reduced sleeping times were observed in white-footed mice and raccoons provided diets containing 25–100 ppm Aroclor 1254 for up to 3 weeks (DOI 1986).

Suppressed avoidance response was observed in Japanese quail fed a diet containing 200 µg Aroclor 1254/g diet for 8 days (Geisy and Kannon 1998). Doves provided an unspecified Aroclor mixture showed altered brain catecholamine levels (Hansen 1987). Altered courtship, reproductive, and nesting behavior were seen in mourning doves at 14–44 days after termination of a 6-week dietary exposure to up to 40 ppm Aroclor 1254 (DOI 1986; WHO 1993). Decreased parental attentiveness was seen in ring doves provided Aroclor 1254 at 10 mg/kg diet (WHO 1993). Nest-building activity was reduced in pigeons orally dosed with 15 mg Aroclor 1254/day by gelatin capsule throughout a courtship cycle (WHO 1993). Avoidance response was significantly reduced in Japanese quail following dietary exposure to 200 ppm Aroclor 1254 (unspecified duration), compared to pre-exposure response levels, and persisted for 6 days after exposure (WHO 1993). Increased migratory restlessness was observed in

European robins and redstarts provided diets of mealworms containing Clophen A50 for 11–13 days (WHO 1993).

Guppies appeared sluggish and uncoordinated after consuming diets containing up to 150 mg PCB 133 or PCB 197/kg diet for up to 247 days, or 550–1,400 mg/kg diet for 65 days (DOI 1996). Poor muscle coordination, tetany, and lateral or ventral caudal flexion were observed in salmon and trout after 1 week of dietary exposure to unspecified PCB mixtures (DOI 1986). Whole-brain noradrenalin amd dopamine levels were significantly reduced and swimming activity was increased in Gulf killifish exposed to 4 mg Aroclor 1242/L for 24 hours; increased swimming activity persisted for 2 days after exposure (WHO 1993).

Positive findings in wild mammals, birds, or fish that contribute to the weight of evidence for immunological effects included morphological changes in organs related to the immune system, as well as functional impairment of humoral- and cell-mediated immune responses. Reduced antibody production in response to SRBC erythrocyte challenge was observed in monkeys exposed to Aroclor 1254 and Kanechlor 400 (Safe 1994). Absolute and relative spleen weights were increased in female minks fed diets containing 1 ppm total weathered PCBs (in carp) for approximately 6 months compared to a control group; no such changes were observed in males (Restum et al. 1998). Increased disease susceptibility in California sea lions has been positively associated with tissue PCB residue (Hansen 1987b). Impaired natural killer cell activity and T-lymphocyte function were observed in harbor seals fed diets of Baltic Sea herring containing relatively high levels of organochlorine-compounds, including PCBs, compared to seals fed diets of fish with lower levels of contamination (Johnson et al. 1998). High blubber PCB levels (94 to 670 µg/g) were observed in a population of western Mediterranean striped dolphins affected by a mobillivirus epizootic (Kannan et al. 1993).

In a survey of herring gulls and Caspian terns (piscivorous birds) in the Great Lakes region, suppression of T-cell-mediated immunity was associated with level of prenatal exposure to unspecified PCBs (van der Schalie et al. 1999). Splenic atrophy was observed in groups of cockerels fed diets containing 400 mg/kg Phenochlor DP6, Clophen A60, or Aroclor 1260 for 60 days (WHO 1993). PCB-induced atrophy of lymphoid tissues in chickens and pheasants and increased susceptibility of ducklings to hepatitis virus have been associated with the immunosuppressive effect of PCBs (WHO 1993). Thymic involution and edema were observed in 1-day-old domestic chickens fed diets containing 400 mg/kg PCB 169 for 21 days (DOI 1996). A lymphoid depletion of the spleen was observed in nestling American kestrels administered daily oral doses of 50 µg/kg PCB 126 for 10 days (DOI 1996).

In channel catfish, 100% mortality was observed in an immunized group that was intraperitoneally injected with 70 mg/kg Aroclor 1232 and then challenged with the virulent bacterium *Aeromonas hydrophila*, while an immunized control group had no mortality. The PCB-treated group also showed significantly decreased serum β-globulin levels, slightly elevated serum α-globulin levels, and decreased peritoneal macrophage activity compared to the control group (Jones et al. 1979). Rainbow trout showed a reduction in the lymphatic elements of the spleen after dietary exposure to 10 or 100 mg/kg Aroclor 1254 for 330 days (WHO 1993). Increased disease susceptibility was observed in pinfish and spot fish (marine/estuarine species) exposed to Aroclor 1254 at concentrations as low as 0.005 ppm (Hansen 1987b).

Wildlife findings that contribute to the weight of evidence for dermal/ocular effects include dermal changes in several taxa. Dose- and time-dependent increases in chloracne and histological changes in the sebaceous glands were observed in Rhesus macaques fed diets containing 0.3 to 3.0 ppm PCB 77 for 1 to 6 months (DOI 1996). Scaly skin, hair loss, and abnormal nail growth were observed in cotton top marmoset monkeys orally administered 0.1 to 3.0 mg PCB 77/kg body weight twice/week for 18–28 weeks (DOI 1996). Enlarged, thickened, and deformed toe nails, hyperkeratosis at the junction of the skin and sponchium, and dysplasia of the root and matrix of the nail were observed in ferrets fed a diet containing 20 ppm Aroclor 1242 for 8 months; Aroclor 1016 similarly administered did not cause these effects (WHO 1993). Bilateral epidermal thinning, hyperkeratosis, cystic dilations of hair follicles, and deformations and fractures of the claws were observed in grey seals of the Baltic Sea suspected of having significant exposure to weathered PCBs (Bergman and Olsson 1985). Flagfish (*Jordanella floridae*) exposed to water concentrations of 5.1 and 18 µg Aroclor 1248/L “almost completely lost their fins and tails”, while sheepshead minnow fry exposed to 0.1–10 µg/L showed increased fin rot (WHO 1993).

Regarding the weight of evidence for reproductive and developmental toxicity, embryo/fetal loss is one effect among a suite of developmental effects observed repeatedly in Great Lakes wildlife that have been characterized as the Great Lakes embryo mortality, edema, and deformity syndrome (GLEMEDs syndrome) (Giesy et al. 1994; Hansen 1987b). The wildlife database outlined in Table 3-6 includes observations of increased postimplantation embryo/fetal loss in several taxa (e.g., nonhuman primates and mustelids), as well as additional effects that may be indicative of PCB-induced embryo/fetal death (e.g., reduced egg hatchability in bird and fish eggs, and reduced numbers of live births in mammals).

In summary, the wildlife toxicity database for PCBs summarized in Table 3-6 contributes to the weight of evidence supporting the critical health effects used in the MRL derivations (i.e., neurological/behavioral,

immunological, and dermal/ocular effects), as well as other PCB-induced effects that are particularly relevant to human health (e.g., reproductive and developmental toxicity).

3.4 TOXICOKINETICS

Although most toxicological data reviewed in this profile have been obtained from studies in which PCB mixtures were used, Section 3.4.3 (Metabolism) in particular, contains descriptions of studies in which individual PCB congeners were used. This supplemental information is considered necessary since most of the current knowledge regarding biotransformation of chlorinated biphenyls in experimental animals has been derived from such studies. Table 4-2 lists the IUPAC number of the congeners and the corresponding chlorine substitution.

Data regarding toxicokinetics of PCBs in humans are limited to information derived from cases of ingestion of food contaminated with PCBs and cases of occupational exposure by the inhalation and dermal routes. Humans can absorb PCBs by the inhalation, oral, and dermal routes of exposure. PCBs, when administered orally, are well absorbed by experimental animals, but they are absorbed less efficiently when administered by the dermal route. Inhalation absorption data are insufficient for estimating absorption rates. In the gastrointestinal tract, PCBs are absorbed on a congener specific basis by passive diffusion. A high diffusion gradient and nearly complete absorption occurs when the PCB level in the gut contents (lipid basis) is much greater than the concentration in serum lipids. The predominant PCB carriers in human plasma are in the lipoprotein fraction. Due to their lipophilic nature, PCBs, especially the highly chlorinated congeners, tend to accumulate in lipid-rich tissues. Greater relative amounts of PCBs are usually found in the liver, adipose, skin, and breast milk. PCBs are metabolized by the microsomal monooxygenase system catalyzed by cytochrome P-450 to polar metabolites that can undergo conjugation with glutathione and glucuronic acid. State of the art PCB exposure assessment utilizing human serum, milk, and/or tissues should not only include congener specific PCB analysis, but also analyze persistent PCB metabolites. Since certain hydroxylated and methylsulfonyl ($MeSO_2$) PCB metabolites are present in some cases at levels higher than their respective parent compounds, it is necessary to further investigate the potential biological and/or toxicological activities of these persistent metabolites. The major routes of excretion of PCBs are fecal and, especially for metabolites, urinary. Mainly metabolites are found in urine and bile, although small amounts of parent compound may appear in the feces. Some PCB congeners are relatively poorly metabolized and thus can remain in the body for long periods of time (months to years). A flow-limited pharmacokinetic model was constructed to describe disposition of some PCB congeners in adults of various animal

species. In general, the model predicted the experimental data well, although some deviations were apparent. Knowledge of the metabolic rates for PCBs is crucial for meaningful interpretation of data. Enzyme induction over long-term occupational and/or environmental exposure can render some PCBs less persistent in exposed humans than in the general population.

3.4.1 Absorption

3.4.1.1 Inhalation Exposure

Inhalation exposure is considered to be a major route of occupational exposure to PCBs (Wolff 1985). Indirect evidence of absorption of PCBs by this route in humans is based on the fact that individual congeners have been detected in tissues and body fluids of subjects exposed in occupational settings where air concentrations have also been measured. From data summarized by Wolff (1985), a maximum of 80% of the levels commonly seen in adipose tissue of exposed capacitor workers may have been absorbed by the inhalation route. A maximum of 20% would have been derived from dermal or oral exposure. Exposure to PCBs in air was positively related to mean serum PCB levels in subjects involved in clean-up operations following a PCB transformer fire (Fitzgerald et al. 1986); the relative contribution of the dermal route was not determined. Duarte-Davidson and Jones (1994) estimated that the average total background PCB exposure for the contemporary UK population was 0.53 µg/person/day, with food consumption accounting for 97% of the total PCB exposure, air contributing 3.4%, and water only 0.04%. 2,4,4'-TriCB (PCB 28) was the most abundant congener detected in air samples and accounted for 3.7% of the total exposure to this congener. More highly chlorinated congeners, such as PCB 180 were detected at lower levels in air, with air borne exposure accounting for only 1.7% of the total daily exposure to this congener. Further information regarding tissue levels in occupationally-exposed subjects can be found in Section 3.4.2.

Information regarding absorption of PCBs in animals following inhalation exposure is limited. Male rats were exposed (whole body) to an aerosol of a PCB mixture, Pydraul A200 (42% chlorine), at a concentration of 30 g/m^3 (0.5–3 µm particle diameter) for #2 hours (Benthe et al. 1972). After a 15-minute exposure, the PCB concentration in the liver was . 40 µg/g tissue, and reached a maximum of 70 µg/g after 2 hours of exposure. These results provide qualitative information regarding absorption of this specific PCB mixture, but the data were not sufficient for estimating the amount or rate of absorption. It must be also mentioned that since exposure was not nose-only, the dermal route may have contributed to absorption.

A recent study in ferrets by Apfelbach et al. (1998) reported for the first time that the olfactory system may be a potentially significant portal for the entry of airborne PCBs. Ferrets were exposed for 5 years to low levels of PCBs (total PCBs, 260 ng/m^3 air) in the ambient air of an animal care room which had PCB containing sealants. Tetra-chlorinated PCBs dominated the congener profile of ambient air, with PCB 52 being found at the highest concentration. The PCB congener pattern in the olfactory bulbs resembled that found in the ambient air, with the less chlorinated, more volatile PCBs (52) being retained at the higher levels. In contrast, the congener profile in adipose tissue resembles that of most exposed or unexposed animals, with hexa- and hepta-substituted congeners being the major congeners present. The olfactory bulbs of the exposed animals had the highest total PCB concentration (642 ng/g lipids), while the liver, adipose tissue, and brain had levels of 202, 303, and 170 ng/g lipids, respectively. The data suggest that inhaled PCBs pass into the dentrites of olfactory sensory neurons and are transported via olfactory axons directly to the bulbs where they accumulate. While the olfactory system appears to be a significant site for the disposition of airborne PCBs, further studies are needed to confirm this observation and assess whether greater disposition in the brain is associated with inhalation exposure.

3.4.1.2 Oral Exposure

Oral exposure through consumption of contaminated food is presumed to be the major route of exposure to PCB mixtures for the general population (Duarte-Davidson and Jones 1994; Hansen 1999). Furthermore, oral exposure through ingestion of contaminated water or soil represents a possible additional source of exposure for populations in the vicinity of hazardous waste sites. Duarte-Davidson and Jones (1994) estimated that the average total PCB exposure for the contemporary UK population was 0.53 µg/person/day, with food consumption accounting for 97% of the total PCB exposure, air contributing 3.4%, and water only 0.04%. PCB contaminated fish, milk and dairy products, vegetables, and meat and animal fat were estimated to account for 32, 26, 18, and 16% of the respective exposure. The congener pattern for different food products varied, with vegetables accounting for a major part of the intake of lower chlorinated PCB congeners, while fatty foods, such as fish, dairy products, and meat, played a greater role with exposure to higher chlorinated congeners. For example, vegetables accounted for 78% of the total dietary exposure to PCB 28 and only 0.2% of the exposure to PCB 180. In contrast, freshwater fish account for 1.2 and 27% of the total dietary exposure to PCBs 28 and 180, respectively.

Direct evidence of absorption of PCBs in humans after oral exposure was provided in a study in which a volunteer ingested 329 µg of a ^{13}C-PCB mixture/kg body weight in a single dose dissolved in edible oil (Buhler et al. 1988). The PCB mixture, which was prepared by the investigators, contained 54% chlorine

(mainly penta-, hexa-, and heptachlorobiphenyls). Use of ^{13}C-PCBs allowed the investigators to distinguish between the administered PCBs and ^{12}C-PCBs already present in the body from diet or other exposure. Blood samples obtained during a period of 260 days after dosing revealed the presence of mostly penta- and hexachlorobiphenyls and smaller amounts of heptachlorobiphenyls. From data presented in the report, it appears that the maximum concentration in blood was reached within 2 days of dosing (the first time point examined). Concentrations of PCBs in whole blood declined rapidly, but strong fluctuations were apparent. This was attributed to changes in blood lipids, which depended on factors such as diet, physical activity, and time of day. Also, additional exposure is also possible since diet was unrestricted and ^{12}C-PCB was more than the administered ^{13}C-PCB. The investigators estimated that an intake of 26 µg/kg of PCB 153 would lead to a total concentration in whole blood of 0.5 ppb 1 month later.

A more recent study in a 19-week-old nursing infant provided quantitative data on absorption (McLachlan 1993). Absorption was estimated as the difference between ingestion and the amount of PCBs found in the feces over a period of 12 days. The mother was 32 years old and nursing for the first time. Several PCB congeners were determined in the milk: 2,2N,4,4N,5-pentaCB (PCB 99), 2,2N,4,4N,5,5N-hexaCB (PCB 153), 2,2N,3,4,4N,5N-hexaCB (PCB 138), and 2,2N,3,4,4N,5,5N-heptaCB (PCB 180). The percentage of dose absorbed was estimated at 96–98% after corrections were made for background levels in the diapers. Similar results were observed in a group of four nursing infants where absorption of 56 PCB congeners in milk was measured over two 48-hour periods, 1–3 months apart (Dahl et al. 1995). Absorption of the coplanar congeners 126 and 169 was estimated to be from 93 to 100%, while the absorption of PCB 77 ranged from 71 to 98%. Absorption of non-coplanar tetra- and higher chlorinated congeners was from 90–100%, while absorption of trichlorinated congeners was 60–98%. However, the authors noted that it was difficult to draw conclusions on the absorption of trichlorinated PCBs due to their low levels and the analytical methodology. The primipara mother's milk had the highest levels of generally all PCBs, and the level in milk generally decreased with months of nursing. Absorption of PCBs was unaffected by the age of the infant (1–6 months).

Indirect evidence of absorption results from studies regarding ingestion of contaminated food by the general population. Elevated levels of PCBs were found in the serum and breast milk of women who ate PCB-contaminated fish from Lake Michigan (Schwartz et al. 1983). Blood levels of PCBs were positively correlated with the amount of fish consumed. Two volunteers who consumed a total of 0.181 and 0.128 mg, respectively, of PCBs (mixture of 42, 48, 54, and 60% chlorine content) in contaminated fish showed a maximum 52–60% increase (2.5–4.0 and 2.3–3.5 ppb) in blood levels of total

PCBs . 5 hours after ingesting the meal (Kuwabara et al. 1979). The concentration of PCBs in blood returned to premeal levels 17 hours later. Levels of PCBs in adipose tissues were not determined.

Schlummer et al. (1998), used a mass balance approach to assess the gastrointestinal absorption of PCBs from food in seven individuals, 24–81 years of age, with different contaminant body burdens (Table 3-7). The net absorption is calculated as the difference between contaminant input with food and contaminant output with feces, normalized to the contaminant intake. Positive values in Table 3-7 indicate net absorption and negative values indicate net excretion, with absorption or excretion expressed as a percentage of daily intake. Nearly complete net absorption was observed for PCBs 28, 52, 77, 101, and 126. Incomplete net absorption and/or net excretion (in older subjects) was observed for PCBs 105, 138, 153, 180, and 202. In the case of the coplanar PCBs, 77 and 126, the congener specific levels in blood lipids of the subjects (given in parentheses) were very low and absorption was nearly complete (90% or greater for PCB 126 in all but two subjects). In the 76- and 81-year-old subjects, PCB 126 was found at higher levels in the blood lipids and the estimated net absorption of this congener was 77 and 53%, respectively. Net excretion or limited absorption was observed for PCBs 138, 153, and 180 in the three older subjects which had the highest levels of these congeners in their blood lipids. Thus, the gastrointestinal absorption or excretion of PCBs from food in humans is not only congener dependent, but is directly related to the concentration of a given PCB in blood, or the congener specific body burden. In most cases of background dietary exposures to PCBs, the PCB blood level or body burden increases with the age of the individual.

Table 3-7 illustrates that compounds showing nearly complete net absorption had low levels in the serum lipids, and for other congeners, there was a trend for decreasing net absorption/increasing net excretion with increasing congener concentration in serum lipids. Together, the data support the passive diffusion model for gastrointestinal absorption, where the concentration of the contaminant in the blood is the major factor determining absorption. In addition, the results suggest that the ingestion of highly contaminated food should result in nearly complete absorption due to the high diffusion gradient associated with high levels of PCBs in the gut contents. This may also be the case for the ingestion of PCB contaminated soil and water near hazardous waste sites. It should not be assumed that PCB absorption involves intestinal transfer to the hepatic portal system. As with fats and other fat-soluble chemicals, PCBs are most likely absorbed from the gut via lymphatic circulation and consequently avoid first-pass metabolism in the liver (Hansen 1999).

Table 3-7. Net Gastrointestinal Absorption or Excretion of PCBs in Humans and Dependence on Congener-Specific Blood Lipid Levels[a,b]

Gender (age in years)[c]	F(24)	M (25)	M (28)	M (36)	M (53)	F (76)	F (81)
PCB 28[d]	65	84	85	87	64	89	84
	(5.0)	(2.8)	(1.9)	(1.9)	(4.5)	(7.8)	(6.6)
PCB 52	73	82	90	89	69	93	92
	(1.52)	(1.09)	(0.89)	(<0.46)	(0.75)	(1.84)	(<0.69)
PCB 77	>91[e]	83	>90	>90	>82	>93	92
	(n.d.)[f]	(n.d.)	(n.d.)	(n.d.)	(0.007)	(0.064)	(<0.02)
PCB 101	56	81	91	90	48	92	82
	(1.50)	(1.11)	(1.22)	(0.78)	(1.38)	(2.3)	(1.43)
PCB 105	78	87	99	90	63	3	61
	(2.2)	(1.21)	(1.65)	(0.86)	(2.9)	(3.2)	(5.7)
PCB 126	90	93	95	96	92	77	53
	(0.066)	(0.068)	(0.042)	(0.029)	(0.082)	(0.174)	(0.39)
PCB 138	80	72	70	87	6	33	6
	(55)	(63)	(131)	(50)	(174)	(133)	(270)
PCB 153	74	60	65	85	-54	31	-42
	(89)	(135)	(230)	(84)	(410)	(250)	(600)
PCB 180	83	70	59	82	-41	34	-75
	(51)	(115)	(171)	(67)	(330)	(175)	(380)
PCB 202	51	36	2	19	-324	-63	-123
	(0.69)	(0.97)	(2.3)	(1.59)	(3.4)	(1.53)	(3.3)

[a]From Schlummer et al. 1998

[b]Net absorption is calculated as the difference between contaminant input with food and contaminant output with feces, normalized to the contaminant intake and is expressed as a percentage of the daily intake. Positive values indicate net absorption and negative values indicate net excretion with absorption or excretion expressed as a percentage of daily intake. Congener-specific levels in blood lipids are given in parentheses.

[c]M or F = sex of volunteer with age in parentheses

[d]PCB blood levels: nanograms per gram of blood lipids, shown in parentheses.

[e]< = values did not exceed three times blank values.

[f]n.d. = not determined due to detection problems.

In a related, but much older study, Price et al. (1972) estimated the gastrointestinal absorption efficiency by monitoring the daily PCB intake through food, and the excretion through feces and urine in 7–9 year old girls. They found that 88% of the ingested PCBs were not excreted, and were therefore assumed to be retained in the body. This estimate of PCB absorption in young girls is supported by the more comprehensive, congener specific mass balance study of Schlummer et al. (1998) discussed above.

In experimental animals, the absorption of PCBs by the gastrointestinal tract is well documented; however, few studies provided quantitative estimates. In rats, individual congeners (mono- to hexa-chlorobiphenyls) were readily absorbed when administered by gavage (vehicle not reported) in doses between 5 and 100 mg/kg (Albro and Fishbein 1972). Retention was >90% of the administered dose over a 4-day period, and was apparently dose independent. No relationship between substitution pattern and degree of absorption could be established due to the low levels of excretion, although a later study reported that absorption efficiency decreased in rats as the number of chlorine atoms increased such that dichlorobiphenyls were absorbed with a 95% efficiency, whereas octachlorobiphenyls had an absorption efficiency of only 75% (Tanabe et al. 1981). Results similar to those obtained in rats were reported in monkeys administered a single dose of 1.5 or 3.0 g Aroclor 1248/kg by gavage (Allen et al. 1974b) and in ferrets given 0.05 mg ^{14}C-labeled Aroclor 1254 in the food on days 14 and 35 of gestation (Bleavins et al. 1984). Retention was estimated to be >90% and 85.4% of the administered dose in the monkeys and ferrets, respectively. Over 90% of a single dose of 10 mg PCB 105 was absorbed by minks (Klasson-Wehler et al. 1993). In mice, absorption of a gavage dose of 8 mg/kg of PCB 52 or 100 mg/kg of PCB 77 was rapid, with serum concentrations increasing 4–7-fold in 30–60 minutes; peak serum concentrations were achieved .2 hours after dosing (Clevenger et al. 1989).

Following a single oral dose of 15 mg/kg Aroclor 1254 to 55-kg growing swine, total PCBs reached maximum blood concentrations (9.8% of dose) in 5 hours (Borchard et al. 1975). The 11 packed-column peaks containing multiple congeners were also calculated individually, reaching maximum levels of 8–20% of the dose in 4–8 hours. In mature ewes receiving 30 mg/kg of the same Aroclor in a single oral dose, absorption was slower and maximum blood levels of 2.2% of the total PCB dose were achieved in 12 hours (Borchard et al. 1975). The absorption half-life for total PCBs was 1.13 hours in swine and 3.83 hours in the ruminants. Maximum blood levels of individual peaks were 3–6 times higher in swine than in sheep; however, sheep readily eliminated the peak containing mainly 2,3,3N,4N,6-pentaCB so that maximum blood levels were 11-fold lower in sheep than in swine for this peak (Borchard et al. 1975).

In weanling swine administered 15 mg/kg Aroclor 1254 in 7 daily oral doses of 2.14 mg/kg, whole blood concentrations peaked near 0.3 ppm between 7 and 28 days (Hansen and Welborn 1977). The concentration in dissectable backfat was about 15 ppm on day 7 and 10 ppm on day 14; however, the body composition was determined by noninvasive means so that the actual amount in body fat was estimated at 70 mg on day 7 and 60 mg on day 14. The slower decline in amount than in concentration was due to disproportionate expansion of the fat compartment in these rapidly growing animals (Hansen and Welborn 1977). The initial decline of total PCBs in fat was due to rapid clearance of lower chlorinated peaks and slower distribution of higher chlorinated peaks from centralized fat to the more peripheral backfat. Simultaneous studies with individual PCBs demonstrated greater amounts of PCB 52 on days 7 and 14 than of PCB 153 if fat PCB content was calculated on the basis of backfat PCB concentration. The total amount of tetrachlorobiphenyl declined linearly through 118 days, but the amount of hexachlorobiphenyl estimated in total fat based on backfat concentration increased gradually so that the 118-day amount was only slightly lower than the 7-day amount (Hansen and Welborn 1977). Further evidence, although indirect, regarding absorption of PCBs after oral exposure in several species can be found in studies on tissue distribution of these chemicals, which are presented in Section 3.4.2.2.

3.4.1.3 Dermal Exposure

The dermal route of exposure has been recognized as a significant contributor to the accumulation of PCBs in adipose tissue of workers in the capacitor manufacturing industry (Maroni et al. 1981a, 1981b; Smith et al. 1982; Wolff 1985). For example, it was reported that the concentration of PCBs in wipe samples from the face and hands of two employees at a private utility company varied from 0.05 to 5 $\mu g/cm^2$ (Smith et al. 1982). Assuming 100% dermal absorption into the main body reservoir (10 kg adipose), Wolff (1985) estimated that the figure of 5 $\mu g/cm^2$ would represent 0.2–20% of a 50-µg/g adipose level, which is commonly seen among capacitor workers.

In addition to contributing to exposure in occupational settings, the dermal route, through skin contact with contaminated water or soil, represents a potential route of exposure to PCB mixtures for populations in the vicinity of hazardous waste sites.

Experimental data on the percutaneous absorption of PCBs in humans is limited to *in vitro* studies thatused human cadaver skin (Wester et al. 1990, 1993). These studies utilized ^{14}C-labeled Aroclor 1242 and 1254 (mixtures containing 42 or 54% chlorine by mass) in soil, mineral oil, and water. Over a 24-hour period, 2.6, 10, and 43% of the dose was retained in human skin when the Aroclor 1242 was

formulated in soil, mineral oil, or water, respectively. Similar results were observed with Aroclor 1254, with 1.6, 6.4, and 44.3% of the dose retained in human skin, following PCB exposure in a soil, mineral oil, or water vehicle, respectively. The *in vitro* data indicate that PCBs readily enter human skin and are available for systemic absorption, and that the dosing vehicle has a major role in regulating the relative retention of PCBs in human skin.

In a related study, Wester et al. (1990, 1993) assessed the *in vivo* percutaneous absorption of PCBs in adult female Rhesus monkeys. ^{14}C-Labeled Aroclor 1242 and 1254 were separately administered iv and topically to Rhesus monkeys and urinary and fecal excretion of radioactivity was measured for the next 30 days. Following iv administration, the 30-day cumulative excretion was 55% of the administered dose (39% urine, 16% feces) for Aroclor 1242 and 27% (7% urine, 20% feces) for Aroclor 1254. The percentage of the dose absorbed following topical administration to abdominal skin (after light clipping of hair) was estimated from the ratio of the total urinary and fecal excretion following topical and iv administration. Topical administration of Aroclor 1242 in soil, mineral oil, trichlorobenzene, or acetone resulted in 14, 20, 18, and 21% absorption of the administered dose, respectively. In contrast to the above *in vitro* results with human skin, the vehicle had little effect on the systemic absorption of the PCBs applied to the skin of monkeys. This may be due to the uncertain viability of the human skin used in the *in vitro* studies and the fact that the *in vitro* study primarily assessed retention of PCBs in human skin and could not estimate systemic absorption.

The effectiveness of methods for decontaminating or removing Aroclor 1242 from Rhesus monkey skin was also investigated by Wester et al. (1990). Use of soap and water was similar in effectiveness to washing with trichlorobenzene, mineral oil, or ethanol. At 15 minutes following dermal exposure, 93% of the applied dose was removed from skin by washing with soap and water. At 24 hours following dermal exposure, only 26% of the dose was removed from skin by washing with soap and water, suggesting that with time, most of the PCB dose undergoes systemic absorption and/or retention in the skin. Thus, washing with soap and water is an effective method for removing PCBs from skin, particularly when washing immediately following a known dermal exposure.

Dermal absorption of PCBs has been measured in monkeys and guinea pigs by comparing excretion following topical administration to excretion following parenteral administration. Single doses of ^{14}C-labeled PCBs (42% chlorine content) in benzene/hexane were applied to the abdominal skin of four Rhesus monkeys and to the lightly clipped skin behind the ear of three guinea pigs (Wester et al. 1983). To an additional group of three guinea pigs, PCB with 54% chlorine content was applied. The

application amount ranged between 4.1 and 19.3 μg/cm^2 skin. The application sites were washed with water and acetone after 24 hours, and radioactivity was monitored in the urine for several weeks postdosing. Absorption efficiency ranged from .15 to 34% of the applied radioactivity in the monkeys and averaged .33% (42% chlorine) and 56% (54% chlorine) of the applied radioactivity in the guinea pigs. Washing the skin immediately after PCB application removed 59% of the applied dose. However, only 1% of the applied label from the PCB containing 42% chlorine and 20% of the label from the PCB containing 54% chlorine could be recovered from the application site when the skin was washed 24 hours after dosing. Dermal absorption of PCBs (48% chlorine) has also been demonstrated in rats (Nishizumi 1976); however, quantitative data were not provided.

Dermal penetration rate constants have been measured in male Fischer 344 rats after single 0.4 mg/kg dermal doses of ^{14}C mono-, di-, tetra-, and hexachlorobiphenyls applied for 48 hours to shaved back skin (Garner and Matthews 1998). Congeners used were 4-chlorobiphenyl (PCB 3), 4,4'-dichlorobiphenyl (PCB 15), 2,2',4,4'-tetraCB (PCB 47), and 2,2',4,4',6,6'-hexaCB (PCB 155). Penetration rate and degree of penetration (defined as penetration through the stratum corneum into the viable epidermis) were inversely related to degree of chlorination. Rate constants for penetration were 0.14, 0.074, 0.028, and 0.0058 hour^{-1} for the mono-, di-, tetra-, and hexachlorinated forms, respectively. Rate constants correlated strongly with the logarithm of the octanol-water partition coefficient. Jackson et al. (1993) also reported a strong inverse correlation between octanol-water partition coefficient estimates and the dermal absorption of several halogenated aromatic hydrocarbons, including 3,3',4,4'-tetraCB (PCB 77). Cumulative penetration at 48 hours was near 100% for the mono-, 95% for the di-, 75% for the tetra-, and 30% for the hexachlorinated forms. Absorption of the tetra- and hexachlorinated forms continued after washing the site with acetone at 48 hours, indicating that the viable epidermis served as a reservoir for these higher chlorinated forms. The rate of systemic absorption of radioactivity was kinetically complex and not a first-order process like penetration into the skin. This may be due to metabolism and partitioning within the skin.

The dermal absorption of ^{14}C-3,3',4,4'-tetraCB (PCB 77) and 2,2',4,4',5,5'-tetraCB (PCB 153) in female F344 rats was assessed under conditions where the PCB was applied as either a solid, aqueous paste, aqueous suspension, or dissolved in ethanol (Hughes et al. 1992). The chemicals were applied to the clipped mid-dorsal region of the rat. The treatment area was then occluded, and urine and feces were collected and analyzed for radioactivity. At 24-hours postexposure, the treatment area was washed with soap and water, recovering 61–91% of PCB 77 and 81–92% of PCB 153. The percentage of the dose absorbed ranged from 6 to 8% for PCB 77 and from 5 to 8% for PCB 153, while the treated skin retained

from 3 to 31% of the PCB 77 and from 3 to 12% of the PCB 153. Although significantly greater absorption of PCB 153 was observed when administered as a solid, compared to using the ethanol vehicle, the remainder of the results indicate that the dermal absorption of PCBs 77 and 153 was similar even when the PCBs were applied in four different physical forms.

3.4.2 Distribution

Quantitative data regarding distribution of PCBs to specific organs or tissues of humans after route-specific exposure were not located. However, relevant information regarding the distribution of PCBs in humans following environmental, dietary, and/or occupational exposures are presented below.

Data regarding distribution of PCBs in human tissues and body fluids are derived mainly from the study of populations exposed in occupational settings or from those who have consumed contaminated food. It is generally agreed that the inhalation and dermal routes are the main exposure routes to PCBs in occupational settings (Wolff 1985). For the general population, the oral route is the major route for PCB exposure (Humphrey 1983).

In humans, PCBs are found in highest concentration in adipose tissue. Due to its high fat content, human milk can accumulate a large amount of PCBs, which can then transferred to children through breast-feeding (Ando et al. 1985; Jacobson et al. 1984b; McLachlan 1993). The PCB congener composition in milk differs from that of the commercially produced PCB formulations (Safe et al. 1985b) (see Section 3.8.1). Offspring can be also exposed to PCBs through transplacental transfer. In a sample of 313 women and their newborn infants, placental passage of PCBs was evidenced by a significant maternal to cord serum correlation (Jacobson et al. 1984b). Additional information on the prenatal and postnatal exposure to PCBs are included in Section 3.7.

Average measured concentrations of 0.5–4 ppm total PCBs have been reported for human milk fat, <5 ppb for blood plasma, and 0.5–10 ppm for adipose tissue (Jensen 1987). However, as pointed out by Jensen (1987), due to the heterogeneity of the study populations, the differences in sampling, and the analytical techniques used, the PCB levels reported by different studies may not be comparable. The levels of several di-*ortho*-substituted congeners in human milk (on a lipid basis) ranged from not detected to >300 ppb (Schecter et al. 1994). For comparable exposure levels, PCB levels in plasma and adipose tissue are generally higher in males than in females (Jensen 1987; Wolff et al. 1982a). PCBs have also been detected in ovarian follicular fluid in concentrations ranging from 0.5 to 24.2 μg/kg, in sperm fluid

ranging from 1.8 to 58.6 µg/kg (Schlebusch et al. 1989), and in bone marrow ranging from 2 to 4 mg/kg based on dry lipid weight (Scheele et al. 1994). Analytical techniques for determining PCBs in biological materials are presented in Chapter 7.

The major components in plasma and adipose tissue (subcutaneous) of occupationally exposed individuals were PCB congeners with chlorine atoms in the 4 and 4N positions (Wolff et al. 1982b, 1992), whereas PCBs with unsubstituted 3,4 positions on at least one ring were observed at lower concentrations. On a wet weight basis, the adipose/plasma partition ratio for Aroclor 1248 residues was 185/1; the partition ratio for Aroclor 1254 residues was 190/1. In a study of 173 workers of the same population, adipose/plasma partition ratios of 210/1, 190/1, and 200/1 were determined for residues of Aroclors 1242, 1254, and 1260, respectively (Brown and Lawton 1984). The partition ratios were significantly dependent on the levels of lipids in the serum, but not on albumin content. A 1989 study determined the concentration of individual PCB congeners in both serum and adipose tissue of 35 currently exposed workers, 17 former workers, and 56 control individuals who were never occupationally exposed to PCB mixtures (Fait et al. 1989). Among all exposure categories, the homolog groups present in the highest concentrations were the hexa- and heptachlorobiphenyls, both in sera and adipose tissue, as expected from the highly chlorinated Aroclor 1260. Mono-, di-, tri-, and nonachlorobiphenyls were found at very low levels in adipose tissue, as expected, and no differences were observed among the exposure categories. Currently exposed workers had significantly higher levels of penta-, hepta-, and octachlorobiphenyls than those in both formerly exposed and control groups. The concentration of tetrachlorobiphenyls was significantly higher among currently exposed individuals than among the other groups. No significant differences were seen in serum for tetra- and nonachlorobiphenyls. Mono-, tri-, penta-, hexa-, hepta-, and octachlorobiphenyls were found at significantly higher concentrations ($p<0.0167$) in currently exposed workers than in comparison groups. Hepta- and octa-concentrations were significantly higher ($p<0.0167$) in serum of formerly exposed subjects than in the serum of the other comparison groups. The relative distribution of individual congeners was similar in the three groups, but the amounts varied. The seven major peaks in serum and adipose tissue were mainly penta-, hexa-, hepta-, and octachlorobiphenyls. More standards became available after the study was published and some congener (but not homolog) identifications were corrected (see Hansen 1999).

Mean PCB concentrations of 5.1, 3.2, and 0.76 mg/kg of extractable fat were determined in samples of abdominal fat, liver, and brain, respectively, obtained from autopsies performed in Denmark (Kraul and Karlog 1976). None of the 82 subjects were occupationally exposed to PCBs. The PCB values in liver could be best correlated with those in adipose tissue. A more recent study described 14 different PCB

isomers in tissues and organs obtained at autopsy of three individuals in North America (Schecter et al. 1989). The large differences observed in isomer distribution within a given tissue and between the various tissues of the donors do not allow generalizations to be made on general population isomer distribution. When expressed as the sum of the 14 isomers on a lipid basis, PCB concentrations ranged from 101–573 ng/g (ppb) of fat in adipose, 89–517 ppb in liver, 30–409 ppb in kidney, 83–354 ppb in muscle, 80–42 ppb in adrenal (two patients), 131–193 (ppb) in lung (two patients), 102–341 ppb in spleen (two patients), 103 ppb in bone marrow (one patient), and 102 ppb in testis (one patient).

The existing information regarding distribution of PCBs in humans is limited. Nevertheless, based on experimental data obtained in animals (see Section 3.4.2.2) and the known physicochemical properties of PCBs, it is reasonable to assume that the lipid soluble PCBs, once cleared from the bloodstream, will accumulate in highest concentration in fatty tissues. Initially, however, PCBs could accumulate in the liver due to its high blood perfusion rate. The availability of PCBs for retention in fatty tissues is intimately linked to metabolism (see Section 3.4.3); therefore, it would be expected that the higher chlorinated PCBs would persist for longer periods of time solubilized in fatty tissues.

As with other organisms, PCB residue levels in humans reflects multiple exposure pathways, and congener-specific elimination. PCB profiles in human serum immediately following exposures reflect the profiles in the exposure sources, however, selective metabolism, excretion, and deposition begin to alter the congener profile within 4–24 hours (Hansen 1999). Thus, in most cases, the PCB profile in adults represents a steady state body burden which does not match the profile of commercial PCB formulations (Aroclors, etc.). For example, neither the PCB profile of human adipose nor of a composite human milk sample resemble the pattern of any commercial PCB formulation (Jensen and Sundstrom 1974; Safe et al. 1985). Abbreviated PCB residue analysis indicates that humans, aquatic mammals, birds, fish and other biota retain a similar profiles of the 4–6 more dominant PCBs, but more complete analyses demonstrate unique patterns among the remaining congeners.

Consumption of PCB contaminated freshwater fish is an example of an excess dietary exposure, which can elevate and/or modify serum PCB profiles. Non-coplanar, *ortho*-substituted, PCB congeners were measured in the serum (collected in 1993–1995) from a group of Lake Michigan residents over 50 years of age who eat fish (fisheaters) and an age- and region-matched comparison group that did not eat fish (nonfisheater) (Humphrey et al. 2000). The same general PCB profile was observed in both groups, with the fisheaters having a mean total PCB level in serum of 14.26 ng/g (n=101), and the nonfisheaters having a mean serum level of 4.56 ng/g (n=78). Four congeners, 138/163 (2,2',3,4,4',5/2,3,3',4',5,6),

180 (2,2',3,4,4',5,5') and 153 (2,2',4,4',5,5'), accounted for 55–64% of the total PCB load. Although 90 congener peaks were quantitated, the analysis found that 22 peaks representing 25 PCB congeners comprised 99% of the total PCBs in both the fisheater and nonfisheater groups.

Anderson et al. (1998) assessed serum levels of coplanar and noncoplanar PCBs in 32 anglers that consumed an average of 49 Great Lakes sportfish meals per year for a mean of 33 years. Highly persistent coplanar PCB 126 (3,3',4,4',5) and PCB 169 (3,3'4,4',5,5') were elevated 8- and 5-fold above the respective levels of these congeners in the control unexposed comparison group from Jacksonville, Arkansas. The less persistent coplanar PCB 77 (3,3',4,4') was found at similar levels in the Great Lakes fish consumer and control groups. The highly persistent, most abundant noncoplanar PCB 138 and PCB 153 were 2- and 3-fold higher in the fish consumers, relative to the respective control group. Thus, the long-term consumption of Great Lakes fish results in an increase in the serum levels of persistent PCBs, and particularly the coplanar PCBs 126 and 169. In subsequent studies from these investigators, Falk et al. (1999) found that consumption of lake trout and salmon significantly predicted serum log (total coplanar PCB) levels, and Hanrahan et al. (1999) found that PCB levels were significantly correlated to age, body mass index, and sportfish and Great Lakes sportfish consumption histories. Regression analysis identified years of consuming sport caught fish as the most robust predictor of serum PCB levels.

The above PCB residue data in humans and other animals suggests that tissue or body burdens of PCBs should be based on individual congeners or groups of congeners and not based on profiles of commercial PCB formulations. The simplest approach involves using one congener as a marker of total PCBs in a biological specimen. Levels of PCB 153 (2,2',4,4',5,5'), a very stable and often the most abundant congener, have been shown to correlate with the total amount of PCBs in human breast milk (Johansen et al. 1994) and human plasma, with a correlation coefficient of r=0.99 (Grimvall et al. 1997). PCB 153 was highly correlated (r=0.95) with total PCBs in 460 serum samples from Swedish men and women (Atuma and Aune 1999). PCB 153 was also highly correlated with total PCBs in serum (r=0.99) and follicular fluid (r=0.99) (Pauwels et al. 1999). In addition, PCB 153 levels correlated (r=0.91) with the total PCB-TEQs in human plasma (Grimvall et al. 1997). However, if a more complete profile of congeners is considered, the correlations are lower (Bachour et al. 1998; Hansen 1998, 1999). Total PCBs or PCB 153 as a marker of the total could be a misleading indicator of the differential exposure to other individual or groups of congeners of toxicological significance.

Another important issue related to exposure assessment is whether analysis of PCBs in serum and adipose tissue provide comparable information on body burden. Stellman et al. (1998) measured 14 PCB

congeners in adipose tissue and serum from 293 women with nonoccupational exposure. The relative patterns of the 14 PCB congeners were similar to those reported in other human studies. Significant positive serum to adipose correlation coefficients were obtained for PCBs 74, 99, 118, 138, 146, 153, 156, 167, 170, 180, 183, and 187, while PCBs 172 and 178 did not reach statistical significance. Thus, this study supports the conclusion that either serum or adipose tissue PCB levels may serve as useful biomarkers of body burden and/or exposure.

Recently, Dewailly et al. (1999) measured the concentration of 14 PCB congeners in subcutaneous fat, omental fat, brain, and liver from autopsy tissue samples collected from Greenlanders between 1992 and 1994 (Table 3-8). The PCB body burden of the Inuit population of Greenland is presently among the highest resulting from environmental exposure. The levels of PCB 138, 153, and 180 were 19, 21, and 16-fold higher than the respective congeners measured by the same analytical method in Canadians from Quebec City. The sum of the three most abundant PCB congeners (138, 153, 180) represents 63–68% of the total PCBs in the Greenlander tissue samples. Since PCBs primarily distribute with the tissue lipids, the tissue PCB concentrations were expressed relative to the lipid content (ng/g lipid) of each tissue.

Mean lipid contents were 62% in subcutaneous fat, 59.6% in omental fat, 8.3% in brain, and 4.5% in liver. Table 3-8 summarizes the mean concentration (µg/kg lipid) for the 14 PCBs in the four tissues. PCB concentrations (lipid basis) were similar in omental fat and subcutaneous abdominal fat, while the hepatic concentrations were generally about 20% lower than fat. PCB levels in brain (lipid basis) were about 10–20% of the levels found in subcutaneous fat. The lower concentration in brain cannot be explained by the presence of the blood-brain barrier because PCBs are highly lipophilic and are therefore expected to freely diffuse across this barrier. The difference in accumulation may be due to the nature of more polar brain lipids, which are mainly phospholipids. PCBs may partition to a greater extent into the triglycerides found in adipose tissue.

In support of the above observations, Weistrand and Noren (1998) found that the concentration of PCB congeners (ng/g lipid basis) were similar in paired human intra-abdominal adipose tissue and liver autopsy samples from seven Swedish subjects. The ratio of the sum of PCBs in liver to that in adipose tissue ranged from 0.8 to 1.0 (median 0.8), which was very similar to that reported in more highly exposed human samples from Greenland (Dewailly et al. 1999).

The concentration of PCBs (ng/g lipid) was measured in brain, liver, muscle, and lung tissue from 25 deceased male and female individuals with environmental exposure to PCBs (Bachour et al. 1998).

Table 3-8. Mean PCB Concentrations (Microgram Per Kilogram Lipid Basis) in Autopsy Tissue Samples from Greenlanders

PCB Congeners (IUPAC no.)	Subcutaneous fat (n=26)			Omental fat (n=41)			Brain (n=17)			Liver (n=26)		
	Mean[a]	Range	Per-cent[b]	Mean	Range	Per-cent	Mean	Range	Per-cent	Mean	Range	Per-cent
28	10	(0.2–185)	100	8	(2.3–156)	96	2.4	(0.5–33)	41	4	(0.5–79)	57
52	10	(2–150)	100	13	(1.9–200)	100	1.8	(0.3–19)	29	8[c]	(0.5–92)	65
99	238	(32–857)	100	215	(33–620)	100	31	(15–74)	100	154	(21–486)	100
101	26	(7–100)	100	18	(4–90)	100	8	(2–24)	94	19	(3–92)	92
105	47	(10–152)	100	50	(7–140)	100	3	(0.5–29)	53	18	(0.9–124)	77
118	257	(41–811)	100	267	(46–764)	100	38	(8–127)	100	209	(32–478)	100
128	9	(0.8–70)	85	3	(0.1–27)	100	1.0	(0.5–2.4)	6	2.1	(0.6–15)	15
138	1,103	(273–3,870)	100	1,098	(190–2,450)	100	134	(34–296)	100	855	(161–2,120)	100
153	1,689	(531–5,580)	100	1,582	(280–3,800)	100	198	(53–397)	100	1,177	(242–3,770)	100
156	173	(57–625)	100	195	(27–497)	100	30	(5–88)	100	143	(51–270)	100
170	385	(112–1,550)	100	422	(61–1,100)	100	46	(7–154)	100	327	(105–886)	100
180	1,147	(239–4,420)	100	1,136	(170–3,000)	100	145	(27–378)	100	791	(234–2,310)	100
183	92	(14–413)	100	93	(19–318)	100	10	(0.5–29)	88	69	(11–241)	100
187	499	(113–2,200)	100	507	(99–1,330)	100	82	(14–175)	100	445	(110–1,030)	100

Source: Dewailly et al. 1999

[a]Geometric mean; in calculating mean values, results not detected were attributed a value equal to half of the detection limit.
[b]Percentage of analyzed samples in which the substance was detected.
[c]Mean lipid content of tissues were 62.0% in subcutaneous fat, 59.6% in omental fate, 8.3% in brain, and 4.5% in liver.

Liver and muscle lipids generally contained similar levels of each of the PCB congeners, while the brain contained consistently lower levels. The lung generally had the highest concentration of the lower chlorinated congeners, including PCBs 28, 31, 49, 52, and 101. The levels in the lung were greater than brain and less than muscle or liver for PCBs 138, 153, 156, 170, 180, and 183. The higher levels of the lower chlorinated congeners in the lung may be related to the greater volatility and greater direct pulmonary exposure to these congeners. It is also possible that PCB binding proteins in the lung contribute to the enhanced pulmonary disposition of these congeners (Brandt et al. 1981).

3.4.2.1 Inhalation Exposure

No studies were located regarding distribution in humans following controlled inhalation exposure to PCBs. Occupational data are presented in the preceding section.

Information regarding PCB distribution in animals after inhalation exposure is limited. Rats exposed to 30 g/m^3 of an aerosol of a PCB mixture (Pydraul A 200, 42% chlorine) had 52, 14, and 9 µg PCB/g tissue in the liver, adipose, and brain, respectively, after 30 minutes of exposure (Benthe et al. 1972). The concentration of total PCBs attained in the liver after 2 hours of exposure was 70 µg/g. PCB levels in the liver reached a maximum 2 hours after exposure and slowly declined to less than half of the maximum 12 hours after exposure. Analysis of retroperitoneal adipose tissue revealed only trace amounts of PCBs immediately after exposure; only a slight increase in concentration was detected after 12 hours. Maximum concentration in adipose tissue was attained 36 hours after exposure. In contrast to adipose tissue, PCBs were detected in the brain immediately after exposure, reached a maximum 24 hours after exposure, and slowly declined thereafter.

A recent study in ferrets by Apfelbach et al. (1998) reported for the first time that the olfactory system may be a potentially significant portal for the entry of airborne PCBs. Ferrets were exposed for 5 years to low levels of PCBs (total PCBs, 260 ng/m^3 air) in the ambient air of an animal care room that had PCB-containing sealants. Tetra-chlorinated PCBs dominated the congener profile of ambient air, with PCB 52 being found at the highest concentration. The PCB congener pattern in the olfactory bulbs resembled that found in the ambient air, with the less chlorinated, more volatile PCBs (52) being retained at the higher levels. In contrast, the congener profile in adipose tissue resembles that of most exposed or unexposed animals, with PCBs 153, 138, and 180 being the major congeners present. The olfactory bulbs of the exposed animals had the highest total PCB concentration (642 ng/g lipids), while the liver, adipose tissue, and brain had levels of 202, 303, and 170 ng/g lipids, respectively. The data suggest that inhaled PCBs

pass into the dentrites of olfactory sensory neurons and are transported via olfactory axons directly to the bulbs where they accumulate. While the olfactory system appears to be a significant site for the disposition of airborne PCBs, further studies are needed to confirm this observation and assess whether greater disposition in the brain is associated with inhalation exposure.

3.4.2.2 Oral Exposure

Retention of individual PCB components from commercial mixtures depends upon species and organ, degree of chlorination, and substitution pattern (Hansen 1979, 1987b). The metabolism of specific PCB congeners by different species is influenced by existing residues and can result in considerable variations in metabolite distribution (Hansen 1987b; Safe 1989a) (see Section 3.4.3, Metabolism). Representative examples in various animal species are described below.

In adult mice, repeated administration of 100 mg/kg 2,2N,5,5N-tetraCB or 8 mg/kg 3,3N,4,4N-tetraCB achieved apparent steady-state levels in 8–10 days (Clevenger et al. 1989). Steady-state concentrations in adipose tissue were much higher than in liver and thymus. Liver concentrations increased from steady-state levels for 2 hours after the final dose before beginning to decline. The distribution ratio of the 3,3N,4,4N-isomer for adipose tissue was 2-fold higher than that of the 2,2N,5,5N-isomer, and the ratios for thymus and liver were 3- and 10-fold higher, respectively. The decline in concentration of both isomers in the three tissues followed first-order kinetics. Tissue elimination half-lives for adipose tissue, thymus, and liver ranged from 1.07 to 2.9 days. Similar values were reported in mice for six other tetra-substituted PCBs (Mizutani et al. 1977). No apparent relationship between a substitution pattern and biological half-life could be observed. Preferential accumulation in mice of 3,3N,4,4N-tetraCB in liver and adipose tissue relative to kidney and lung was also observed (Klasson-Wehler et al. 1989a). Results from whole-body autoradiography experiments showed high concentration of radioactivity in adipose tissue of mice administered ^{14}C-2,3,3N,4,4N-pentaCB (Klasson-Wehler et al. 1993); lower, but significant amounts were detected in the liver. No selective tissue retention was observed over a 30-day period that followed dosing. Physical exercise has been shown to increase the PCB levels by a factor of 10 in mice livers due to mobilization of fat deposits from adipose tissue (Kurachi and Mio 1983a).

In monkeys, a single dose of 1.5 or 3.0 g/kg Aroclor 1248 resulted in a dose-dependent liver concentration of Aroclor 1248 (25 or 53 µg/g) 2 times higher than that found in the kidney (12 or 27 µg/g) and brain (17 or 28 µg/g) 4 days after dosing (Allen et al. 1974b). This difference was greatly increased 14 days after treatment due to both a reduction in kidney and brain concentration and an

increase in liver concentration. The blood levels of Aroclor 1254 increased rapidly in monkeys during 10 months of treatment (from approximately 1.2 µg/g at time zero to about 100 µg/g in the high-dose group) with doses between 20 and 80 µg/kg/day (Mes et al. 1989), but this increase leveled off during the remaining 27 months of treatment. A dose of 5 µg/kg/day induced only a slight increase in blood PCB levels during the total treatment period of 37 months. When the data were expressed as relative concentration, it appeared that absorption and bioaccumulation were dose-dependent. However, the concentration/dose levels were, to some extent, affected by background PCB levels of the control group, which would have a greater impact on the relative concentrations of the lowest dose groups rather than on the higher dose groups.

Information regarding distribution of PCB residues in monkeys after long-term feeding of Aroclor 1254 is available. In that study (Mes et al. 1995b), female monkeys were dosed with Aroclor 1254 (0.005, 0.02, 0.04, or 0.08 mg/kg/day) for over 6 years. Throughout the treatment period the monkeys were bred to untreated males and the resulting offspring were nursed by their mother. In dams, PCB residues in blood and tissues (on a lipid basis) increased with dose. On a wet tissue basis and at all dose levels, adipose tissue contained the most PCBs (10 times that found in the liver). At the highest dose, blood had 274 µg/g, followed by the liver (190 µg/g), adipose (171 µg/g), kidney (156 µg/g), and brain (22 µg/g) based on the lipid content in each tissue. Monkeys that were sacrificed before termination of treatment because of poor health had higher PCB levels in their tissues than monkeys killed at termination.

Following a 7-day total dose of 15 mg/kg Aroclor 1254 to growing pigs, lower chlorinated congeners reached peripheral fat (dissectable backfat) more rapidly than did higher chlorinated congeners; however, redistribution (from more central fat) between 35 and 80 days resulted in total estimated amounts of higher chlorinated congeners (Hansen and Welborn 1977). Total PCBs in noninvasively determined total fat were estimated by multiplying body weight times percent body fat times the concentration of PCBs in backfat. Long-term declines in fat concentrations for higher chlorinated PCBs were due mainly to dilution by growth and expansion of the fat compartment.

Administration of a total dose of . 7 mg/kg of PCBs (tetra- and hexachlorobiphenyls) to rats over #50 weeks resulted in the highest PCB concentration being detected in adipose tissue regardless of the treatment duration (Hashimoto et al. 1976). Intermediate concentrations were detected in the skin, adrenal gland, aorta, and sciatic nerve; all other major organs and tissues had lower PCB concentrations. In each tissue, PCBs were preferentially distributed to the lipid fraction. Similar results were observed in rats following administration of a single dose of hexachlorobiphenyl followed by a long-term observation

period (Mühlebach and Bickel 1981). The highest concentration of chlorinated hydrocarbon residues 24 hours following administration by gavage of a single dose of 1,600 mg/kg Aroclor 1254 or 3,200 mg/kg Aroclor 1260 to female Sherman rats was found in fat tissue, followed by kidney, liver, and brain; plasma and muscle contained the least (Curley et al. 1971). Despite the difference in administered doses, the concentration of residues in tissues, derived from both Aroclors, were comparable. When male and female rats were given an oral dose of 73 mg/kg/day Aroclor 1254 for 98 days, fat tissue again had the highest concentration, followed by muscle and liver (Curley et al. 1971). No significant differences were observed between adult male and female rats.

A number of animal studies have demonstrated that PCB mixtures and specific congeners and isomers can cross the placental barrier and enter the fetus. High levels of lipid soluble PCBs accumulate in the fat portion of the milk, also resulting in high exposure of suckling animals. Significantly increased PCB residues were detected in blastocytes (day 6 postcoitum) from female rabbits administered Aroclor 1260 before insemination (Seiler et al. 1994), but no residues could be detected in cleavage stage embryos (day 1 postcoitum). In pregnant mice fed PCBs through the first 18 days of gestation, the highest levels of serum PCBs were found in 1–2-week-old offspring compared with 18-day fetuses or with older offspring (Masuda et al. 1979). In studies that exposed monkeys prior to and during gestation, signs of PCB-induced intoxication were observed in suckling offspring, but not in neonates (Allen and Barsotti 1976; Iatropoulus et al. 1978). PCB blood levels in the offspring continuously increased during lactation, but decreased just before or immediately upon weaning (Mes et al. 1994, 1995c). In rats administered PCBs before gestation, 0.003% of the PCBs accumulated in the dams was transferred to the fetus through the placenta; however, the amount transferred to sucklings increased to 5% of the maternal PCB (Takagi et al. 1986). Similar results have been reported in ferrets administered single doses of PCBs early or late during gestation (Bleavins et al. 1984). Results such as these have led to the conclusion that suckling may account for higher exposure of young offspring than does placental transfer; the fetus, however, may be more sensitive.

Experiments in monkeys have suggested that fetal tissue may be unable to metabolize and excrete certain PCB congeners that are more readily metabolized and eliminated by adults and older infants (Barsotti and Van Miller 1984; Mes et al. 1995b, 1995c).

3.4.2.3 Dermal Exposure

No studies were located regarding distribution in humans following dermal exposure to PCBs. However, there is no evidence suggesting that distribution is route-dependent. Because of the lipophilic nature of these compounds, it would be reasonable to assume that once they are absorbed, PCBs will distribute to various tissues in proportion to their lipid contents. However, data from humans at autopsy suggest that the disposition of PCBs is congener and tissue specific and not based exclusively on the lipid content of tissues (Bachour et al. 1998; Dewailly et al. 1999; Schecter et al. 1994). Data regarding occupational exposure are discussed in Section 3.4.2.

Total tissue radioactivity has been measured in male Fischer 344 rats after single 0.4 mg/kg dermal doses of ^{14}C mono-, di-, tetra-, and hexachlorobiphenyls applied for 48 hours to shaved back skin (Garner and Matthews 1998). Congeners used were 4-CB, 4,4'-diCB, 2,2',4,4'-tetraCB, and 2,2',4,4',6,6'-hexaCB. Peak total radioactivity in the tissues (excised dose site, samples of blood, adipose tissue, muscle, skin [ears], and the entire liver and kidney) occurred at progressively later times depending on the degree of chlorination. For example, the monochlorinated form reached maximal concentrations (37% of the absorbed dose) in blood and other tissues at 4 hours postadministration and was almost absent (0.2%) at 2 weeks. In contrast, peak tissue concentrations of the tetrachlorinated form (80% of the absorbed dose) occurred at 72 hours and approximately 45% remained in the tissues after 2 weeks. Absorption of the tetra- and hexachlorinated forms continued after washing the site with acetone at 48 hours, indicating that the viable epidermis retained these forms and served as a reservoir. This may be due to partitioning into lipophilic sites in the skin or adsorption to epithelial proteins.

3.4.2.4 Other Routes of Exposure

In general, the results reported by studies in which PCBs were administered to experimental animals by parenteral routes are consistent with those derived from oral administration.

In adult rats with a constant adipose tissue mass, 2,2N,4,4N,5,5N-hexaCB (PCB 153) administered intravenously distributed preferentially to adipose tissue (about 5 µg/g), followed by the skin, lung, and liver (all about 0.3 µg/g) (Wyss et al. 1986). Four days after dosing, only adipose tissue, skin, and muscle contained significant amounts of the PCBs (. 75% of the dose). Between 2 and 4 weeks later, PCB levels in adipose tissue and skin reached a maximum, corresponding to 68 and 15% of the administered dose, respectively, which indicates a slow redistribution process of the chemical. In rats given 3,3N,5,5N-tetra-

CB intravenously, 20–40% of the administered dose was found in adipose tissue over 42 days; the blood, liver, muscle, and skin had <20% (Tuey and Matthews 1977).

In pregnant mice injected intravenously with ^{14}C-labeled 3,3′,4,4′-tetraCB (PCB 77), most of the radioactivity was localized in the fetus and consisted mainly of a hydroxylated metabolite (Klasson-Wehler et al. 1989b). However, after a dose of 3,3′,4,4′,5-pentaCB (PCB 126), no radioactivity was found in the fetus, except for traces in the liver. This differential distribution is probably due to differences in maternal metabolism for the tetra- and pentachlorobiphenyls (see Section 3.4.3, Metabolism).

3.4.3 Metabolism

The metabolism of PCBs has been extensively reviewed (Hansen 1999; Hu and Bunce 1999; Safe 1989a, 1993). Differential accumulation and retention of PCBs is related to exposure and the relative biological stability (rate of biotransformation) of each congener. Limited excretion of parent PCBs does occur (see Table 3-7), but biotransformation is necessary for the majority of PCB excretion.

The initial step in the biotransformation of PCBs involves CYP enzyme (cytochrome P-450) (CYP1A1, 1A2, and CYP2B1/2B2) mediated oxidation of arene oxides, which readily undergo further metabolism (Matthews 1982; Preston et al. 1984). CYPs of the 3A family are also very likely to participate and, perhaps, are more important than CYPs of the 2B family (see Hansen 1998, 1999). Coplanar (non*ortho*) PCBs are dioxin-like inducers of CYP1A and PCB 77 is preferentially oxidized by these isozymes. Many *ortho*-substituted, nonplanar, PCBs are inducers of CYP 2B isozymes and are also metabolized by these enzymes (Brown 1994). Mono-*ortho* PCBs are often mixed inducers of CYP1A and CYP2B isozymes (Safe et al. 1985a). Some congeners induce P-450s from the 3A and 4A families, but the structure-activity relationships are incomplete (Huang and Gibson 1992; Schuetz et al. 1986). Arene oxides are mainly transformed to hydroxylated aromatic compounds but also to sulfur-containing metabolites via the mercapturic acid pathway (Haraguchi et al. 1999b; Matthews 1982). Depending on the number and position of the chlorine-substitutions, one or more arene oxide intermediate may be formed from a given PCB. Unsubstituted *meta* and *para* carbon atoms are the preferred site for oxidation (Borlakoglu and Wilkins 1993b). Hydroxylation of coplanar PCBs usually predominates at the para position in the least chlorinated phenyl ring, and the rate of metabolism generally decreases with increasing chlorine substitution (Hu and Bunce 1999). Nonplanar PCBs are generally hydroxylated at an open *meta* position. The presence of vicinyl hydrogens (adjacent unchlorinated carbons) favors the metabolism of higher

chlorinated PCBs. Comparison of the molecular structures of the biologically persistent congeners reveals different molecular weights, substitution patterns, feasibility to rotate on the phenyl-phenyl bond, and intramolecular distances determined by nonelectrostatic forces (Borlakoglu and Walker 1989). Despite these physicochemical differences, it appears that higher chlorinated PCBs and congeners that lack unsubstituted *meta-para*-vicinal positions are better candidates for bioaccumulation.

Information regarding the metabolism of PCBs in humans is limited. Chromatographic analysis of adipose tissue samples of volunteers revealed almost 60 individual PCB components (Jensen and Sundström 1974). Examination of these results showed that <12 congeners accounted for . 80% of the total PCBs. For example, 2,2N,4,4N,5,5N-hexaCB (PCB 153) was the congener found in the highest concentration, whereas 2,2N,4,4N,6,6N-hexaCB (PCB 155) was not detected. PCB 155 is neither in commercial PCB mixtures nor in the environment at appreciable levels (Table 4-5). As PCB 153 is found in commercial PCB mixtures and in the environment, the presence of this congener in adipose tissue appears to be related to biologic persistence and/or to metabolism. The results of *in vitro* metabolism with human liver microsomes entirely support the conclusions drawn above. Human liver microsomes did not metabolize PCB 153 under various conditions, but did metabolize PCB 136 (Schnellmann et al. 1983). The major metabolites identified, 2,2N,3,3N,6,6N-hexachloro-4-biphenylol, and 2,2N,3,3N,6,6N-hexachloro-5-biphenylol, suggest that this congener is metabolized through an arene oxide. PCB 153 is often the most prevalent PCB detected in humans, due to exposure and the slow rate of biotransformation of this congener. More recently, 3-hydroxy-2,4,5,2',4',5'-hexaCB was identified as the major metabolite of PCB 153 formed by human CYP2B6 (Ariyoshi et al. 1995). CYP2B6 is constitutively expressed in humans, but only accounts for a maximum of 1–2% of the total CYPs in human liver. Approximately 75% of the subjects examined had no detectable level of CYP2B6 protein by immunoblotting (Mimura et al. 1993). This may be the reason why no metabolite of PCB 153 was detected in an earlier *in vitro* study using human liver microsomes (Schnellmann et al. 1983). The *in vitro* metabolism of 4,4N-diCB by human liver microsomes produced six metabolites, with 4,4N-dichloro-2-biphenylol being the most abundant (Schnellmann et al. 1984). These data also suggested that 4,4N-diCB is metabolized through an arene oxide and that migration of substituents from the site of hydroxylation to the adjacent carbon atom (NIH shift) occurs (Gardner et al. 1973; Safe et al. 1975). The major hydroxylated PCB metabolite in human plasma from unexposed people is 4-hydroxy-2,2N,3,4N,5,5N,6-heptaCB, originating from either 2,2N,3,4N,5,5N,6-hepta- and/or 2,2N,3,4,4N,5N,6-heptaCB (Bergman et al. 1994). All major 1-*ortho*-PCBs are transformed to 4-hydroxy-chlorobiphenyl metabolites, retained in plasma or blood. Changes in residue patterns, including actual increases over

time, indicating possible dechlorination products were observed by Wolff et al. (1992). Brown (1994) further suggested that humans were capable of very slow PCB dechlorination.

Most hydroxylated PCB metabolites are excreted in feces and/or in urine, or are conjugated to glucuronic acid or sulfate. However, several hydroxylated PCB metabolites are retained in the body, either due to their high lipophilicity or reversible binding to proteins. Although several of the OH-PCBs are present in rat plasma and seal blood, the spectrum of OH-PCBs is different in human plasma, compared to that in rats or seals (Bergman et al. 1994). For example, 4-OH-2,3,5,6,2',4',5'-hepta PCB, the major OH-PCB in human plasma (Bergman et al. 1994; Sandau et al. 1998), was found in seal blood but was not detected in rat plasma. This major metabolite may originate from 2,3,5,6,2',4',5'-heptaCB (PCB 187) and/or 2,3,4,6,2',4',5'-heptaCB (PCB 183), which are present in human milk. The 4-OH-2,3,5,3',4'-pentachloro-biphenylol, the major hydroxy PCB metabolite detected in rat plasma, is also a major contributor to hydroxy-PCBs in seal blood and in human plasma (Bergman et al. 1994). This metabolite is formed after a 1,2-shift of a chlorine in the para position in the 2,3,4-trichlorinated phenyl ring of PCB 105. In addition to PCB 105 and 118, this metabolite could arise from the PCB 156 3,4- or 4,5-arene oxide with the loss of a chlorine. A similar rearrangement is also observed to occur in the 3,4-dichloro-substituted phenyl rings of PCBs 77, 105, 118, and 156 (Klasson-Wehler et al. 1993). Thus, all of the major 1-*ortho*-PCBs can be biotransformed to 4-OH PCB metabolites that are retained in plasma or blood. Hydroxylated PCBs are retained in lung, liver and kidney tissue, which may be explained by the blood residues in these tissues. It is important to note that the concentration of the 4-OH metabolite of PCB 105 in rat liver or lung is similar to that of PCB 153, one of the most persistent and abundant PCB congeners. Persistent 3-OH PCB metabolites have also been identified at lower levels. In general, the persistent OH-PCBs have chlorine atoms on the adjacent carbons to the OH-group and contain five or more chlorine atoms.

Methylsulfonyl ($MeSO_2$) metabolites of PCBs have been widely detected in the tissues of marine mammals (Bergman et al. 1994; Letcher et al. 1995) and of humans (Haraguchi et al. 1986; Noren et al. 1996; Weistrand and Noren 1997). The $MeSO_2$-PCBs are formed via P-450 dependent epoxidation (Safe 1989a, 1989b) and subsequently via the mercapturic acid pathway (Preston et al. 1984). The most abundant $MeSO_2$ metabolites in wildlife and humans are originated from 2,2',4,5,5'-pentaCB (PCB 101) or 2,2',3,4',5',6-hexa (PCB 149) (Bergman et al. 1994; Haraguchi et al. 1992; Noren et al. 1996). Human milk in Sweden was analyzed for methylsulfonyl metabolites of PCBs, which decreased from approximately 9 to 2 ng/g milk lipid from 1972 to 1992 (Noren et al. 1996). The levels of these metabolites also correlated with the levels of total PCBs. The major $MeSO_2$-PCBs in the milk were

4-$MeSO_2$-2,5,2',3',4'-PCB (87) and 4-$MeSO_2$-2,3,6,2',4',5'-PCB (149). Methylsulfonyl metabolites of PCBs were also analyzed in autopsy tissues from seven Swedish individuals (Weistrand and Noren 1997). Twenty $MeSO_2$-PCBs were detected in liver and adipose tissues, with 4-$MeSO_2$-2,5,2',3',4'-PCB (87) and 4-$MeSO_2$-2,3,6,2',4',5'- PCB (149) also being found at the highest levels in these tissues. 3-$MeSO_2$ metabolites were detected in adipose tissue, but at lower levels. However, in the liver, the 3-$MeSO_2$ metabolites were most abundant, with 3-$MeSO_2$-2,2',3',4',5,6-PCB (132) contributing 61–82% of the sum of all $MeSO_2$-PCBs in the liver. The methylsulfonyl metabolite profile in one lung sample was similar to that observed in adipose tissue, with 4-$MeSO_2$ metabolites dominating the profile. The ratios of the sum of all methylsulfonyl metabolites to total PCB levels were 1/250 and 1/28 in adipose tissue and liver, respectively, calculated for the median values. Thus, methylsulfonyl metabolites of PCBs are selectively retained in different human tissues and therefore require further study regarding their biological and/or toxicological activity.

The metabolism of PCBs in experimental animals has been extensively reviewed (Safe 1980, 1989a; Sipes and Schnellmann 1987; Sundstrum et al. 1976a, 1976b). Many substrates have been tested, and the PCBs were usually administered by the oral or parenteral routes. In general, these studies showed that the metabolism rate of PCBs depends on the number and position of chlorine atoms on the phenyl ring and on the animal species. In rats, the elimination half-lives of four PCBs with one, two, five, or six chlorines increased as the number of chlorines increased (Matthews and Anderson 1975). The decreased excretion rate with increasing chlorination was directly related to the decreased rate of metabolism of the more highly chlorinated congeners. Sheep liver microsomes converted 2,2N,5-triCB to at least 5 more polar metabolites within 1 minute and at least 10 metabolites by 15 minutes; however, within the homologous series, 2,2N,5,5N-tetraCB and 2,2N,4,5,5N-pentaCB were oxidized to only 3 metabolites at rates 7- and 14-fold slower, respectively (Hansen 1987b; Hansen et al. 1977). Not only does the number of chlorines affect the rate of biotransformation, but the position of the chlorines on the phenyl rings is also critical. This was demonstrated in rats, which excreted four symmetrical hexachlorobiphenyls at different rates depending on the chlorine positions (Kato et al. 1980). As the number of unsubstituted *meta* positions or adjacent unsubstituted carbon atoms increases, the percentage of the dose excreted increases. The major hydroxylated PCB metabolite in rat plasma after administration of 25 mg/kg Aroclor 1254 in peanut oil by gavage is 4-hydroxy-2,3,3N,4N,5-pentaCB. From days 1 to 14 after exposure, this metabolite is found at concentrations 7–10 times the concentration of the major PCB 153 (Bergman et al. 1994).

The following generalizations based mostly on data obtained from experimental animals can be made (Safe 1980):

1. Hydroxylation is favored at the para position in the least chlorinated phenyl ring unless this site is sterically hindered (i.e., 3,5-dichloro substitution).

2. In the lower chlorinated biphenyls, the para position of both biphenyl rings and carbon atoms that are para to the chloro substituent are all readily hydroxylated.

3. The availability of two vicinal (adjacent) unsubstituted carbons atoms (particularly C5 and C4) also facilitates oxidative metabolism of the PCB substrate, but it is not a necessary requirement for metabolism.

4. As the degree of chlorination increases on both phenyl rings, the rate of metabolism decreases.

5. The metabolism of specific PCB isomers by different species can result in considerable variations in metabolite distribution.

The major PCB metabolites are phenolic products; however, sulphur-containing metabolites (Klasson-Wehler et al. 1987), trans-dihydrodiols (Norback et al. 1976), polyhydroxylated congeners, and methyl ether derivatives (Koga et al. 1989) have also been identified. The occurrence of trans-dihydrodiol metabolites suggests that the metabolism of PCB congeners proceeds through formation of arene oxide intermediates (Gardner et al. 1973). Due to their high reactivity, arene oxide intermediates are difficult to detect. They hydrate to give trans-dihydrodiols and spontaneously rearrange to phenols with the concomitant 1,2-migration of substituents from the site of hydroxylation to the adjacent carbon atom (NIH shift) (Daly et al. 1972). Arene oxides are potential electrophiles, and have been implicated in cellular necrosis, mutagenicity, and carcinogenicity (Safe 1989b). Experimental evidence using P^{32}-postlabeling supports the hypothesis that lower chlorinated biphenyls are metabolically activated to electrophilic species which bind to DNA (McLean et al. 1996; Oakley et al. 1996). The incubation of 2-chloro-, 4-chloro-, 3-chloro-, 3,4-dichloro-, and 3,4,5-trichlorobiphenyl with calf thymus DNA and rat liver microsomes followed by oxidation with a peroxidase, produced 1–3 major DNA adducts. The reactive metabolites may result for arene oxides and/or catechol and p-hydroquinone species, which are oxidized to semiquinones and/or quinones. The results raise the possibility that lower chlorinated biphenyls may be genotoxic and may explain the fact that commercial PCB mixtures are complete rodent carcinogens.

The formation of 3-hydroxy-2,2N5,5N-tetraCB as a metabolic product of 2,2N,5,5N-tetraCB suggested that a nonarene oxide direct hydroxylation mechanism is an alternative metabolic route for some chlorinated biphenyl congeners (Billings and McMahon 1978; Preston et al. 1983). Sipes and Schnellmann (1987) review and confirm additional routes for PCB oxidative metabolism.

Methylsulphonyl metabolites of PCBs have received considerable attention since these compounds are possibly etiologically connected to the respiratory toxicity described in *Yusho* victims (Brandt and Bergman 1987; Haraguchi et al. 1986). PCB methyl sulfones are formed as follows: products of the reaction between arene oxides and glutathione are degraded and excreted in the bile into the large intestine where they undergo cleavage by a microbial C-S lyase. The thiols formed are methylated, reabsorbed, and further oxidized on the sulfur to the corresponding methyl sulfones, which are distributed by the blood (Brandt et al. 1985). The methylsulphonyl-PCBs are initially bound to a uteroglobin-like protein found in high concentrations in rat and mouse lung cytosol (Lund et al. 1985) of the Clara and goblet cells. This protein-sulfone complex is subsequently secreted into the airway lumen and spread over the surface lining. It has also been suggested that this complex is transported by the mucociliary system to the pharynx and swallowed (Brandt and Bergman 1987). In the gastrointestinal tract, the complex may be released, reabsorbed, and recirculated to the lung, which could contribute to the long retention times for methylsulphonyl-PCBs in rodents (Brandt and Bergman 1987). Methylsulphonyl-PCBs have also been localized in rodent kidney cortex (Brandt et al. 1985), but the mechanism of accumulation in the proximal tubules appears to be different than that operating in respiratory airways since only trace amounts of the lung binding protein are present in rodent kidney (Lund et al. 1985). Methylsulphonyl metabolites of PCBs are also retained in the fat of seals (Jensen et al. 1979) and in liver and muscle of minks (Bergman et al. 1992).

It has also been shown that the 3-$MeSO_2$ metabolites from PCBs with 2,5-chlorine substitution were selectively retained in the liver of marine mammals (Bergman et al. 1994), whereas the isomeric 4-$MeSO_2$ metabolites were localized in the lung of mice (Bergman et al. 1979; Klasson-Wehler et al. 1996). Although the binding mechanism for 3-$MeSO_2$ metabolites is not clear, the binding protein for 4-$MeSO_2$ metabolites has been identified as a uteroglobulin-like protein present in the nonciliated bronchiolar (Clara) cells of the lung, also referred to as Clara cell secretary protein (CCSP) (Hard et al. 1995; Stripp et al. 1996). Exposure to 4-$MeSO_2$-2,2',4',5,5'-PCB demonstrated that CCSP-deficient mice no longer accumulate this metabolite within lung or kidney tissue (Stripp et al. 1996). These data demonstrate that CCSP is the 4-$MeSO_2$-PCB binding protein in mice and suggests that 4-$MeSO_2$-PCBs will accumulate at sites of CCSP localization, such as the respiratory and reproductive tracts of humans.

Haraguchi et al. (1999a) recently investigated the tissue distribution of methylsulfonyl metabolites derived from PCB 101 and 149 in male Wistar rats. Both congeners are metabolized primarily by hydroxylation at the 3-position and methylthiolation at the 4-position. The 3-/4-$MeSO_2$ metabolite ratios in liver and adipose tissue for both congeners were 0.41–0.61 at day 4, increasing to 0.85–1.00 for up to

42 days. In contrast, the ratios in lung were 0.03–0.04, and then decreased to 0.01 at 42 days. The ratio of metabolite to parent compound in tissues provides an estimate of the relative persistence or abundance of the methylsulfonyl metabolites. In the liver, the ratio of 3-$MeSO_2$ to PCB 101 was 0.46 and the ratio of 3-$MeSO_2$ to PCB 149 was 0.21. 4-$MeSO_2$ metabolites were highly retained in the lung, with metabolite to parent compound ratios of 9.5 and 4.0 for PCBs 101 and 149, respectively.

Persistent $MeSO_2$-PCBs also have been reported to induce hepatic P-450s in rats. 3-$MeSO_2$-PCBs identified in mammals were strong phenobarbital type inducers of CYP2B protiens, while the 4-$MeSO_2$ metabolites were inactive (Kato et al. 1995, 1997). Specifically, the inducing ability of 3-$MeSO_2$-PCB 101 was more than 500 times greater than the parent PCB101.

A summary of the structures of PCB metabolites that have been identified using various substrates and biosystems is presented in Figure 3-3.

3.4.4 Elimination and Excretion

3.4.4.1 Inhalation Exposure

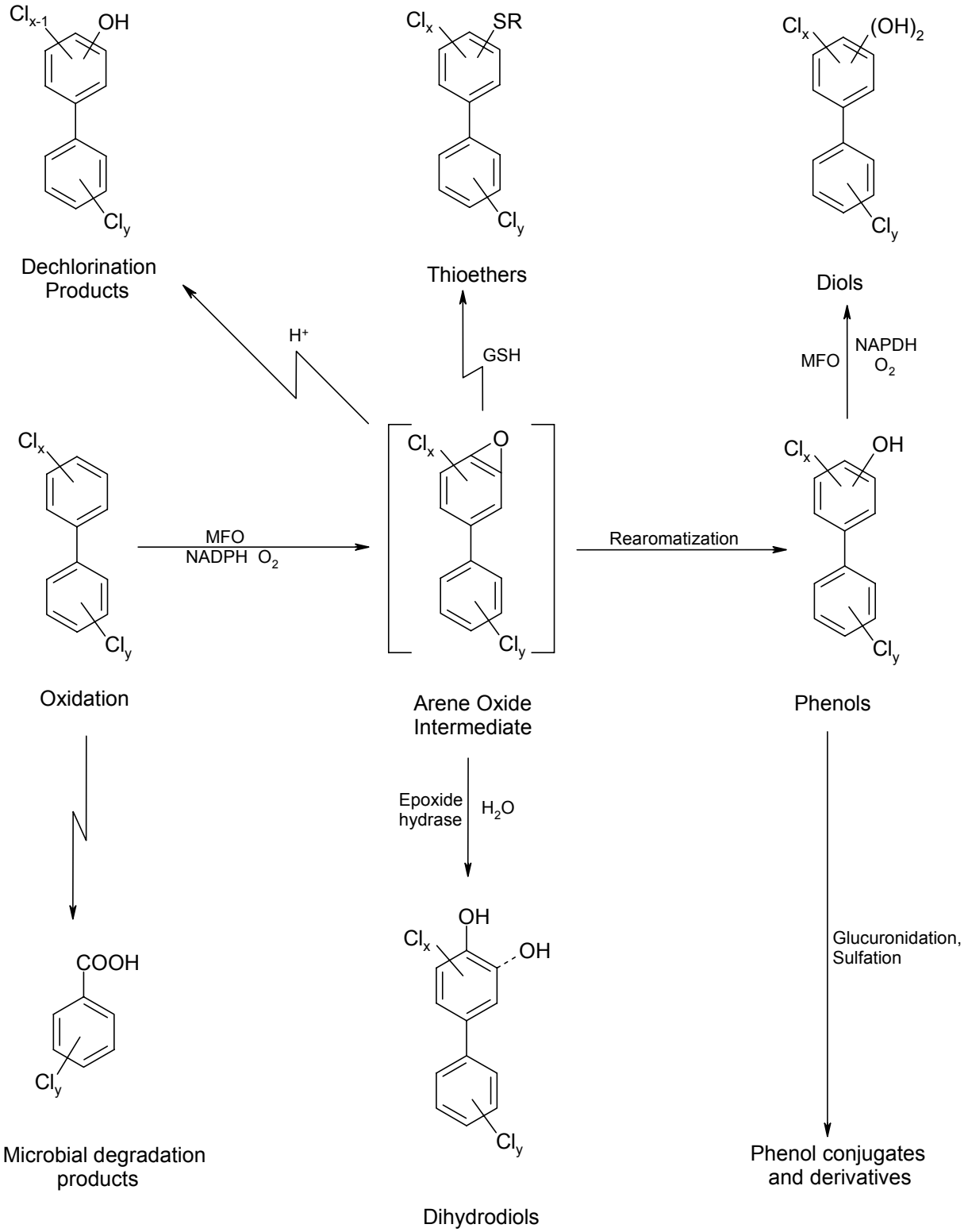
No studies were located regarding excretion in humans or animals following controlled inhalation exposure to PCBs. However, there is no reason to believe that once absorbed by inhalation, the excretion pattern of PCBs will differ from that observed after oral absorption. Much greater amounts of PCBs are excreted in the feces than in the urine following oral absorption.

3.4.4.2 Oral Exposure

Human Studies. Estimates for the half-lives for elimination of PCBs from humans have been based on body burden measurements at two or more time points form the same individual. A simple mass balance approach is commonly employed to characterize the elimination of PCBs from humans. In general, a simplified single compartment model is used where only intake and first order elimination are assumed to occur. In most cases, the intake is assumed to be negligible and the following equation is used to estimate k, the first order loss or rate constant (day $^{-1}$), where C_o and C_t are the initial and final tissue concentrations, respectively, and t is the time between sampling.

$$C_t = C_0 e^{-kt} \qquad (1)$$

Figure 3-3. Metabolic Pathways for Polychlorinated Biphenyls



Source: Safe 1984

This relationship assumes that the mass of the adipose tissue compartment and daily intake remaining constant over the observed time period and that daily intake is negligible. If these conditions are not met or are unknown, the k in the above equation represents an apparent rate constant (k'). The apparent half-life from the above equation can then be expressed as follows:

$$t_{1/2} = \frac{-ln2 \cdot t}{\ln \frac{C_t}{C_0}} \qquad (2)$$

Equation (2) was used to calculate apparent half-lives when human body burden data, such as blood, serum, plasma, or adipose tissue concentrations of PCBs are available at two or more time points.

It is often useful for the purposes of risk assessment to estimate the daily intake of PCBs needed to maintain a steady state body burden or tissue level of PCBs. The daily intake, I, required to produce a steady state body burden of $C \cdot M_{fat}$ is as follows:

$$I = k \cdot (C \cdot M)_{fat} \qquad (3a)$$

$$I = \frac{ln2}{t_{1/2}} \cdot (C \cdot M)_{fat} \qquad (3b)$$

Tables 3-9 and 3-10 summarize data from multiple studies on the apparent half-lives (years) of PCB congeners and PCB mixtures in humans. Although analysis of human blood, serum, plasma, adipose tissue, or other tissues does not match the specific profile of commercially produced PCB mixtures (Aroclors, etc.), the studies in Table 3-10 did not use congener specific analysis and estimated apparent half lives for PCB profiles with varying degrees of chlorination. Most of the studies cited in Table 3-9 utilized congener specific analysis; however, this was not the case for several studies. The studies report apparent half-life estimates as low as 0.02 years and as long as infinity, with no apparent loss in body burden despite removal of a known source of exposure. Thus, it is important to evaluate the variability between studies and use caution in interpreting results from a given study. Absolute values for PCB congeners and mixtures must also be interpreted with caution, since methods for analysis have improved in recent years. However, even with high resolution gas chromatography and electron capture detection (congener specific analysis), a given peak may represent more than one chemical (see Table 3-9).

Extensive sample preparation followed by gas chromatography/mass spectrometry (GC/MS) analysis is necessary to validate that a given peak is a PCB. This is the method of choice for the quantitation of very low levels of coplanar PCBs. However, for comparison of half-life estimates, it is not necessary to compare absolute PCB concentrations in different studies. Half-life estimates for a given study do depend on a consistent analytical method used to quantify PCB levels in biological samples obtained at two or more time points. The studies cited in Tables 3-9 and 3-10 are often limited by small sample size, short sampling intervals, and low initial body burdens.

Half-life estimates of less than one year have been reported by several studies in Tables 3-9 and 3-10. Buhler (1989) reported elimination in a single volunteer that ingested a single dose of a uniformly ^{13}C-labeled PCB mixture similar to Aroclor 1254 (329 µg/kg). Blood samples were taken during a 260 day period and analyzed for the concentration of ^{13}C- and ^{12}C-PCB using GC/electron capture detection (ECD) and GC/MS. Elimination of congeners followed first order kinetics and resulted in half lives of <1 year in this individual. These short apparent half-life estimates may be measuring a redistribution of the PCBs among body compartments during this relatively short sampling time rather than actual elimination from the body. Luotamo et al. (1991) estimated very short apparent PCB half-lives of 0.02–0.13 years in 12 individuals involved in a capacitor accident at a pulp mill. Apparent half-life estimates were based on blood and adipose tissue taken 1 and 30 days following the accident. Since the first sample was taken only 1 day following the accident, it is likely that equilibrium had not yet been reached and that absorption and/or distribution of the PCB exposure/dose was still occurring at that early time point. The sampling interval (29 days) was also very short in this study, particularly for highly persistent PCB congeners. These limitations must be considered in interpreting the half-life data from this study, which is much shorter than other studies.

Wolff and Schecter (1991) investigated an accident where four children (2–6 years of age) were exposed to PCBs while playing with parts of a capacitor. The excess exposure resulted in a serum PCB concentration of lower chlorinated congeners, similar to Aroclor 1242, that was about 4-fold above the reference group. Half-life estimates in Table 3-9 were based on serum samples obtained on 1–4 children at 5 and/or 11 months following the accident. Packed column GC of the serum samples resulted in detected peaks that often contained more than one PCB congener. Infinite half-lives were reported for seven congeners in Table 3-9, based on the fact that the levels of these congeners did not decline over time. One explanation for this observation was that the accidental exposure did not markedly increase the levels of these congeners, which appear to be at steady state. Furthermore, there was no correction for the growth of the children, which would dilute the PCB concentration over time. The remaining four

Table 3-9. Apparent Half-lives (Years) of PCB Congeners from Multiple Studies

Congener	Brown et al. 1989	Buhler et al. 1988	Chen et al. 1982[a,b]	Chen et al. 1982[a,c]	Luotamo et al. 1991[d]	Luotamo et al. 1991[e]	Ryan et al. 1993[f]	Wolff and Schecter 1991[g]	Wolff et al. 1992[h]	Yakushiji et al. 1984
18					0.02	0.03				
31								0.5[i]		
28	1.4				0.05	0.12		0.5[i]	4.8	3.0
33					0.02	0.02				
52								0.3[j]	5.5[k]	
47					0.2			0.3[j]	5.5[k]	
44								4	1.6	
72								4	1.2	
74	3.2				4	4		4[l]	4[m]	8.4
70								4[l]		
66						0.03	2.5	4[l]	4[m]	
95								0.4[n]	3[n]	
60									3[n]	
56								4	3[n]	
101					0.02	0.04		4[o]	5.7[o]	
99	3.3							4[o]	5.7[o]	
108		0.3–0.8[p]								
118	5.8	0.3–0.8[p]	0.83	0.77			1.2		9.6	

Table 3-9. Apparent Half-lives (Years) of PCB Congeners from Multiple Studies *(continued)*

Congener	Brown et al. 1989	Buhler et al. 1988	Chen et al. 1982[a,b]	Chen et al. 1982[a,c]	Luotamo et al. 1991[d]	Luotamo et al. 1991[e]	Ryan et al. 1993[f]	Wolff and Schecter 1991[g]	Wolff et al. 1992[h]	Yakushiji et al. 1984
153	12.4	0.93	47	26		4	3.8		4[q]	27.5
105	3.9		0.58	0.51					4[q]	
138	6–7	0.88	32	20			3.4		16.7	16.3
163	>20									
183						0.13			7.9[r]	
128			5.2	5.4					7.9[r]	
171						0.08			24	
156			4	4			4.0			
180		0.34	4	4			4.3			9.9
169							10.4			
170			47	71			3.9			
n	39	1	17[s]	7[s]	12	12	1, 3	1–4	18–165	8
Data[t]	Geomean	nr	Median	Median	Mean	Mean	Median	Mean	Geomean	Mean

Source: Modified from Shirai and Kissel (1996)

[a]Recalculated using median concentration ratios
[b]First and second samples
[c]First and third samples
[d]Serum
[e]Adipose
[f]Half-life of congener 169 was not recalculated due to inadequate data.
[g]Does not include adjustment for growth
[h]Based on a 46-month interval
[i]Co-eluting 28/31
[j]Co-eluting 47/48/52
[k]Co-eluting 47/49/52
[l]Co-eluting 74/66/70
[m]Co-eluting 74/66
[n]Co-eluting 95/56/60
[o]Co-eluting 99/101
[p]Co-eluting 108/118
[q]Co-eluting 153/105
[r]Co-eluting 183–128
[s]For congener 105, the n's were 14 and 6
[t]Mean = arithmetic mean; geomean = geometric mean; nr - not reported

Table 3-10. Apparent Half-lives (Years) of PCB Mixtures from Multiple Studies

Mixture	Elo et al. 1985	Hara 1985	Phillips et al. 1989	Steele et al. 1986[a]	Taylor and Lawrence 1992	Wolff and Schecter 1991[b]	Wolff et al. 1992[c]	Yakushiji et al. 1984	Yakushiji et al. 1984
Clophen A30	0.02								
Kanechlors									
300		5.1							
300/500		>15						0.67	7.1, 2.8[d]
Aroclors									
1242			2.6	2.0	1.8	0.9, 4[e]			
1248							8.6		
1254			4.8		3.3		65		
1260				27.6	4.1	1.2, 0.5[e]			
n	12	20, 14	58	5	148, 148, 121	4–5	18–165	1	8, 18
Data[f]	nr	Mean	Median	Median	Geomean	Mean	Geomean	Mean	Mean

Source: Modified from Shirai and Kissel (1996)

[a]Recalculated using median concentration ratio
[b]Not adjusted for growth rate
[c]Based on a 46- month interval
[d]Mothers and children, respectively
[e]Direct and indirect exposure groups, respectively
[f]Mean = arithmetic mean; geomean = geometric mean; nr = not reported

reported congeners had apparent half-lives of 0.3–0.5 years, and due to lack of chromatographic resolution, represent at least eight PCB congeners. Thus, there are serious limitations of the apparent half-life estimates from the congener data of Wolff and Schecter (1991) (Table 3-9). Apparent half-life estimates for PCB mixtures (Aroclor 1242 and 1260) are presented in Table 3-10 for these four children and five adults and teenagers that had an opportunity for indirect exposure, since they lived in the same households as the children (Wolff and Schecter 1991). Half-lives of 0.9 and 1.2 years were reported in the children for lower and higher chlorinated congeners, respectively. The PCB levels in the five teenagers and adults were not different from unexposed individuals, and were likely to be at steady state. Thus, although apparent half-lives were reported for the five teenagers and adults, the lack of excess exposure in this group makes half-life estimates of limited value.

Chen et al. (1982) measured blood PCB levels in 17 subjects from Taiwan that consumed PCB contaminated rice-bran oil in March and April of 1979 (*Yu-Cheng* episode). Blood was sampled for PCB analysis in 1980 and 1981. The authors estimated apparent half-lives of 0.83 and 0.58 years for PCB 118 and 105, respectively. The authors stated that half-lives of other congeners in blood were not calculated because they were either too long or too short to calculate or because the concentrations of these PCBs in blood were too small to accurately measure. Shirai and Kissel (1996) estimated the apparent half-lives for the other congeners reported by Chen et al. (1982), which ranged from 5.2 years to infinity. PCB exposures in this study were relatively low (0.7–4.7 ppb initially) and thus, the long half-lives may reflect near steady state conditions over this relatively short sampling period and, should be interpreted cautiously.

Wolff et al. (1992) estimated the apparent half-lives of PCBs in up to 165 capacitor manufacturing workers with initial serum total PCB levels of 1.2–24 ppb. Blood samples were taken in March of 1976 and in December of 1979 and the serum PCB levels were measured by packed column GC, which often results in two or more PCBs co-eluting in the same peak. Apparent half-lives in Table 3-9 range from 1.2 years to infinity. One explanation for the longer half-lives of certain congeners is that excess exposure to PCBs occurred in some individuals during the interval between 1976 and 1979. Excess occupational exposure could have occurred from 1976 to 1977, when all PCB use stopped. Although PCBs may not have been in use in the manufacturing facilities from 1977 to 1979, residual contamination at and around the work site could have contributed to additional occupational exposure. Secondary exposures from nonoccupational sources such as high fish consumption rates, and/or exposure in homes may also impact on loss rates. The apparent half-life estimates in Table 3-9 do not consider excess exposure to PCBs during the sampling interval and thus may be an over estimate of the relative

persistence of particularly the congeners with half-lives in excess of 10 years. The above factors also contribute to the long apparent half-lives reported by Wolff et al. (1992) for the low and particularly the highly chlorinated PCBs (Table 3-10).

Brown et al. (1989) investigated a subgroup of 39 individuals, originally from the study of Lawton et al. (1985a, 1985b), who had occupational exposure to PCBs from two capacitor plants. Inhalation and dermal contact were considered the main routes of occupational exposure. PCBs were measured in serum from blood samples obtained in 1976 and 1983. Apparent half-lives ranged from 1.4 years for PCB 28 to >20 years for PCB 163. Once again, excess occupational exposure to PCBs was possible over the sampling period, particularly from 1976 to 1977, which would increase the apparent half-life estimates. However, strengths of the study include the 7-year sampling period and the 39 subjects. Brown et al. (1989) made an interesting comparison between this population and that reported for a Taiwan population (*Yu-Cheng*) which was accidentally exposed via ingestion of contaminated rice oil (Chen et al. 1982). It was found that the mono-*ortho* congeners (PCBs 74, 118, 105) were cleared 3–7 times faster in the *Yu-Cheng* population than the capacitor workers, while the di-*ortho* congeners (PCBs 99, 153, 138) were eliminated 3–7 times more slowly in the *Yu-Cheng* population. Brown et al. (1989) speculated that these differences may have been related to alterations in the cytochrome P-450 mediated metabolism of PCBs in the *Yu-Cheng* population that was exposed to PCBs and PCDFs. Specifically, they speculated that PCDF exposure in the *Yu-Cheng* population may have produced an increase in CYP1A and depression of CYP2 forms.

Ryan et al. (1993) estimated the apparent half-lives of the seven most abundant PCBs in three individuals who ingested PCB contaminated rice oil in Taiwan (*Yu-Cheng*) in 1979. Blood samples were obtained 171, 425, 1,049, 2,025, and 3,502 days following the first sampling in May of 1980. Total PCBs levels in 1980 ranged from 150 to 400 ppb and 9 years later, decreased to about 30–35 ppb. PCB 180 was the most persistent, with apparent half-lives ranging from 3.7 to 5.7 years (median of 4.3 years). PCB 118 was the least persistent of the measured congeners, with apparent half-lives ranging from 1.1 to 1.3 years (median of 1.2 years). The apparent half-life for total PCBs ranged from 3.2 to 4.6 years (median of 3.5 years). This study was not limited by short sampling intervals, low initial body burdens, or the method for PCB analysis. Although the results were obtained from only three subjects, this study provides a good estimate of the apparent half-lives of the most abundant PCB congeners in humans (Table 3-9). Half-lives may still have been extended from ambient exposures, though.

Yakushiji et al. (1984) studied PCB elimination over a 3-year period in eight Japanese women occupationally exposed to Kanechlor 300 (similar to Aroclor 1242). Apparent half-lives for this mixture of 7.1 and 2.8 years were reported for these eight women and their children (1–13 years of age), respectively (Table 3-10). Initial whole blood mean PCB concentrations were 42 and 30 ppb for the women and children, respectively. The shorter half-life in children may be related to growth over the 3-year sampling period and subsequent dilution of PCB blood levels with increasing mass. Apparent half-lives for four PCB congeners in the eight occupationally-exposed women were also reported in Table 3-9.

In 1977 and 1985, serum PCB concentrations were determined for 58 workers in a factory that used PCBs in capacitor manufacturing until 1977 (Phillips et al. 1989a). This study expanded upon the earlier investigation in five members of this cohort (Steele et al. 1986). Less chlorinated PCBs were quantitated as Aroclor 1242, and more highly chlorinated congeners were quantitated as Aroclor 1254. The workers had excess occupational exposure, as documented by serum PCB levels of 2–3,300 and 5–250 ppb in 1977 for Aroclors 1242 and 1254, respectively. Median apparent half-lives of 2.6 and 4.8 years for Aroclors 1242 and 1254, respectively (Table 3-10). The half-lives of the respective mixtures in each individual varied inversely with the initial (1977) serum concentrations, with more rapid elimination occurring at higher PCB levels. This relationship may be a result of continued low level PCB exposure, variations in the time of exposure, and/or cytochrome P-450 induction, with the resulting increase in PCB metabolism and elimination at high initial PCB body burdens. Strengths of the study by Phillips et al. (1989a) are the population size (n=58) and the 8-year sampling interval. The study was limited by not providing congener specific analysis.

Taylor and Lawrence (1992) reported apparent half-lives in another occupational cohort, where serum PCB levels were available from 1979 and 1983 on 148 workers for Aroclors 1242 and 1254, and 121 workers for Aroclor 1260. The range of concentrations in serum in 1979 were 0–3,133, 4–639, and 4–377 ppb for Aroclors 1242, 1254, and 1260, respectively. The apparent half-lives in this study (Table 3-10) were similar to those reported by Phillips et al. (1989a) for another occupationally exposed group. As in the earlier report by Phillips et al. (1989), this study observed more rapid elimination of PCBs in individuals with higher initial (Phillips et al. 1979) serum PCB levels. Again, this relationship may be a result of continued low level PCB exposure, variations in the time of exposure, and/or cytochrome P-450 induction, with the resulting increase in PCB metabolism and elimination at high initial PCB body burdens.

In summary, the studies by Phillips et al. (1989a) and Taylor and Lawrence (1992) on apparent half-lives of PCB mixtures (Table 3-10) are in general agreement. These are well designed studies in two different occupational cohorts that are not limited by small sample size, short sampling intervals, or low initial body burdens. The main limitation of these studies was that congener specific PCB analysis was not conducted. Nevertheless, these studies provide the best estimates of the apparent half-lives of PCB mixtures following occupational exposure.

Animal Studies. In experimental animals, the major PCB excretion pathways were the fecal and urinary routes (Lutz and Dedrick 1987; Sipes and Schnellmann 1987), although trace amounts were reported in expired air of rats 24 hours after gavage administration of hexa- and tetrachlorobiphenyl (Hashimoto et al. 1976). Biliary excretion represents a major source of the PCB compounds found in the feces (Allen et al. 1974b; Norback et al. 1976). Significant amounts of PCBs can also be eliminated through lactation (see Section 3.7, Children's Susceptibility). At equilibrium, chlorobiphenyl congeners are eliminated from tissues according to individual kinetic parameters. For example, rats that received six weekly doses of PCBs showed three general patterns of elimination (Tanabe et al. 1981). One group of compounds, primarily di- and trichlorobiphenyls, had elimination half-lives of 1–2 days; a second group, primarily tetrachlorobiphenyls, had two elimination constants: one between 2 and 10 days and a second one of #90 days. A third group, composed mostly of penta- and hexachlorobiphenyls, had single elimination half-lives of >90 days. Thus, highly chlorinated PCBs are preferentially retained, probably because of a lower metabolism rate.

A 2-phase elimination process was also described in monkeys fed 2,4N,5-triCB for 84 days. When dosing was discontinued, the initial rapid phase had a whole body elimination half-life of 30–32 hours and was followed by a slower process, with a combined urinary and fecal excretion half-life of 4.5–4.8 days (Felt et al. 1977).

Elimination half-lives from blood between 0.3 and 7.6 years were estimated for a group of mono-*ortho*-chlorine-substituted PCB congeners in monkeys dosed with Aroclor 1254 for over 6 years (Mes et al. 1995a). In these monkeys, steady-state in adipose tissue had apparently been achieved after 37 months of dosing. Half-lives were estimated from blood measurements every 2 weeks after treatment ceased and monthly during the subsequent 2 years. The half-lives did not seem to be dose-dependent over the dose range tested (0.005, 0.02, 0.04, or 0.08 mg/kg/day). The wide range estimates were probably due to the small sample size (n=3) and the great degree of variation among the individual monkeys.

The very long half-lives of some PCBs introduces a dilution-by-growth variable that must be considered in making comparisons. Weanling pigs were given seven daily oral doses of one of three single-congener PCBs, or Aroclor 1254 for a total of 15 mg/kg (Hansen and Welborn 1977). Blood and biopsied backfat were monitored for up to 118 days, and the increase in total body fat was determined. If elimination was based on concentration in the backfat, half-lives of 24, 24, 63, and 42 days were determined for the three single congeners and Aroclor 1254, respectively. Half-lives based on estimated total body PCBs increased to 108, 268, >300, and 284 days, respectively. Body weights increased 370–560% in these rapidly growing animals and percent fat increased disproportionally from about 21% body weight to near 28% body weight during the duration of the study. The long total body half-lives in swine compared to rats (Anderson et al. 1993; Mühlebach and Bickel 1981) reflect sequestering in a larger and expanding compartment, making less PCB available for metabolism as well as elimination. This may be more relevant to some humans with fluctuating body weight and composition, contributing to the long half-lives reported by Wolff et al. (1992a, 1992b).

An important factor in the elimination process of PCBs is the location of the chlorine atoms in the phenyl rings. This was studied in mice administered a series of PCBs with different molecular configurations (Gage and Holm 1976). For the series of PCB congeners tested, the results show that differential biotransformation results in compounds having at least one pair of unsubstituted *ortho-meta* vicinal carbon atoms (positions two and three) being excreted much faster than those with other configurations, but this was greatly diminished by chlorines in the 2,2'- or 2',2'-positions.

The chemical identity of the PCB metabolites excreted by different species greatly depends on the structure of the parent compound. This has been studied by numerous investigators. In rats, 85% of the fecal excretion of metabolites derived from a hexachlorobiphenyl was hexane-extractable, indicating the presence of nonpolar compounds as opposed to urinary metabolites, which are usually polar derivatives (Mühlebach and Bickel 1981). Analysis of the feces of rats dosed with 3,3N,4,4N-tetraCB revealed parent compound (indicative of incomplete absorption), 5-hydroxy-tetraCB, 6-hydroxy-tetraCB, and 4-hydroxy-3,3N,4N,5-tetraCB, whereas the urine contained mainly conjugated hydroxylated metabolites (Klasson-Wehler et al. 1989a, 1989b). Rats treated with 2,3-diCB and 2,4,6,-triCB excreted compounds hydroxylated in the 4-position in the feces; a dihydroxy metabolite in position 3- and 4- was also identified (Goto et al. 1974). The major metabolites found in the urine of monkeys treated with 2,5,4N-triCB were free and conjugated monohydroxy derivatives; the feces were not examined (Felt et al. 1977). The urine of rabbits administered 2,2N,5,5N-tetraCB revealed 3-hydroxy-2,2N,5,5N-tetraCB, 4-hydroxy-2,2N,5,5N-tetraCB, and a trans 3,4-dihydro-3,4-dihydroxy-2,2N,5,5N-tetraCB (Gardner et al.

1973). Excretion of radioactive material in the urine of ferrets treated with ^{14}C-labeled Aroclor 1254 accounted for <10% of the amount excreted in the feces (Bleavins et al. 1984). During the first week after dosing, 22.1 and 1.8% of the dose was excreted in the feces and urine, respectively. Total excretion diminished considerably during subsequent weeks.

3.4.4.3 Dermal Exposure

Limited data were found regarding the excretion of PCBs in experimental animals following dermal exposure. The urinary excretion half-life of an undefined PCB containing 42% chlorine applied to the abdominal skin was 6.9 days in monkeys (Wester et al. 1983). In guinea pigs in which the same mixture was applied to the back of the ear, a 2-phase urinary excretion process was observed. The first phase was rapid, with an elimination half-life of 1.9 days, and was followed by a slower phase, with an elimination half-life of 12.6 days. However, the elimination half-life of a PCB containing 54% chlorine was 2.9 days and was linear for the duration (16 days) of the urine collection (Wester et al. 1983). Wester et al. (1990) reported that following percutaneous application of 4 µg ^{14}C-labeled Aroclor 1242/cm^2 to the abdominal skin of monkeys (four per group), a maximum of 11% of the dose (as ^{14}C-derived radioactivity) was excreted over a 30-day period when the solvent was mineral oil, while 10% was excreted when the solvent was trichlorobenzene (unspecified isomer). Excretion was virtually complete after the first 10 days. Urinary excretion of ^{14}C-derived radioactivity was approximately 2 times fecal excretion. Following application of 4.8 µg ^{14}C-labeled Aroclor 1254/cm^2 in mineral oil or trichlorobenzene, 5.5 and 3.9% of the dose, respectively, was excreted over a 30-day period. The probability that the authors measured only excretion for the most readily metabolized components is increased because in the most recent study, which specified Aroclor mixtures, urinary excretion was 2 times fecal excretion and urinary excretion was virtually complete after 10 days.

3.4.4.4 Other Routes of Exposure

Excretion of PCBs and metabolites after parenteral administration follows the same general pattern as after oral administration: much greater amounts are excreted in the feces than in the urine. Furthermore, nonpolar derivatives are found in the feces, whereas polar metabolites are preferentially found in the urine.

In rats, 80% of an intravenous dose of ^{14}C-labeled 3,3N,5,5N-tetraCB was excreted in the feces over a 42-day period, whereas only 6.1% was excreted in the urine (Tuey and Matthews 1977). Less than 10%

of the radioactivity in bile, feces, and urine was parent compound. The terminal half-life for whole-body elimination was 9.8 days. In contrast to the tetrachlorobiphenyl, excretion of ^{14}C-labeled 2,2N,4,4N,5,5N-hexaCB was minimal after intravenous injection in rats. Only 16% of the dose was excreted in the feces over 40 weeks, while urinary excretion accounted for 0.8% of the dose (Mühlebach and Bickel 1981). It has been suggested that the hexachlorobiphenyl, which is minimally metabolized, was stored after redistribution (Hansen and Welborn 1977; Mühlebach and Bickel 1981). The higher lipid solubility of the hexachlorobiphenyl may have also contributed to the greater retention of this congener. The significance of the chlorine substitution in the phenyl rings was examined by Kato et al. (1980), who injected four symmetrical hexachlorobiphenyls intravenously in rats. Most of the administered doses underwent predominantly fecal elimination. The 2,2N,3,3N,6,6N-hexaCB congener, which has unsubstituted vicinal carbon atoms, was rapidly and extensively (90%) excreted over a 7-day period. For the other isomers, <15% was excreted over the 7-day period.

Following intravenous injection of 32.7 µg Aroclor 1242 to Rhesus monkeys, 39.4% of the administered dose was excreted in the urine, and 16.1% was excreted in the feces over a 34-day period (Wester et al. 1990); the bulk of the dose (>90%) was excreted within the first 10 days. For Aroclor 1254, 7% of the administered dose (47.4 µg) was excreted in the urine and 19.7% in the feces over a 30-day period. The duration of the study probably accounted for only the most rapidly metabolized components of the mixtures (Kato et al. 1980; Lutz and Dedrick 1987).

In rats with ligated bile ducts, unchanged 2,3N,4,4N-tetraCB appeared in the small intestine 1 hour after intravenous injection suggesting that the wall of the small intestine is an important site of PCB excretion (Yoshimura and Yamamoto 1975). Lactation was also shown to be a major route of excretion of PCBs from postpartum mice administered the compounds before pregnancy (Gallenberg and Vodicnik 1987).

The time-course of elimination of several PCB congeners (mono and di-*ortho*-substituted penta-, hexa-, and heptachlorobiphenyls) was examined in the liver, lungs, and carcass of mice over a period of several weeks after a single intraperitoneal dose of 500 mg Aroclor 1254/kg at 8 days of age (Anderson et al. 1993). The concentration of total PCBs decreased in the three compartments as a function of time. Analyses of elimination half-times for total PCBs from carcass and liver, and of changes in body weight indicated excretion as well as dilution. All congeners were efficiently retained in the lung and decreased only as a function of dilution due to growth. In general, congeners with pairs of unsubstituted carbons were eliminated faster than those without unsubstituted carbon pairs. The changes in liver over time were complex: there was retention of all congeners during the prepubertal growth phase, with specific

enrichment of 2,3,3N,4,4N-pentaCB. This was followed by more rapid elimination of certain congeners at a later time. The investigators suggested that changes in the liver may have reflected differences or changes in amounts or types of lipids, in binding proteins, and/or in metabolizing enzymes.

3.4.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

Physiologically based pharmacokinetic (PBPK) models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic end points.

PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen et al. 1987; Andersen and Krishnan 1994). These models are biologically and mechanistically based and can be used to extrapolate the pharmacokinetic behavior of chemical substances from high to low dose, from route to route, between species, and between subpopulations within a species. The biological basis of PBPK models results in more meaningful extrapolations than those generated with the more conventional use of uncertainty factors.

The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parametrization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-specific physicochemical parameters, and species-specific physiological and biological parameters. The numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) is adequately described, however, this simplification is desirable because data are often unavailable for many biological processes. A simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

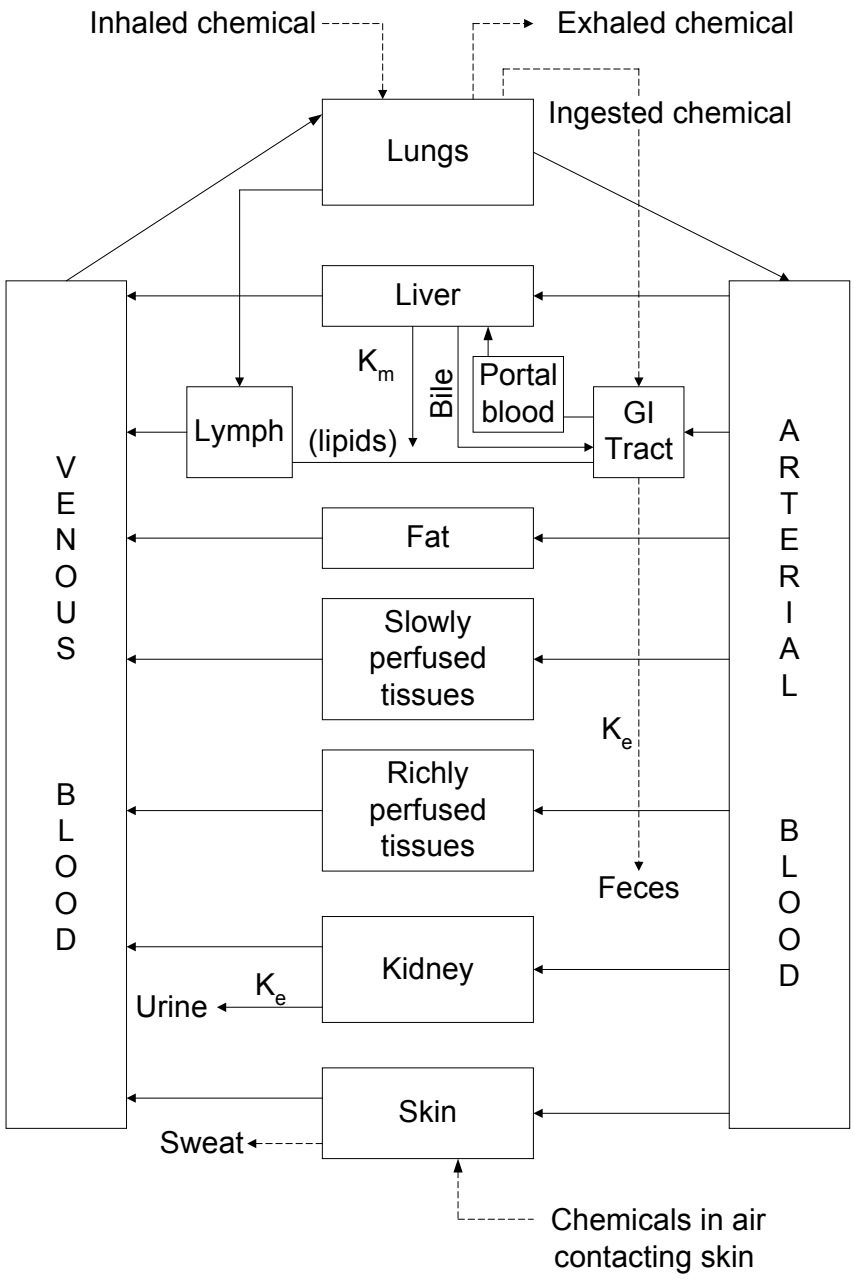
PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste sites) based on the results of studies where doses were higher or were administered in different species. Figure 3-4 shows a conceptualized representation of a PBPK model.

3.4.5.1 Summary of the PBPK Model

A flow-limited physiologic pharmacokinetic model was formulated to describe the individual kinetics of the tissue distribution, metabolism, and excretion of several PCB congeners in the rat, mouse, dog, and monkey. In general, the model predicted well the experimental data, but some deviations were apparent. Rates of metabolism were species specific, and there was no apparent scaling factor, such as body weight or surface area, for predicting metabolic rates from species to species. Meaningful extrapolation of data among species is dependent on accurate estimates of the rates of PCB congener metabolism.

The information presented below has been extracted from studies by Lutz et al. (1977), Tuey and Matthews (1980), and Lutz et al. (1984), and from reviews of those studies by Matthews and Dedrick (1984) and Lutz and Dedrick (1987). Models for the prediction of congener-specific PBPK parameters (tissue:blood partition coefficients, rates of metabolism) from structural information are discussed at the end of this section.

Figure 3-4. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



Source: adapted from Krishnan et al. (1994) and Hansen (1999)

Note: This is a conceptual representation of a physiologically based pharmacokinetic (PBPK) model for a hypothetical chemical substance. The chemical substance is shown to be absorbed by inhalation, by ingestion, or via the skin; metabolized in the liver; and excreted in the urine, bile, feces, sweat, or by exhalation. Lymphatic absorption from the gastrointestinal tract avoids the first-pass effect of liver metabolism and is very important for lipophilic chemicals (e.g., PCBs). Important first-order rate constants are K_e (elimination) and K_m (metabolism).

3.4.5.2 Description of the Model

The PBPK model for PCBs includes several compartments (Figure 3-4) thought to represent regions of substantial chemical uptake, regions involved in the clearance process, or regions of interest due to their toxic response to PCBs. Transport of the PCBs occurs by the inflow and outflow of blood through the compartment, by transcapillary transport, and by transport across cell membranes. The concept of blood flow-limited uptake was used because experimental data appeared to indicate that PCBs leave the blood and enter tissues very rapidly. Therefore, the assumption was made that the uptake is flowlimited. In Figure 3-4, dashed lines within compartments represent rapid equilibrium partitioning between blood and tissue space. In order to simplify the model, metabolism of PCBs was assumed to occur in the liver compartment as a single step leading to the formation of one metabolite that is excreted in the urine and bile as the glucuronide conjugate. The mathematical model consists of a set of differential equations describing mass balances on each PCB congener in each compartment. For example, for a tissue in which metabolism may occur, such as the liver, the mass balance takes the form

$$d(V_L C_L)/dt = Q_L(C_B - C_L/R_L) - (k_m \times C_L/R_L)$$

where t = time, V = tissue volume or mass, C = concentration, Q = blood flow rate, k_m = metabolic rate constant, and R = tissue/blood distribution coefficient. The subscripts L and B refer to liver and blood, respectively.

The PCB model was initially constructed to describe the distribution of 4-monoCB, 4,4N-diCB, 2,2N,4,5,5N-pentaCB and 2,2N,4,4N,5,5N-hexaCB in Sprague-Dawley rats following a single intravenous dose (0.6 mg/kg) of the PCB congeners (Lutz et al. 1977). The same model was subsequently applied to these congeners in CD-1 mice administered a single 0.6 mg/kg intravenous dose of the same PCB congeners (Tuey and Matthews 1980). In the dog (beagle) and monkey (Cynomolgus), 4,4N-diCB, 2,2N,4,4N,5,5N-hexaCB, and 2,2N,3,3N,6,6N-hexaCB were modeled, also after a single intravenous dose of 0.6 mg/kg (Lutz et al. 1984). The latter two congeners were chosen since they have the same number of chlorines, but 2,2N,3,3N,6,6N-hexaCB has two adjacent unsubstituted carbons, which has been shown to enhance metabolism.

Many of the parameters used in the model, in particular anatomical parameters and blood flow rates, were available from the literature. The skin flow rate used in the model had to be reduced by 10-fold in order to simulate the behavior of PCBs in the skin. Use of measured values or literature values resulted in

overprediction of the skin PCB concentration at early times by a considerable amount. This may have reflected a deviation from a flow-limited transport mechanism in the skin. Distribution coefficients (R) for both parent and metabolite were estimated for each compartment by taking the respective ratios of the specific tissue concentration to the blood concentrations at times when the compartments (tissues) were assumed to be in equilibrium with effluent venous blood. This implies that elimination is sufficiently slow so that the venous blood is a fair representation of the effluent venous blood from each tissue. Metabolic rate constants (k_m) for rapidly metabolized PCB congeners were estimated by dividing the amount of PCB appearing in the urine, feces, and tissue, by area under the blood concentration-time curve for a given time interval. For slowly metabolized PCB congeners, k_m was estimated by dividing the average rate of excretion of metabolite by the average blood concentration of metabolite. The rate of urinary excretion was assumed to be proportional to the blood metabolite concentration. Urinary clearance (k_K) was estimated by dividing the amount of PCB metabolite collected in the urine by the area under the metabolite blood curve. Biliary excretion (k_B) was estimated from direct cannulation of the bile duct or calculated from fecal excretion rates. Liver equations for the dog and monkeys carried an additional term that allowed for preferential and rapid removal of a specified fraction (F) of the PCB from the liver blood pool; this fraction was rapidly and irreversibly transferred to the bile fluid. Some metabolism parameters (metabolism rate, kidney clearance rate, biliary clearance rate) for the mouse were scaled from values reported for the rat.

3.4.5.3 Discussion of the Model

Anatomical parameters used in the model are listed in Table 3-11. Metabolism and clearance parameters are listed in Tables 3-12, 3-13, and 3-14. Table 3-12 shows that in the animal species examined k_m decrease as chlorination increases, but the chlorine position also determines the rate of metabolism. This is suggested by the difference in k_m values between 2,2N,3,3N,6,6N-hexaCB and 2,2N,4,4N,5,5N-hexaCB in the rat, dog, and monkey. It appears that the *meta* and *para* positions are preferred sites for arene oxide formation, which would explain why the k_m for 2,2N,3,3N,6,6N-hexaCB is even larger than for 4,4N-diCB. Table 3-12 also shows no apparent interspecies correlation of k_m with body weight or surface area. As body size increased, k_m increased, but when the parameters are normalized by either body weight or body surface area, no consistent pattern of k_m is evident. On any basis, k_m values for the dog are greater than those for the mouse, rat, or monkey. Based on these data alone, it cannot be ascertained which species, if any, would predict PCB metabolism in humans; however, the collective data integrated with human residue data have been developed into what appears to be a reliable model (Brown 1994).

Table 3-11. Volumes and Flow Rates in Several Tissues of Four Species[a]

	Volumes (mL)				Blood flow rates (mL/minute)			
	Mouse 30 g	Rat 250 g	Monkey 5 kg	Dog 12 kg	Mouse 30 g	Rat 250 g	Monkey 5 kg	Dog 12 kg
Blood	2.89	22.5	300	1000	–	–	–	–
Muscle	17.1	125	2068	5530	1.42	7.5	103	275
Liver	2.24	10	118	480	3.1	16	125	342
Skin	5.51	40	470	1680	0.12	0.5	2.7	11.7
Fat	3.72	17.5	389	777	0.1	0.4	10.7	17.9

[a]Values were used in physiologic pharmacokinetic model.

Source: Lutz and Dedrick 1987

Table 3-12. Metabolism Rate Constant (k) from the Physiologic Model[a,b]

	4-MCB	4,4'-DCB	2,2',4,5,5'-PCB	2,4,5-HCB	2,3,6-HCB
k_m mL/min					
Mouse (30 g)	2.4	0.37	0.095	0.01	–
Rat (250 g)	10	2	0.39	0.045	5
Monkey (5 kg)	–	7	–	0.67	15
Dog (12 kg)	–	470	–	16	183
k_m mL/min/kg					
Mouse	68.5	9.7	2.5	0.25	–
Rat	40	8	1.56	0.18	20
Monkey	–	1.4	–	0.13	3
Dog	–	39	–	1.33	15.2
k_m mL/min/$kg^{0.7}$					
Mouse	25	3.7	0.94	0.1	–
Rat	26.4	5.2	1.02	0.12	13
Monkey	–	2.3	–	0.22	5
Dog	–	82	–	2.8	32

[a]Source: Lutz and Dedrick 1987

[b]Middle set of numbers is per-unit animal body weight, mL/min/kg. Bottom set is mL/min/$kg^{0.7}$, since body surface area is approximately proportional to body weight to the 0.7 power.

DCB = dichlorobiphenyl; HCB = hexachlorobiphenyl; MCB = monochlorobiphenyl; PCB = pentachlorobiphenyl

Table 3-13. Tissue-to-blood Distribution Coefficients for Parent Polychlorinated Biphenyls (R) and Metabolites (R')

	4-Monochlorobiphenyl				4,4′-Dichlorobiphenyl				2,2′4,5,5′-Pentachlorobiphenyl			
	Mouse	Rat	Monkey	Dog	Mouse	Rat	Monkey	Dog	Mouse	Rat	Monkey	Dog
Parent, R												
Muscle	1	1	–	–	2	2	5	4	5	1	–	–
Skin	10	10	–	–	10	10	50	12	20	7	–	–
Fat	30	30	–	–	70	70	300	40	200	70	–	–
Liver	1	1	–	–	5	3	20	6	14	12	–	–
Metabolite, R′												
Muscle	0.14	0.14	–	–	0.4	0.4			0.1	0.1	–	–
Skin	0.25	0.25	–	–	0.8	0.3			0.1	0.1	–	–
Fat	0.4	0.4	–	–	1	0.6			0.4	0.4	–	–
Liver	2	2	–	–	4	5			2	2	–	–

	2,4,5-Hexachlorobiphenyl				2,3,6-Hexachlorobiphenyl			
	Mouse	Rat	Monkey	Dog	Mouse	Rat	Monkey	Dog
Parent, R								
Muscle	5	4	7	6	–	–	4	4
Skin	35	30	70	30	–	–	40	8
Fat	300	400	500	300	–	–	250	30
Liver	10	12	30	10	–	–	20	2
Metabolite, R′								
Muscle	3	0.3	1	0.2	–	–	0.1	0.1
Skin	5	2	3	0.7	–	–	0.5	0.2
Fat	1	2	9	2	–	–	1	0.25
Liver	10	4	5	10	–	–	5	10

Source: Lutz and Dedrick 1987

Table 3-14. Kidney Clearance (k_k) and Biliary Clearance (k_g) for Selected Polychlorinated Biphenyls in Several Species[a,b]

	4-MCB		4,4N-DCB		2,2N,4,5,5N-PCB		2,4,5-HCB		2,3,6-HCB	
	k_g	k_k	k_g	k_k	k_g	k_k	k_g	k_k	k_g	k_k
Mouse (30 g)	0.05	0.05	0.15	0.069	0.10	0.009	0.074	0.018	–	–
Rat (250 g)	0.2	0.2	0.35	0.133	0.3	0.033	0.30	0.03	1.0	0.03
Monkey (5 kg)	–	–	0.083	1.5	–	–	0.70	0.041	0.5	0.4
Dog (12 kg)	–	–	10.2	2.7	–	–	1.8	0.15	7.0	2.0

[a]Source: Lutz and Dedrick 1987
[b]Clearance values are in mL/minute.

DCB = dichlorobiphenyl; HCB = hexachlorobiphenyl; MCB = monochlorobiphenyl; PCB = pentachlorobiphenyl

Table 3-13 lists tissue/blood distribution coefficients (R) for parent compounds and metabolites. In the four species tested, and for all the PCB congeners modeled, the rank order for R is fat >skin >liver >muscle >blood. The large R for parent PCBs in adipose tissue is not unexpected considering the lipophilic nature of the compounds. Also, as expected, Rs for metabolites were considerably lower than those for the parent compounds. This is likely because glucuronide conjugates of the parent compound are less lipophilic and more water soluble. The largest R for metabolites were found in the liver, reflecting the fact that metabolism of PCBs occurs primarily in the liver. Clearance parameters for biliary and urinary elimination listed in Table 3-14 do not show any apparent interspecies scaling correlations.

3.4.5.4 Validation of the Model

The model used to simulate the data described well the kinetics of distribution, metabolism, and excretion of the PCB congeners studied in adult animals. However, some deviations were apparent. For example, in the rat the model predicted a faster rate of clearance from blood and tissues for lower chlorinated biphenyls beyond 48 hours. This was tentatively attributed to the formation of minor metabolites, which have different pharmacokinetic behavior than the major metabolites. Formation of metabolites covalently bound to tissue macromolecules also was a possibility. The model overpredicted the concentration of PCBs in the skin at early times, suggesting that the use of measured or literature values of skin-flow rate in the flow-limited skin compartment may not have been appropriate. Among the possibilities offered to explain this phenomenon were: the existence of an additional barrier in the skin that reduced uptake, inaccurate measurement of skin blood-flow rates because of shunt flow, and presence of subcutaneous skin fat serving as reservoir for the diffusion of PCBs from the skin tissue. Also, in order to obtain a good simulation of the behavior of the poorly metabolized 2,2N,4,4N,5,5N-hexaCB in rats, a term describing changes in fat volume of the growing rats had to be incorporated into the model. Without this term, the simulations overpredicted the long-term data. Apparent elimination of the PCB congener from blood or adipose tissue was in reality a dilution effect due to the increased fat volume. When the physiological compartment model for the rat was scaled to the mouse, the disposition of PCBs in the latter was in reasonable agreement with the experimental data. An exception was the finding that greater biliary clearance rates than the corresponding rate scaled from the rat for the di-, penta-, and hexachlorobiphenyl were observed. Model simulations of tissue disposition of parent compounds in the monkey were in reasonably good agreement with the experimental data. This was not the case for the dog, a species in which, except for 2,2N,4,4N,5,5N-hexaCB, the simulations underpredicted the experimental data at longer time points. The data showed that the dog metabolized the three PCB congeners tested considerably faster than the monkey. As with the rat, simulations of blood-flow rate to

the skin in the dog and monkey had to be reduced to one-tenth its physiologic value in order to fit the experimental data.

The results of these studies show that pharmacokinetic modeling is a valuable tool for predicting PCB disposition in one animal species by extrapolation of data from other animal species. However, while many similarities exist from species to species, some important differences were also apparent. The most important parameter in the model appeared to be the k_m. Knowledge of this parameter in a species of interest is crucial if reliable predictions of PCB disposition are to be made. Lutz et al. (1984) recognized that “without additional information about metabolism, extrapolation of the present model to simulation of human disposition would be suspect." The model constructed by Lutz and co-workers provides kinetic and metabolic information regarding a very small number of PCB congeners, but not toxicity information that could eventually be used for developing risk assessment approaches for PCBs. The most extensively studied PCB congeners from the point of view of their toxic properties are the dioxin-like congeners. These congeners lack chlorine substitution in the *ortho* position and are isostereomers of 2,3,7,8-TCDD (dioxin). The mechanism of toxicity for these congeners is related to the enhancement of gene expression triggered by initial binding to a cytosol receptor (Ah receptor) (see Section 2.5.2 for further details). Although no PBPK model has been constructed for the dioxin-like congeners, information exists for 2,3,7,8-TCDD. PBPK models for 2,3,7,8-TCDD account not only for determinants of disposition, such as tissue partitioning, biotransformation rates, and protein-binding constants, but also describe pharmacodynamic events related to the induction of specific dioxin-binding proteins in the liver (Leung et al. 1988). Recent refinements of the model incorporate information on the interactions of the dioxin-Ah receptor complex with regulatory regions of specific genes (Andersen et al. 1993). This level of information is expected to provide a basis for investigating the scaling across species of the PBPK model for dioxin and chemicals with common-mediated mechanisms of toxicity.

3.4.5.5 Prediction of Congener Specific PBPK Model Parameters.

Since the toxicity of a given PCB mixture is related to its congener composition, congener-specific information on kinetic parameters is necessary if PBPK models are to be used as part of risk assessment. Methods to predict the tissue:blood partition coefficient (Parham et al. 1997) and metabolic rate constants (Parham and Portier 1998) for all 209 congeners based on structural information are discussed below.

Data from a study of occupationally exposed humans allowed the authors to calculate the adipose:plasma partition coefficient for 24 PCB congeners (Wolff et al. 1982b). In an effort to predict these results by

modeling (Parham et al. 1997), a total of 27 structural descriptors were identified for PCBs (e.g., total number of chlorines, number of *ortho*-chlorines, number of chlorines on most-substituted ring, etc.). A stepwise regression method was used to identify a small set of descriptors that would adequately predict the observed adipose:plasma partition coefficients for each congener. It was found that the three most important structural descriptors for the prediction of the adipose:plasma coefficient were (1) whether the congener had a ring with unsubstituted adjacent *meta* and *para* carbons, (2) the congeners nonplanarity, and (3) the polarity of the congener. The adipose:blood partition coefficient can be derived from the adipose:plasma partition coefficient if the ratio between plasma and whole blood is known for the congener. Stepwise regression was similarly used to predict a data set for the distribution between plasma and the cellular component of blood of eight PCB congeners and biphenyl in the rat (Matthews et al. 1984). In this case, a good fit with the experimental data was obtained with the use of only one structural descriptor, the number of adjacent unsubstituted *meta* and *para* carbons. Conversion from rat blood to human blood was accomplished by adjusting for the higher proportionate volume of the cellular component in rat blood and assuming that all PCBs in plasma are bound to protein in both species. A factor was derived for conversion of the predicted adipose:plasma partition coefficient to the adipose:blood partition in humans; this factor depends on the number of unsubstituted *meta*-*para* carbon pairs (0-4) in the specific congener. Adjustment factors for partition coefficients for other tissues (liver, muscle, and skin) were developed based on lipid fraction in the tissue, percent nonneutral lipid, and percent neutral lipid.

A similar stepwise regression process has been applied to the prediction of metabolic rate constants for specific PCB congeners from structural descriptors (Parham and Portier 1998). The metabolic rates used as the input for the stepwise regression were derived from *in vitro* rates of formation of metabolites for 25 congeners in rat liver microsomes (Borlakoglu and Wilkins 1993a, 1993b) and from a modification of the Lutz et al. (1977) PBPK model using data from four intravenous injection studies in rats employing nine congeners (Abdel-Hamid et al. 1977; Matthews and Anderson 1975; Tuey and Matthews 1977, 1980). The *in vitro* data included 14 congeners tested with microsomes from Aroclor 1254-induced rats and 11 congeners from noninduced rats. The stepwise regression resulted in seven descriptors included in the model. Five were structural descriptors (degree of chlorination, noncoplanarity, and three that described the presence of adjacent unsubstituted carbon atoms) and two were nonstructural (whether the data was from [1] induced or noninduced rats and [2] *in vitro* or *in vivo* experiments). The final model was used to predict the blood radioactivity data from intravenous injection studies and appeared to fit the experimental data well. Some individual misfits could be attributed to the fact that Borlakoglu and Wilkins (1993a, 1993b) measured only primary metabolites but used prolonged incubation times.

3.5 MECHANISMS OF ACTION

PCBs are lipophilic compounds that are readily absorbed from the gastrointestinal tract. While PCBs in many cases entered the environment as commercial formulations containing a relatively defined mixture of specific PCB congeners, the accumulation and retention of specific PCB congeners in various environmental matrices, wildlife, and humans does not directly reflect the PCB profile of the commercial mixtures. Therefore, it is important to consider the biological fate and activity of individual PCB congeners when assessing the risk that PCBs pose to human health. Although PCBs are found in all tissues analyzed to date, they are stored in high concentration in adipose tissue since they are lipophilic. PCB congeners are metabolized in the liver by microsomal cytochromes P-450 to less lipophilic metabolites that can undergo conjugation with glutathione or glucuronic acid. The rate of congener metabolism is highly dependent on the chlorine substitution pattern in the biphenyl ring. Strong evidence suggests that the mechanism of toxicity for dioxin-like congeners is related to the enhancement of gene expression triggered by initial binding to the same cytosol receptor (Ah) involved in 2,3,7,8-TCDD toxicity. The mechanism(s) of toxicities for other groups of PCB congeners, such as those showing estrogenic or neurotoxic activity, and the mechanism of PCB carcinogenicity, has not been elucidated. Similarly, disruption of neutrophil function and calcium homeostasis appear to be mediated by mechanisms other than the Ah receptor. Disruption in thyroid hormone homeostasis occurs through mechanisms that transcend all congener groups of PCBs.

3.5.1 Pharmacokinetic Mechanisms

The mechanism of absorption of PCBs by the inhalation and dermal routes of exposure is not known. PCBs are well absorbed from the gastrointestinal tract. Diet is the main source of background human exposures to persistent lipophilic organic pollutants, such as PCBs (Duarte-Davidson and Jones 1994; Hansen 1999). Because PCBs are lipid soluble, transfer from the aqueous environment of the intestine across cell membranes is a passive process (Albro and Fischbein 1972; Gage and Holm 1976; Matthews and Anderson 1975). The concentration gradient favors partitioning across the cells into blood serum or lymph. As with other fat-soluble chemicals, PCBs are most likely absorbed from the gut via lymphatic circulation rather than by transfer to the hepatic portal system (Hansen 1999). Absorption efficiency appears to increase with the degree of ring chlorination up to a certain point. Schlummer et al. (1998) calculated the net gastrointestinal absorption of PCBs in humans as the difference between contaminant input with food and contaminant output with feces, normalized to the contaminant intake. PCB congeners showing nearly complete net absorption had very low or nondetectable levels in the serum lipids, while

for other congeners, there was a trend for decreasing net absorption and /or increasing net excretion with increasing congener concentration in serum lipids. Together, the data support the passive diffusion model for gastrointestinal absorption, where the concentration of the contaminant in the blood is the major factor determining absorption. This suggests that the ingestion of more highly contaminated food should result in nearly complete absorption due to the high diffusion gradient associated with high levels of PCBs in the gut contents. In blood, PCBs are associated with red blood cells, albumin, and lipoproteins (Matthews et al. 1984). Distribution in plasma is determined primarily by partition among the various proteins according to lipid solubility and concentration (Matthews and Dedrick 1984). As the degree of halogenation increased, the binding to lipoproteins also increased (Matthews et al. 1984). Partition of PCBs between blood and tissues also seems determined primarily by lipid content and concentration gradient. The fraction associated with red blood cells is more rapidly removed from the blood by the tissues than fractions associated with plasma proteins (Matthews et al. 1984).

Borlakoglu et al. (1990) proposed a model for the transport and cellular uptake of PCBs following oral exposure. Following the ingestion of PCBs, the absorbed congeners are secreted into the bloodstream in association with chylomicrons and then are associated with the VLDLs synthesized in the liver. As the congeners come in contact with the lipoprotein lipase located on the surface of the capillary endothelial cells of adipose tissue, the PCB congeners are transferred into the adipocytes. Mobilization of PCBs from adipose tissue will release PCBs into the bloodstream, where they will associate with HDL and plasma proteins, such as albumin, by non-covalent binding. Noren et al. (1999) found PCBs mainly associated with the lipoprotein depleted (LPDP) fractions (containing primarily albumin). On average, 44% of the PCBs and 61% of the methylsulfonyl metabolites of PCBs ($MeSO_2$-CBs) were distributed in the LPDP fraction. This may be expected due to the more polar character of the $MeSO_2$-CBs. Among the lipoprotein fractions, LDL was the main carrier of PCBs, while $MeSO_2$-CBs were distributed equally between the LDL and HDL fractions. When the concentrations of PCBs were calculated in relation to apolipoprotein B, the levels were about 10 times higher in VLDL than LDL.

As with other organisms, PCB residue levels in humans reflects multiple exposure pathways, and congener-specific elimination. PCB profiles in human serum immediately following exposures reflect the profiles in the exposure sources; however, selective metabolism and excretion begin to alter the congener profile within 4–24 hours (Hansen 1999). Thus, in most cases, the PCB profile in adults represents a steady state body burden which does not match the profile of commercial PCB formulations (Aroclors, etc.). For example, neither the PCB profile of human adipose nor of a composite human milk sample resemble the pattern of any commercial PCB formulation (Jensen and Sundstrom 1974; Safe et al. 1985).

Humans, aquatic mammals, birds, fish, and other biota retain unique profiles of PCB congeners consistent with exposures and toxicokinetic principles. Borlakoglu and Walker (1989) reported that fish-eating sea birds, human fat, American breast milk, and German breast milk have similar PCB congener profiles, reflecting fish residues, which differ from Aroclor 1260 or Clophen A60. Hansen (1999) cites several studies reporting diet-dependent PCB profiles in various birds, and a larger breast milk study shows regional differences in congener profiles in Canadian breast milk (Newsome et al. 1995).

PCBs are rapidly (minutes to hours) cleared from the blood of adult animals and accumulated in the liver and muscle (Matthews and Dedrick 1984; Safe 1980, 1989a). This appears to be due to the high perfusion in the liver and the relatively large muscle volume. Due to their high affinity for lipophilic tissues, PCBs are subsequently translocated to adipose tissue and skin for storage. Subcutaneous fat accumulates PCBs more slowly than central fat stores (Hansen and Welborn 1977). Stored residues are less available for elimination or metabolism by the liver. A dynamic equilibrium of PCB concentrations is established among all tissues for each PCB homolog (Matthews and Dedrick 1984). As previously discussed, mathematical models that incorporate anatomical as well as pharmacokinetic parameters have been developed to describe distribution and body burden of PCBs in adults of species such as mice, rats, dogs, and monkeys (Lutz and Dedrick 1987). Pharmacokinetic modeling of PCB disposition predicts that, at equilibrium, changes in the PCB concentration or changes in tissue volume of any tissue will lead to a corresponding change in all tissues (Matthews and Dedrick 1984). For instance, if the concentration of a PCB congener in the liver is reduced by metabolism or excretion, then the concentration of that PCB congener in all tissues will be reduced proportionally. Congeners that cannot be metabolized or excreted will concentrate in adipose tissue, but will still circulate to other tissues. Exposure to other tissues will be proportional to the respective tissue/blood ratios and the concentration in the main storage tissues. This dynamic distribution results in accumulation of persistent congeners in all tissues and depletion from all tissues of those congeners that can be cleared (Matthews and Dedrick 1984). Metabolites, however, may accumulate in specific tissues due to solubility differences as well as tissue binding (Section 3.4.3). Relatively little is known regarding the biological and toxicological activity of these persistent PCB metabolites.

A possible explanation for the highly selective retention of the OH-PCBs in blood may be their structural resemblance with thyroxin. Both rats and mice metabolize PCB 77 by CYP1A to the 1,2-shift metabolite, 4-OH-3,5,3',4'-PCB, 5-OH 3,3',4,4'-PCB, and 6-OH-3,3',4,4'-PCB (McKinley et al. 1993; Morse et al. 1995). Only the 4-OH metabolite was selectively retained, with blood containing 4-OH-3,5,3',4'-PCB at a concentration 15 times higher than the parent compound, 5 days after oral exposure to PCB 77 in mice

(Bergman et al. 1994). This metabolite was found to be bound to a thyroxin-transporting protein (transthyretin) in the blood (Brouwer et al. 1986). Competitive binding studies of OH-PCBs relative to T_4 and computer modeling showed that OH-PCBs with the substituents in *meta* or *para* positions were much more effective competitors for T_4 than if the substituents were bound in an *ortho* position (Rickenbacher et al. 1986).

Methylsulfonyl ($MeSO_2$) metabolites of PCBs have been widely detected in the tissues of marine mammals (Bergman et al. 1994; Letcher et al. 1995) and of humans (Haraguchi et al. 1986; Noren et al. 1996; Weistrand and Noren 1997). Although the binding mechanism for 3-$MeSO_2$ metabolites is not clear, the binding protein for 4-$MeSO_2$ metabolites has been identified as a uteroglobulin-like protein present in the nonciliated bronchiolar (Clara) cells of the lung, also referred to as Clara cell secretary protein (CCSP) (Hard et al. 1995; Stripp et al. 1996). Exposure to 4-$MeSO_2$-2,2',4',5,5'-PCB demonstrated that CCSP-deficient mice no longer accumulate this metabolite within lung or kidney tissue (Stripp et al. 1996). These data demonstrate that CCSP is the 4-$MeSO_2$-PCB binding protein in mice and suggests that 4-$MeSO_2$-PCBs will accumulate at sites of CCSP localization, such as the respiratory and reproductive tracts of humans.

Experimental evidence using P^{32}-postlabeling supports the hypothesis that lower chlorinated biphenyls are metabolically activated to electrophilic species which bind to DNA (McLean et al. 1996; Oakley et al. 1996). The incubation of 2-chloro-, 4-chloro-, 3-chloro-, 3,4-dichloro-, and 3,4,5-trichlorobiphenyl with calf thymus DNA and rat liver microsomes followed by oxidation with a peroxidase, produced 1–3 major DNA adducts. The reactive metabolites may result for arene oxides and/or catechol and p-hydroquinone species, which are oxidized to semiquinones and/or quinones. The results raise the possibility that lower chlorinated biphenyls may be genotoxic and may explain the fact that commercial PCB mixtures are complete rodent carcinogens.

The major routes of excretion of PCBs are fecal and urinary. For higher chlorinated congeners such as penta- and hexachlorobiphenyls, the predominant route of excretion is via the feces (up to 60% of total excretion); for lower chlorinated congeners, the situation seems to reverse (Lutz and Dedrick 1987). Mainly metabolites are found in urine and bile, although small amounts of parent compound may appear in the feces, in particular congeners that are poorly metabolized such as 2,2N,4,4N,5,5N-hexaCB. Elimination kinetics tend to follow first-order processes with elimination rates directly related to their metabolic rates (Gage and Holm 1976). An important route of PCB elimination is milk. This varies considerably with the species due to volume and lipid content of the milk, but the basic mechanisms are

the same for all species. Because PCBs are in dynamic equilibrium with all tissues, they move passively from blood to milk at the beginning of lactation to maintain their respective tissue/blood ratios.

PCBs are metabolized by microsomal cytochrome P-450 to polar metabolites that can undergo conjugation with glutathione and/or glucuronic acid. The rate of metabolism of some PCB congeners depends on (1) the degree of ring chlorination, (2) the chlorine ring substitution pattern, and (3) the pattern and levels of P-450 isozymes and other enzymes in the target tissue. PCB congeners of low chlorine content are transformed into hydroxylated derivatives that are predominately eliminated in the urine. Highly chlorinated congeners with nonsusceptible substitution patterns are either retained or excreted unchanged in the feces. Extensive information regarding the mechanism of metabolism of PCBs is provided in Section 3.4.3.

Because of the many factors that may determine the toxic response associated with exposure to PCBs, caution should be exercised when extrapolating high-dose response to low-dose responses, and/or single-dose exposures to chronic exposures. Caution is warranted for two main reasons. First, the dynamic mechanism involved in the distribution of PCB congeners, in which lipophilicity plays a crucial role, will influence the amount of circulating PCBs. For example, one can predict that because lean individuals have a smaller fat compartment, all of their body tissues will have higher concentrations of PCBs than those in fatter individuals of the same exposure. Also, the dosing schedule (single compared to repeated) will determine whether steady-state is achieved. Secondly, because PCBs can induce their own metabolism, data obtained with exposure levels associated with a significant induction of CYP1A1 and CYP1A2 may not necessarily reflect toxicokinetic behavior at low exposure levels. This has been illustrated in the model proposed by Brown (1994) in comparing high-exposed humans to low-exposed humans. In addition, serum residues of 2,2N,4,4N,5,5N-hexaCB are lower in prepubertal rats when the total of two doses is high enough to induce P-450 2B (Li et al. 1994). Toxicokinetic data for PCBs do not suggest route-dependent toxicity.

3.5.2 Mechanisms of Toxicity

Mechanisms by which the broad array of toxic effects observed in animals orally exposed to PCB mixtures develop are incompletely understood, but there is evidence to suggest that PCB congeners differ qualitatively and quantitatively in biological activities and that multiple and diverse mechanisms are involved in responses to PCB mixtures. Research in the 1970s and 1980s focused on mechanistic similarities between PCBs and CDDs involving initial mediation of effects by the Ah receptor (Poland

and Knutson 1982; Safe 1990, 1994), but research through the 1990s has found increasing evidence for the involvement of alternative mechanisms for several PCB-induced effects (Chauhan et al. 2000; Cheek et al. 1999; Fischer et al. 1998; Hansen 1998; Harper et al. 1993a, 1993b; Safe 1994; Tilson and Kodavanti 1998). An in-depth and all-inclusive review of the many recent and ongoing research efforts regarding PCB mechanisms of action is outside of the scope of this profile; rather, an overview of this large body of research is presented with the intent of providing information relevant to public health issues.

PCB Effects Involving Ah-receptor Dependent Mechanisms

Induction of Hepatic CYP1A Oxygenases and Phase II Enzymes. PCBs induce hepatic Phase I enzymes (CYP oxygenases) and Phase II enzymes (e.g., UDP glucuronyltransferases, epoxide hydrolase, or glutathione transferase) to varying degrees and specificities (Connor et al. 1995; Hansen 1998; Safe 1994). The demonstration of relationships between PCB molecular structure and induction of CYP isozymes has provided a framework within which much mechanistic research has been conducted. In general, commercial mixtures of PCBs induce both 3-methylcholanthrene-type (CYP1A1 and 1A2) and phenobarbital-type (CYP2B1, 2B2, and 3A) CYPs. Strong structure-activity relationships have been demonstrated between CYP1A1/1A2 induction in rodents and non-*ortho* and mono-*ortho* PCBs, which can assume a coplanar molecular configuration and bind to the Ah receptor (Connor et al. 1995; Hansen 1998; Safe 1994). In structure-activity studies of CYP1A induction in hepatocytes from Cynomolgus monkeys by 20 PCBs varying in degree and pattern of chlorine substitution (4–7 chlorines), the most potent inducers were without *ortho* chlorines (van der Burght et al. 1999). Many PCBs with *ortho* chlorines (mono-, di-, tri-, and tetra-*ortho* congeners) displayed no CYP1A induction activity, but a few mono-*ortho* and multiple-*ortho* congeners displayed activities that were about 1,000- and 10,000-fold less than the most potent non-*ortho* congeners, respectively (van der Burght et al. 1999). A working mechanistic hypothesis involves initial binding of coplanar PCBs to the Ah receptor in the cytosol of target cells, transport of the ligand-receptor complex to the nucleus, and subsequent changes in gene expression (e.g., induction of CYP1A1/1A2) leading to toxic responses via subsequent and/or parallel molecular mechanisms that are largely unexplored. Support for this hypothesis comes from the similarity in the array of PCB effects compared with the array produced by 2,3,7,8-TCDD and related halogenated aromatic hydrocarbons via initial Ah-receptor mediation, results from *in vitro* binding studies, and results from congener-specific *in vivo* studies of specific end points (e.g., enzyme induction and down regulation, body weight, and immunological responses to SRBC) in mouse strains and rat genders differing in responsiveness to Ah-receptor mediation (Hori et al. 1997; Safe 1990, 1994).

The complexity of Ah-receptor mediated effects on hepatic enzyme levels is illustrated by results from a study with mouse strains differing in Ah-receptor responsiveness and three PCB congeners (Hori et al. 1997). Ah responsive (C57BL/6) and Ah nonresponsive (DBA/2) mice were given single intraperitoneal doses of 3,3',4,4',5-pentaCB (a congener with high Ah receptor affinity), 3,3',4,4'-tetraCB (a congener with lesser affinity), and 2,2',5,5'-tetraCB (a low-affinity ligand). Only the high-affinity 3,3',4,4',5-congener produced body weight wasting in the dose range tested (up to 50 mg/kg) in Ah-responsive C57BL/6 mice, and this effect was accompanied by a decrease in selenium-dependent glutathione peroxidase and an increase in θ glutathione *S*-transferase. The effect on levels of these Phase II enzymes was not produced by the other congeners in C57BL/6 mice, and did not occur in DBA/2 mice exposed to any of the congeners, indicating the involvement of Ah-receptor mediation. These Phase II enzymes both play protective roles in scavenging intracellularly generated peroxides and the balance of their activities is likely to influence a cell's ability to withstand damage from peroxides.

Body Weight Wasting, Thymic Atrophy, and Porphyria. In addition to induction of hepatic levels of CYP1A1/1A2/1B1 and induction or repression of some Phase II enzymes, PCB-induced effects that appear to predominately involve Ah-receptor initiated mechanisms include body weight wasting and thymic atrophy from acute exposure (Hori et al. 1997; Safe 1994) and porphyria and porphyria cutanea tardea (Franklin et al. 1997; Smith et al. 1990a, 1990b). For example, single intraperitioneal doses of 5 mg/kg 3,3',4,4',5-pentaCB, a potent inducer of CYP1A1 and a high-affinity Ah-receptor agonist (relative to other PCBs), produced marked body weight wasting in Ah-responsive C57BL/6 mice, but not in DBA/2 mice that have a low-affinity Ah-receptor (Hori et al. 1997). Showing a link between Ah-receptor responsiveness and development of uroporphyria, female F344 rats had significantly higher hepatic levels of porphyrins and ethoxyresorufin deethylase activity (an indicator of CYP1A1) in response to exposure to 0.005% Aroclor 1254 in the diet for 15 weeks than did male rats (Smith et al. 1990b). A similar gender-specific correlation between porphyrinogenic response and CYP1A induction was observed in iron-loaded F344 rats exposed to single intraperitoneal doses of 63 mg Aroclor 1254/kg (Franklin et al. 1997). In mice of the Ah-responsive C57BL/6 strain, a single dose of iron-dextran (600 mg Fe/kg), followed by feeding of a diet containing 0.01% Aroclor 1254 for up to 12 months, produced markedly increased hepatic levels of porphyrins and liver enlargement, but this response to iron and Aroclor 1254 was not observed in similarly treated DBA/2 mice (Smith et al. 1990a). Exposure to iron-dextran alone caused a moderate porphyria in C57BL/6 mice, but not in DBA/2 mice, lending support to a postulate that there are constitutive genetic differences between these strains that influence porphyria development and do not involve Ah-receptor mediation (Smith et al. 1990a). One mechanistic hypothesis proposes that induction of CYP1A2 by the Ah-receptor-PCB complex leads to generation of a

competitive inhibitor of uroporphyrinogen decarboxylase in the liver and subsequent accumulation of porphyrins (see Franklin et al. 1997).

Ah Receptor TEF Approach to Health Hazard Assessment. A TEF approach to evaluating health hazards from exposure to complex environmental mixtures containing PCBs, CDDs, and CDFs has been developed and used to some extent to guide public health decisions because humans are exposed to complex and varying mixtures of these halogenated aromatic hydrocarbons and there are limited toxicological data for these complex mixtures and many of their components (ATSDR 1998; Safe 1990, 1994; van den Berg et al. 1998). PCBs were included in this component-based approach because (1) the spectrum of effects in animals exposed to some PCB mixtures and congeners is similar to the spectrum produced by 2,3,7,8-TCDD (via Ah receptor initial mediation) and (2) coplanar PCBs display Ah receptor binding affinities that were related to their potency in producing health effects in rodents such as body weight wasting and inhibition of immunological responses to SRBC (Safe 1990, 1994). The TEF approach compares the relative potency of individual congeners, based on *in vitro* or acute *in vivo* data, with that of 2,3,7,8-TCDD, the best-studied member of this chemical class, so that the TEF for 2,3,7,8-TCDD is 1. The concentration or dose of each active component in a mixture of concern is multiplied by its TEF to arrive at a TEQ, and the TEQs are added to give the total toxic equivalency of the mixture which is compared with reference exposure levels for 2,3,7,8-TCDD expected to be without significant risk for producing health hazards. TEFs have recently been recommended by the World Health Organization for 7 CDD, 10 CDF, and 12 PCB congeners (Van den Berg et al. 1998).

Limitations in using the TEF approach for assessing health hazards from PCB-containing environmental media revolve around the inherent assumptions that the components jointly act in an additive manner through a common Ah-receptor initial mechanism and the evidence that Ah-receptor-binding congeners in PCB-containing environmental mixtures are minor components (Hansen 1998; Safe 1998a, 1998b) Several studies have provided evidence of nonadditive interactions between specific PCB congeners and between some PCB congeners and 2,3,7,8-TCDD (Safe 1998a, 1998b), and there is evidence, discussed below, that several Ah-receptor-independent mechanisms may make contributions to toxic effects from PCB mixtures.

PCB Effects Involving Ah-receptor Independent Mechanisms

Induction of Hepatic CYP2B Oxygenases. In contrast to the distinct relationships between CYP1A1/1A2 induction, PCB molecular structures, and Ah-receptor initiation of toxic effects,

relationships between potency in inducing CYPs 2B1/2B2/3A, PCB structural properties, and toxic effects are less clear (Connor et al. 1995). For example, some PCBs with two *ortho* chlorines and lateral chlorines induce both types of CYPs and display a very small affinity for the Ah receptor, whereas other di-*ortho* PCBs with one or two para chlorines predominately induce CYP2B1/2B2/3A and have no measurable affinity for the Ah receptor (Connor et al. 1995; Hansen 1998). Some noncoplanar congeners, such as 2,2',4,4'-tetraCB, also induce CYP3A through the glucocorticoid-sensitive pregnane X receptor (PXR) (Schuetz et al. 1986, 1998). It is clear that PCB induction of phenobarbital-type CYPs is independent of the Ah receptor and that the most potent inducers of CYP have at least two *ortho* chlorines and one or two para chlorines.

Other PCB-induced effects involving Ah-receptor independent mechanisms include neurological and neurodevelopmental effects involving changes in brain dopamine levels (Seegal 1996b, 1998; Seegal et al. 1989, 1990; Shain et al. 1991); inhibition of dopamine vesicular uptake (Mariussen et al. 1999) and/or changes in brain cell intracellular calcium homeostasis and related signal transduction processes (Kodavanti and Tilson 1997; Tilson and Kodavanti 1997, 1998; Tilson et al. 1998; Wong and Pessah 1996, 1997; Wong et al. 1997); tissue injury related to activation of neutrophils (Brown and Ganey 1995; Ganey et al. 1993; Tithof et al. 1995); thyroid disruptions not involving UDP-GT induction (Chauhan et al. 2000; Cheek et al. 1999; Darnerud et al.1996a; Li and Hansen 1996; Ness et al. 1993; Van Birgelen 1992); and PXR and ryanodine receptor (RyR) mediated mechanisms (Schuetz et al. 1986, 1998).

Brain Dopamine Levels and Neurological Effects. Aroclor 1254 decreased cellular levels of dopamine in cultured pheochromocytoma cells, which synthesize, store, release, and metabolize dopamine in a manner similar to the intact mammalian central nervous system (Seegal et al. 1989). Daily oral exposure of adult nonhuman primates (*Macaca nemestrina*) to Aroclor 1016, a commercial mixture of lightly chlorinated PCB congeners, for 20 weeks, likewise, produced decreased dopamine concentrations in brain regions including the caudate, putamen, substantia nigra, and hypothalamus (Seegal et al. 1990). In these brain regions, only three PCB congeners were detected (2,4,4'-triCB and 2,2',4,4'- and 2,2',5,5'-tetraCB), suggesting that nonplanar PCBs, which are poor Ah receptor agonists, may have been responsible for the effect. Structure-activity studies of 50 PCB congeners in the pheochromocytoma *in vitro* system found that the most active congeners had at least two *ortho* chlorines (e.g., 2,2',4,6-, 2,2',5,5'-, and 2,2',4,5-tetraCB) and that congeners that were relatively strong Ah receptor agonists (e.g., 3,3',4,4'-tetraCB and 3,3',4,4',5-pentaCB) were inactive or had minimal effects on dopamine levels (Shain et al. 1991). However, *ortho* substitution was not the sole determinant of activity in this system; for example, a congener with four *ortho* chlorines (2,2',6,6'-tetraCB) had no effect on dopamine levels in

pheochromocytoma cells (Shain et al. 1991). The effect on dopamine levels has been postulated to involve decreased dopamine synthesis via direct or indirect PCB inhibition of tyrosine hydroxylase (Choksi et al. 1997; Seegal et al. 1996b) or L-aromatic amino acid decarboxylase (Angus et al. 1997) and/or decreased uptake of dopamine into vesicles (Mariussen et al. 1999). For example, several congeners that were inactive in causing dopamine level changes in pheochromocytoma cells (e.g., 2,2',6,6'- and 3,3',4,4'-tetraCB) were much less active in inhibiting vesicular uptake of dopamine than other more active congeners (e.g., 2,2',4,6- and 2,2',4,5'-tetraCB) (Mariussen et al. 1999).

Disruption of Ca^{+2} Homeostasis and Neurological Effects. Neurological and/or neurodevelopmental effects from exposure to PCBs also have been hypothesized to involve interference with calcium homeostatic mechanisms and intracellular second messenger systems by PCB congeners that are not effective Ah receptor agonists (see reviews by Kodavanti and Tilson 1997; Tilson and Kodavanti 1998; Tilson et al. 1998). In agreement with structure-activity relationships observed for PCB effects on dopamine levels in pheochromocytoma cells (Shain et al. 1991), 2,2'-diCB altered intracellular calcium homeostasis in cultured rat cerebellar granule cells (increased free calcium levels and inhibited calcium buffering systems) at noncytotoxic exposure concentrations (higher concentrations were cytotoxic) (Kodavanti et al. 1993). In contrast, 3,3',4,4',5'-pentaCB, one of the most effective Ah receptor agonists among tested PCBs (Safe 1994), was not cytotoxic in the tested concentration range and did not alter calcium homeostasis to as great an extent as 2,2'-diCB (Kodavanti et al. 1993). Using phorbol ester binding in rat cerebellar granule cells as a measure of protein kinase C translocation (which is thought to play key roles in cellular signal transduction in neurons and be regulated by several intracellular factors including intracellular levels of free calcium), commercial mixtures of PCBs (Aroclors 1016, 1254, and 1260) were shown to increase protein kinase C translocation in a concentration-dependent manner with varying potencies (Kodavanti et al. 1995). Aroclors 1016 and 1254 were more potent than Aroclor 1260. Examination of 24 PCB congeners showed that the most potent congeners (e.g., 2,2'-diCB, 2,2',5,5'-tetraCB, and 2,2',4,6,6'-pentaCB) had multiple *ortho* chlorines, whereas congeners without *ortho* chlorines tended to have either no or lower activities (Kodavanti et al. 1995). Similar results were found in structure-activity studies of 24 PCB congeners and their effects on *in vitro* Ca^{+2} sequestration by microsomes and mitochondria from freshly isolated rat cerebellar cells (Kodavanti et al. 1996a). Structure activity relationships for PCB congeners and protein kinase C translocation in rat cerebellar granule cells and Ca^{+2} sequestration were similar to relationships for PCB congener-induced changes in dopamine levels in pheochromocytoma cells. For example, 2,2',5,5'- and 2,2',4,6-tetraCB were among the most potent congeners and 2,2',6,6'- and 3,3',4,4'-tetraCB were inactive in all three systems (Kodavanti et al. 1995, 1996a; Shain et al. 1991).

One proposed molecular target for PCB disruption of calcium homeostasis that may be involved in neurological and neurodevelopmental effects is ryanodine-sensitive Ca^{+2} channels. Commercial PCB mixtures with intermediate to high degrees of chlorination (Aroclors 1248, 1254, and 1260) enhanced ryanodine binding to calcium release channels in sarcoplasmic reticulum membranes from skeletal or cardiac rabbit muscles, and mixtures with lower (Aroclors 1221, 1232) or higher (Aroclor 1268) chlorination showed little enhancement (Wong and Pessah 1996). Examination of selected pentachlorobiphenyls indicated that *ortho* substitution favored activity; 2,2',3,5',6-pentaCB induced the greatest enhancement of ryanodine binding, whereas the 3,3',4,4',5-isomer did not enhance binding (Wong and Pessah 1996). The 2,2',4,6,6'-isomer with full substitution at the *ortho* positions produced less enhancement than the 2,2',3,5',6-isomer, indicating that some degree of rotation about the biphenyl bond may be important for full activity. Results from studies with hippocampal slices from freshly dissected rat brains indicated that perfusion with a tri-*ortho* congener (2,2',3,5',6-pentaCB) enhanced ryanodine binding and inhibited electrophysiological responses to electrical pulse stimulations, but a mono-*ortho* congener (2,3',4,4'-tetraCB) showed no enhancement of ryanodine binding and no inhibition of electrophysiological responses to stimulation (Wong et al. 1997). Offspring of rats exposed to gavage doses of 8 or 32 mg/kg/day 2,2',3,5',6-pentaCB on gestation days 10–16 displayed neurobehavioral changes as adults (depressed open field locomotor activity, faster acquisition on a working memory task, and no changes in a delayed spatial alternation task) and changes in ryanodine binding to calcium channels in specific regions of the brain (e.g., decreased in hippocampus and increased in cerebral cortex) (Schantz et al. 1997). Although it is not understood how these changes in ryanodine binding are specifically related to the observed neurobehavioral changes, the results from this series of studies emphasize the potential importance of Ah receptor independent mechanisms in PCB-induced neurological and neurodevelopmental effects.

Neutrophil Function and Immunological Effects and Tissue Damage. PCB-induced functional changes in neutrophils may be involved in impaired immune defenses against pathogens or enhanced inflammatory responses (e.g., production of reactive oxygen species and cytolytic enzymes) leading to tissue injury. Incubation of quiescent cultured rat peritoneal neutrophils with Aroclor 1242 stimulated neutrophil production of superoxide anion and induced degranulation in a concentration-dependent manner without producing cytotoxicity (Ganey et al. 1993). In neutrophils that were activated for these functions, Aroclor 1242 produced further increases in superoxide anion production, but inhibited the activated degranulation process. Similar effects were observed when neutrophils were incubated with 2,2',4,4'-tetraCB, a congener that has little affinity for the Ah receptor and induces phenobarbital-type CYPs, but 3,3',4,4'-tetraCB, an Ah receptor agonist and inducer of 3-methylcholanthrene-type CYPs, did

not affect neutrophil function (Ganey et al. 1993). The effects of 2,2',4,4'-tetrachlorbiphenyl on *in vitro* production of superoxide anion by neutrophils were inhibited when neutrophils were incubated in the absence of extracellular calcium or in the presence of TMB-8, an antagonist of the intracellular mobilization of calcium (Brown and Ganey 1995). In addition, neutrophil degranulation induced by 2,2',4,4'-tetraCB was enhanced by coexposure with the calcium ionophore A23187 (Brown and Ganey 1995). A mono-*ortho* congener, 2,3,4,5-tetraCB, displayed somewhat different effects on neutrophil functions than those from the 2,2',4,4'-congener: it stimulated degranulation in quiescent and activated neutrophils, but only increased superoxide anion production in activated neutrophils, not in quiescent cells. The results from the neutrophil studies suggest the involvement of an Ah-receptor independent mechanism that involves PCB-induced increases in intracellular calcium or PCB effects on a signal transduction pathway that is dependent on calcium availability (Brown and Ganey 1995). Recent work suggests activation of phospholipase A2, release of arachidonic acid from triglycerides, and production of prostaglandins as a probable mechanism (Tithof et al. 1996; Olivero and Ganey 2000). This mechanism could also contribute to other pathologies.

PCB Effects Involving Ah-receptor Dependent and Independent Mechanisms

PCB-induced effects that may involve both Ah-receptor dependent and independent mechanisms include liver hypertrophy (Hori et al. 1997); neurodevelopmental effects or reproduction effects involving changes in steroid hormone homeostasis (Arcaro et al. 1999; Connor et al. 1997; Gierthy et al. 1997; Fischer et al. 1998; Li and Hansen 1997; Nesaretnam and Dabre 1997; Nesaretnam et al. 1996; Seegal et al. 1997) and/or thyroid hormone disruption (Brouwer et al. 1998b; Hansen 1998; Li and Hansen 1996a, 1996b, 1997); immunological effects (Harper et al. 1993a, 1993b; Silkworth and Grabstein 1982; Stack et al. 1999); and cancer through nongenotoxic mechanisms involving promotion of oncogenic cells (Cogliano 1998; Safe 1994) and/or genotoxic mechanisms (Robertson and Gupta 2000).

Liver Hypertrophy. Liver hypertrophy in animals is produced by oral exposure to commercial PCB mixtures and appears to involve both Ah-receptor dependent and independent mechanisms. An illustration of this phenomenon is the observation that single intraperitoneal doses of any one of three PCB congeners varying in affinity for the Ah receptor produced liver hypertrophy in Ah responsive (C57BL/6) and Ah nonresponsive (DBA/2 mice (Hori et al. 1997). The studied congeners were 3,3',4,4',5-pentaCB, a congener with high Ah receptor affinity, 3,3',4,4'-tetraCB, a congener with lesser affinity, and 2,2',5,5'-tetraCB, a low-affinity Ah-receptor ligand.

Reproductive Effects. There are several studies examining female reproductive function variables in rats (Brezner et al. 1984; Hany et al. 1999b; Linder et al. 1974; Sager and Girard 1994), mice (Welsch 1985), rabbits (Seiler et al. 1994), minks (Aulerich and Ringer 1977; Backlin and Bergman 1995; Kihlstrom et al. 1992), and monkeys (Arnold et al.1995, 1996; Barsotti et al. 1976) repeatedly exposed orally to commercial PCB mixtures, predominately Aroclor 1254. In general, results from these studies identify minks and monkeys as sensitive species.

In minks, repeated exposure to low doses of Aroclor 1254 or Clophen A50 (0.4–1.8 mg/kg/day) caused reproductive failure that has been associated with fetal death following embryo implantation (Aulerich and Ringer 1977; Backlin and Bergman 1995; Backlin et al. 1997; Kihlstrom et al. 1992). This effect may predominately involve Ah-receptor mediation, as evidenced by observations that only 1/10 minks exposed to 2.5 ppm Aroclor 1254 in the diet from 1 month prior to breeding through parturition produced offspring, whereas exposure by a similar protocol to 2,2',4,4',5,5'-hexaCB or 2,2',3,3',6,6'-hexaCB at concentrations up to 5 ppm did not influence reproductive performance (Aulerich et al. 1985). In contrast, exposure to dietary concentrations as low as 0.1 ppm 3,3',4,4',5,5'-hexaCB in this study (Auerlich et al. 1985), and 0.05 ppm in another study (Aulerich et al. 1987), caused mortality and prevented the minks for reproducing. Dietary exposure of minks to a fraction of Aroclor 1254, containing only congeners with no *ortho*-chlorines or a single *ortho*-chlorine and representing <20% of the total weight of Aroclor 1254, reduced litter size and fetal survival, and increased incidence of interrupted pregnancies to a similar degree as doses of the complete Aroclor 1254 mixture (1.3 mg/kg/day) containing the same amount of these congeners (Kihlstrom et al. 1992). These results suggest the importance of Ah-receptor mediation of PCB-induced reproductive impairment in minks.

Another mink study comparing reproductive effects from intraperitoneal doses of 2,2',4,4',5,5'- and 3,3',4,4',5,5'-hexaCB reinforces the idea that congeners with high Ah-receptor affinity are more potent than congeners with low Ah-receptor affinity, but also provides evidence that Ah-receptor independent mechanisms may be involved (Patnode and Curtis 1994). Administration of single 20-mg/kg doses of the 2,2',4,4',5,5'-isomer (a poor Ah-receptor agonist that has been detected in wild mink tissues at concentrations 50-fold greater than the 3,3',4,4',5,5'-isomer) to pregnant minks on the approximate date of implantation did not affect the number of implantation sites (assayed 14 days after dose administration), but significantly decreased the number of embryos and embryonic weight, crown-to-rump length, and head length. The 3,3',4,4',5,5'-isomer (at lower dose levels of 0.4 or 0.8 mg/kg) also did not affect the number of implantation sites, but produced more severe effects on embryo survival and the weight, crown-to-rump length, and head length of surviving embryos (Patnode and Curtis 1994).

The mechanisms involved in PCB-induced reproductive impairment in minks are unknown, but examination of mid- to late-gestation placentae from minks exposed to Clophen A50 by light and electron microscopy revealed degenerative lesions in maternal (endothelial detachment and thrombosis in maternal vessels) and fetal (trophoblastic disintegration and loss of fetal capillary integrity) tissues (Backlin et al. 1998b). Jones et al. (1997) postulated that the mechanisms are likely to be multifactorial given the possibility of direct and/or indirect tissue damaging actions of PCBs and the wide range of reported effects of PCBs on steroid hormone synthesis and functions including PCB regulation of CYP oxygenases that activate or deactivate different endogenous steroid hormones, estrogenic and antiestrogenic effects of PCBs, and PCB regulation of estrogen and progesterone receptor levels (see Battershill 1994; Li and Hansen 1997; Patnode and Curtis 1994).

Impaired ability to conceive and decreased fetal survival have been observed following repeated exposure of female Rhesus monkeys to commercial PCB mixtures. Exposure to dietary levels of 2.5 or 5 ppm Aroclor 1248 (approximately 0.1 or 0.2 mg/kg/day) for 16–19 months (including a 7-month period before breeding with nonexposed males) produced resorptions or abortions in 3/8 and 4/6 impregnated female Rhesus monkeys, compared with 0/12 in a control group (Barsotti et al. 1976). In this study, 12/12, 8/8, and 6/8 females became impregnated in the 0-, 2.5-, and 5-ppm groups, respectively. Another study fed encapsulated Aroclor 1254 at dose levels of 0, 0.005, 0.02, 0.04, or 0.08 mg/kg/day to female Rhesus monkeys for 37 months before breeding with nonexposed males and continued dosing through mating and gestation (Arnold et al. 1995). Incidences of abortions, resorptions, or stillbirths were 2/11, 5/10, 3/4, 2/6, and 4/5 in impregnated monkeys in the control through high-dose groups, respectively; respective incidences for impregnation success were 11/16, 10/16, 4/15, 6/14, and 5/15 (Arnold et al. 1995). Mechanisms for these effects in monkeys are unknown, but microscopic examination of tissues from control and exposed monkeys in the second monkey study found no evidence for an association with endometriosis (Arnold et al. 1996).

The plausibility that PCB effects on reproductive function (and other functions such as neurobehavior and immunological competence) may involve PCB effects on endocrine functions has led to investigations of the estrogenic and anti-estrogenic activities of PCB mixtures and individual congeners, and the effects of PCBs or related halogenated aromatic compounds on steroid hormone metabolism via induction of Phase I or Phase II enzymes. How these PCB effects are specifically related to PCB effects on reproductive function is unknown, but the results of these investigations provide further evidence that reproductive effects from PCB mixtures may not be restricted to Ah-receptor mediation alone and are

likely to involve multiple mechanisms that have yet to be elucidated. Related information on endocrine disruption is discussed in Section 3.6.

The estrogenic and anti-estrogenic activities of some commercial PCB mixtures, PCB congeners, and hydroxylated derivatives of PCB congeners have been assayed by examining uterine variables in immature or ovariectomized female rodents, cell proliferation or gene expression variables in cultured cells including human breast cancer or HeLa cells, and *in vitro* binding to estrogen receptor preparations (see Andersson et al. 1999; Arcaro et al. 1999; Battershill 1994; Connor et al. 1997; Gierthy et al. 1997; Hansen 1998; Kramer et al. 1997; Krishnan and Safe 1993; Li and Hansen 1997; Moore et al. 1997; Safe 1999; Safe et al. 1998b for reviews). In general, (1) PCB-induced estrogenic activities have been characterized as weak compared to the endogenous hormone, 17β-estradiol, (2) a wide variability of responses has been observed across types of PCBs and assays indicating the involvement of multiple mechanisms (e.g., direct binding to the estrogen receptor is not the only way that estrogenic or anti-estrogenic physiological effects may be mediated), (3) anti-estrogenic activities have been most strongly associated with PCBs that are Ah-receptor agonists, and (4) hydroxylated metabolites of PCBs are postulated to be at least partly responsible for physiological responses to PCBs that may involve changes in estrogen receptor-dependent physiological processes. Recent demonstrations that hydroxy PCBs inhibit hydroxy steroid sulfotransferase suggest that PCB metabolites indirectly exert estrogenic activity via inhibition of estradiol metabolism (Kester et al. 2000).

Early studies showed that subcutaneous administration of 8 mg of Aroclors 1221, 1232, 1242, or 1248 increased uterine weight and glycogen content in rats, but similar exposure to Aroclors 1254, 1260, 1262, or 1268 did not produce this estrogenic effect (Bitman and Cecil 1970). More recent studies have provided further evidence that PCB mixtures can produce estrogenic responses (albeit weak) and that PCB congeners with multiple *ortho* chlorines (or their hydroxylated metabolites) may be at least partly responsible for these responses. Intraperitoneal doses of Aroclor 1242 (8 mg/rat on day 20 or 0.08 or 0.32 mg/rat on days 20 and 21) significantly increased uterine wet weight in immature female rats to about 40% of the increase produced by 0.001 mg 17β-estradiol (Jansen et al. 1993). Similar increases in uterine wet weight were produced by exposure to di-*ortho* congeners or hydroxylated derivatives (0.640 mg 2,2',5,5'-tetraCB or 0.250 mg 2,4,6-trichloro-4'-hydroxy-biphenyl on days 20 and 21), but not by exposure to a coplanar congener without *ortho* chlorines (0.160 mg 3,3',4,4'-tetrachlorobiphenyl). In another study, the tetra-*ortho* congener, 2,2',6,6'-tetraCB, displayed similarly weak estrogenic responses in an *in vitro* human breast cancer cell assay and an *in vivo* immature female rat assay (Arcaro et al. 1999). This congener did not competitively bind *in vitro* to recombinant human estrogen receptors α and

β, but a hydroxylated metabolite, 2,2',6,6'-tetrachloro-4'-hydroxy-biphenyl, competitively bound to estrogen receptor α and produced proliferative responses in the breast cancer assay at concentrations about 10-fold lower than effective concentrations of the parent molecule (Arcaro et al. 1999).

Combined exposure of immature rats to 0.32 mg Aroclor 1242 and 0.001 mg 17β-estradiol produced a response similar to estradiol alone, indicating no obvious anti-estrogenic activity, but combined exposure to 0.001 mg estradiol and 0.160 mg 3,3',4,4'-tetraCB markedly diminished the estradiol-induced increase in uterine wet weight (Jansen et al. 1993). Anti-estrogenic effects similar to those from 3,3',4,4'-tetraCB were also observed in rodent uterine tissue (Astroff and Safe 1990) and human breast cancer cells (Krishnan and Safe 1993) by other congeners with no or single *ortho* chlorines (e.g., 3,3',4,4',5-pentaCB, 2',3,3',4,4',5-hexaCB), but commercial PCB mixtures were not anti-estrogenic in the breast cancer cell assay. Whereas the data collected by Krishnan and Safe (1993) suggest that anti-estrogenic activities of PCBs may be related to Ah receptor binding affinity of nonmetabolized PCBs, anti-estrogenic activities of hydroxylated metabolites of PCBs with no *ortho* chlorines, with single *ortho* chlorines, or with multiple *ortho* chlorines have been observed in in several assay systems (Connor et al. 1997; Moore et al. 1997; Nesaretnam et al. 1996; Safe et al. 1998b). Thus, whether or not a specific PCB mixture will be anti-estrogenic appears to be at least partly dependent on the chlorine substitution pattern of the parent PCBs and on the degree of formation of hydroxylated metabolites.

Structure-activity relationships for estrogenic activities of PCB congeners or their metabolites are less clear. Some hydroxylated PCBs (2,4,6-trichloro-4'-hydroxy-biphenyl and 2,3,4,5-tetrachloro-4'-hydroxy-biphenyl) have been demonstrated to competitively bind to mouse estrogen receptor preparations and to increase uterine weight and glycogen in immature mice (Korach et al. 1988). In other estrogenic assays, 2,2',4,4',6-tetraCB, 2,4,4',6-tetrachloro-4-hydroxy-biphenyl, and 2,4,6-trichloro-4'-hydroxy-biphenyl were equally effective in stimulating proliferation of human breast cancer cells, but only 2,4,6-trichloro-4'-hydroxy-biphenyl caused significant induction of vitellogenin in cultured brown trout hepatocytes (Andersson et al. 1999). A structure-activity study of eight hydroxylated PCBs in a series of *in vivo* and *in vitro* estrogenic assays found that structure activity relationships were complex and differed from one assay to the next (Connor et al. 1997; Safe et al. 1998b). For example, all but one of the compounds displayed competitive binding to rat and mouse cytosolic estrogen receptors (affinities ranged from about 10^{-3} to 10^{-5} of 17β-estradiol's affinity), but no estrogenic activities (wet weight, peroxidase activity, progesterone receptor level) were produced in the uteri of immature rats and mice exposed to three consecutive daily doses of the individual hydroxylated PCB congeners at levels of 25, 50, or 100 mg/kg.

In contrast, four of the hydroxylated congeners produced estrogenic effects in cultured human breast cells and HeLa cells (Connor et al. 1997; Safe et al. 1998).

Complex effects on male reproductive organs and functions have been observed in animals exposed to commercial PCB mixtures including reduced testes weight in adult male offspring of guinea pigs exposed during gestation to Clophen A50 (Lundkvist 1990), reduced testes weight in adult male offspring of female rats exposed from 50 days prior to mating through birth of offspring to 4 mg/kg/day Aroclor 1254 or a mixture of PCBs reflective of the composition of human milk samples (Hany et al. 1999b), reduced fertility (without changes in reproductive organ weights, sperm production, or sperm morphology) in adult male offspring of female rats exposed to doses of 8 mg/kg Aroclor 1254 and higher on lactation days 1, 3, 5, 7, and 9 (Sager et al. 1987, 1991), and elevated testes weight and increased sperm production in adult rats exposed to subcutaneous doses of Aroclor 1242 or 1254 (10–80 mg/kg/day) on postnatal days 0–25 (Cooke et al. 1996). Mechanisms involved in these effects on male reproductive organ development are unknown, but have been postulated to involve developmentally specific periods of responsiveness such as long-lasting elevation of testosterone-metabolizing enzymes from *in utero* exposure leading to reduced testes weight (Hany et al. 1999b) and continued depression of thyroid hormone levels during the neonatal period leading to Sertoli cell proliferation and increased testes weight (Cooke et al. 1996).

Disruption of Thyroid Hormone Homeostasis. Concern that the thyroid hormone system may be important in PCBs mechanisms of toxicity stems from mainly two important types of observations (Brouwer et al. 1998b; Porterfield and Hendry 1998): (1) extensively corroborated findings in experimental animals that exposure to PCBs *in utero* and/or during early development (e.g., through breast milk) can deplete levels of circulating thyroid hormone in the fetus or neonate, which may give rise to effectively a hyopothyroid state during development (Collins and Capen 1980c; Cooke et al. 1996; Corey et al. 1996; Darnerud et al. 1996a; Goldey et al. 1995; Juarez de Ku et al. 1994; Li et al. 1998; Morse et al. 1996c; Rice 1999a; Provost et al. 1999; Schuur et al. 1998a; Seo and Meserve 1995; Zoeller et al. 2000); and (2) the recognition of the importance of thyroid hormones in normal development of the brain, as evident from neurodevelopmental disorders and deficits associated with hypothyroidism (Boyages 2000). The latter are typified by iodine deficiency (e.g., endemic cretinism), which can produce a wide range of neurodevelopmental deficits, including auditory, motor, and intellectual deficits. These outcomes suggest an importance of thyroid hormones in the normal development of the fetal cochlea, basal ganglia, and cerebral cortex, which begin to develop in humans during the second trimester of gestation. This is also the time in which the fetal thyroid gland becomes functional.

Evidence for a potential thyroid hormone involvement in PCB toxicity rests largely on observations made in experimental animals, including rodents and nonhuman primates (see Section 3.2.2.8.3). Although the studies differ in design and, in particular, the emerging picture from these studies is that, depending on dose and duration, PCBs can disrupt the production and disposition of thyroid hormones at a variety of levels. The major findings include (1) histological changes in the thyroid gland indicative of both stimulation of the gland (e.g., similar to that induced by TSH or a hypothyroid state) and a disruption of the processing of follicular colloid needed for normal production and secretion thyroid hormone (Chu et al. 1994, 1995, 1996a, 1996b, 1998b; Collins and Capen 1980a; Collins et al. 1977; Hansen et al. 1995; Tryphonas et al. 1986b); (2) depression of serum T_4 and T_3 levels, which may effectively create a hypothyroid state (Byrne et al. 1987; Collins and Capen 1980c; Cooke et al. 1996; Corey et al. 1996; Darnerud et al. 1996a; Desauliniers et al. 1997; Goldey et al. 1995; Gray et al. 1993; Hansen et al. 1995; Hood et al. 1999; Juarez de Ku et al. 1994; Kasza et al. 1978; Li et al. 1998; Morse et al. 1996c; Price et al. 1988; Provost et al. 1999; Rice 1999a; Schuur et al. 1998a; Seo and Meserve 1995; Van Birgelen et al. 1995; Zoeller et al. 2000); (3) increased rates of elimination of T_4 and T_3 from serum (Goldey and Crofton 1998); (4) increased activities of T_4-UDP-GT in liver (Chu et al. 1995; Desauliniers et al. 1997; Morse et al. 1996c; Schuur et al. 1998a; Van Birgelen et al. 1995), which is an important metabolic elimination pathway for T_4 and T_3; (5) decreased activity of iodothyronine sulfotransferases in liver which are also important in the metabolic elimination of iodothyronines (Schuur et al. 1998a, 1998b, 1999); (6) decreased activity of iodothyronine deiodinases including brain Type-2 deiodinase, which provide the major pathways for the production of the active thyroid hormone, T_3 (Morse et al. 1996c; Schuur et al. 1998a); and (7) decreased binding of T_4 to transthyretin an important transport protein for both T_4 and T_3 (Cheek et al. 1999; Darnerud et al.1996a).

The above observations suggest that PCBs can disrupt the production of thyroid hormones, both in the thyroid and in peripheral tissues, can interfere with their transport to peripheral tissues, and can accelerate the metabolic clearance of thyroid hormones. The most convincing evidence that PCBs can exert toxicity by disrupting thyroid hormone system derives from two studies in rats. In one study, neurobehavioral deficits in pups that experienced exposures to Aroclor 1254 *in utero* and during nursing, were significantly attenuated by subcutaneous injections of T_4 that increased serum T_4 and T_3 concentrations that were otherwise depressed in the PCB-exposed animals (Goldey and Crofton 1998). While this study examined relatively high doses of Aroclor 1254 ($1 mg/kg/day), it nevertheless demonstrated neurodevelopmental effects that are directly relevant to observations made in epidemiological studies and to neurological sequelae of fetal hypothyroidism, including motor disturbances and hearing.

In the second study, increased testes weight and sperm production in rats that were administered Aroclor 1254 on postnatal days 1–25 were attenuated by injections of T_4 on postnatal days 1–25, which also prevented the depression in serum T_4 concentrations (Cooke et al. 1996). Here again, although produced by relatively large doses of Aroclor 1254 ($40 mg/kg/day, subcutaneous), similar effects can be produced by other hypothyroid-inducing agents, including PTU. Furthermore, the effects observed may reflect a disruption of the normal sexual maturation process, which is known to be associated with neonatal hypothyroidism in humans (Longcope 2000).

The effects PCBs on thyroid hormone status appear to involve Ah-receptor mediated or modulated actions as well as actions that appear to be independent of the Ah receptor. Depressed levels of serum T_4 have been observed in rats given oral doses of coplanar PCB congeners (Desauliniers et al. 1997; Price et al. 1999; Van Birgelen et al. 1994b) or di-*ortho*-substituted congeners that have relatively low affinity for the Ah receptor (Ness et al. 1993; Van Birgelen 1992). At least one potential Ah-receptor mediated mechanism for this effect is the induction of UDP-GT, which catalyzes the metabolic elimination of T_4 to the T_4-glucuronide conjugate (Desauliniers et al. 1997; Van Birgelen et al. 1995). However, the UDP-GT mechanism does not appear to be important in the depression of T_4 levels produced by non-coplanar PCBs. Li and Hansen (1996) observed depressed serum T_4 levels in rats administered a PCB mixture extracted from soil. Treatment of the mixture with activated charcoal greatly reduced the content of co-planar PCBs in the mixture, substantially decreased the potency of the mixture for inducing UDG-GT and EROD, but had little effect on the potency for depressing T_4 levels. This suggests that an Ah-independent mechanism may exist that is not related to UDP-GT induction.

PCBs, including poly-*ortho*-substituted PCBs, which have a very low affinity for the Ah receptor, inhibit the binding of T_4 to transthyretin, an important transport protein for both T_4 and T_3 (Chauhan et al. 2000; Cheek et al. 1999; Darnerud et al. 1996a). Inhibition of binding of thyroid hormones to transthryetin could alter hormone delivery to target tissues, including the brain, and could also result in depressed levels of serum total TT_4 or TT_3 (Brouwer et al. 1998).

Immunological Effects. Studies with inbred mice strains differing in Ah-receptor responsiveness indicate that immunosuppression from PCB mixtures involves Ah-receptor mediation (e.g., Silkworth and Grabstein 1982; Harper et al. 1993a), but there is evidence that other mechanisms also may contribute to PCB-induced immunological effects (Harper et al. 1993a, 1993b; Stack et al. 1999). Illustrating the importance of Ah-receptor mediation for some PCB congeners, Ah-responsive C57BL/6 mice given single intraperitoneal doses of 100 mg/kg 3,3',4,4'-tetraCB showed marked decreases in the number of

splenic PFCs formed in response to immunization with SRBCs (which are T-cell dependent antigens) compared with similarly treated Ah-nonresponsive DBA/2 mice (Silkworth and Grabstein 1982). In addition, ED_{50} values for 2,3,7,8-TCDD, three CDFs, and two PCBs without *ortho* substitution (3,3',4,4',5-pentaCB and 3,3',4,4',5,5'-hexaCB) in this immunotoxicity assay were lower in C57BL/6 mice than in DBA/2 mice, and the order of immunotoxic potency of these six compounds was the same as that for potency in inducing CYP1A1 (Harper et al. 1993a). In another study, a series of four hexachlorinated biphenyls with differing chlorine substitution patterns displayed varying ED_{50} values in the same immunotoxicity assay as follows: 2, >1,000, 120, and >1,000 µmol/kg for a mono-*ortho*- (2,3,3',4,4',5'-), di-*ortho*- (2,2',4,4',5,5'-), tri-*ortho*- (2,2',4,4',5',6-), and tetra-*ortho* (2,2',4,4',6,6'-)-isomer, respectively (Harper et al. 1993b). Harper et al. (1993b) concluded that immunotoxic potency decreases (i.e., ED_{50}s increase) with increasing *ortho*-chlorine substitution of PCBs, but, as shown above, the decrease was not monotonic with increasing degree of *ortho* chlorination. Furthermore, this relationship did not apply to more highly chlorinated PCBs with three or four *ortho* chlorines that are inactive as Ah-receptor agonists and only minimally induce CYP1A1 (Harper et al. 1993b). Three nonachlorobiphenyls (2,2',3,3',4,4',5,5',6-, 2,2',3,3',4,4',5,6,6'-, and 2,2',3,3',4,5,5',6,6'-nonaCB) and decachlorobiphenyl displayed ED50s for inhibition of the splenic PFC response to SRBC in C57BL/6 mice that were less than those for hexachlorobiphenyl isomers with multiple *ortho* chlorines reported above: 15, 7, 17, and 35 µmol/kg, respectively. These results are consistent with the hypothesis that some PCBs induce immunotoxicity via Ah-receptor *independent* mechanisms. In an *in vitro* assay of cell proliferation in response to lipopolysaccharide (a T-cell independent antigen), Aroclors 1221, 1242, 1254, or 1260 inhibited the proliferative response similarly in splenocytes from either C57BL/6 or DBA/2 mice (Stack et al. 1999). Two non-*ortho* and two mono-*ortho* PCBs that have been demonstrated to be effective Ah-receptor agonists and CYP1A1 inducers did not inhibit the *in vitro* proliferative response to lipopolysaccharide, but two di-*ortho* congeners (2,2',3,4,4',5- and 2,2',4,4',5,5'-hexaCB) significantly inhibited the response. These *in vitro* results provide supporting evidence for the existence of mechanisms of PCB immunotoxic actions that are independent of the Ah receptor.

Cancer. Lifetime oral exposure to any one of four commercial PCB mixtures (Aroclors 1016, 1242, 1254, and 1260) has been demonstrated to produce liver tumors in female rats; Aroclor 1260 also induced liver tumors in male rats (Mayes et al. 1998). Mixtures with high chlorination content (e.g., Aroclor 1254) were generally more potent than mixtures with low chlorine content (e.g., Aroclor 1016) (Mayes et al. 1998). Tumor promotion by commercial PCB mixtures following initiation by a variety of chemical agents also has been investigated in a number of animal systems including rat liver, rat kidney, mouse skin, and newborn mouse liver and lung (see Silberhorn et al. 1990 for review). The tumor

promoting effect of extended exposure to PCB mixtures was demonstrated principally in the liver of rats; there is some evidence that PCB mixtures also can promote tumors in mouse lung and mouse skin, but not in rat kidneys. The mechanism of PCB-induced cancer is poorly understood, but there is evidence to suggest that both Ah-receptor dependent and independent mechanisms may be involved.

PCB promotion of tumors does not appear to be solely an Ah-receptor mediated process, since individual congeners that are not Ah receptor agonists have tumor promotion capabilities in animal systems. For example, 2,2',5,5'-tetraCB, 2,2',4,4'-tetraCB, and 2,2',4,4',5,5'-hexaCB were shown to promote liver tumors in female Sprague-Dawley rats (Hemming et al. 1993; Preston et al. 1985). In addition, 2,2',5,5'-tetraCB, 2,2',3,3',4,4'-hexaCB, and 2,2',4,4',5,5'-hexaCB were potent inhibitors of *in vitro* gap junctional cellular communication, an assay that is indicative of tumor promotion capacity (Bager et al. 1997; De Haan et al. 1996). A general working mechanistic hypothesis for PCB promotion of liver tumors involves indirect stimulation of cell proliferation following cell or tissue injury by reactive metabolites of PCBs (Silberhorn et al. 1990). Alternatively, the cell injury could be caused by increased intracellular concentrations of other reactive species (e.g., superoxide anion or other reactive oxygen species) caused by an overall imbalance from PCB-induced perturbations of cellular biochemical processes, including induction of CYP oxygenases and glutathione S-transferases, repression of selenium-dependent glutathione peroxidases, and/or disruption of calcium homeostatic processes and signal transduction pathways (Silberhorn et al. 1990).

PCB mixtures have not shown consistent tumor initiating activity in animal initiation-promotion protocols (Silberhorn et al. 1990), but demonstration that chronic oral exposure to commercial PCB mixtures induced liver tumors in female rats (Mayes et al. 1998) suggests that PCBs may have both tumor initiating and promoting activities. Although PCB mixtures generally have been found to be inactive as mutagens in *S. typhimurium* strains and in several other tests of genotoxicity that may be predictive of tumor initiation capability (see Silberhorn et al. 1990 for review), *in vitro* studies with rat microsomes have indicated that metabolism of lower chlorinated PCBs (e.g., 4-CB, 3,4-diCB, and 3,4,5-triCB) can lead to covalently modified macromolecules including proteins and DNA (see Robertson and Gupta 2000 for review). Studies demonstrating the Ah-receptor dependence or independence of this potential genotoxic effect from PCBs were not located. The available data indicate that PCBs are not potent genotoxicants, but the possible involvement of genotoxic mechanisms (involving covalent modification of proteins and/or DNA) in the development of PCB-induced cancer is not without some experimental support.

The relative contribution that Ah-receptor dependent and independent mechanisms may make to carcinogenic responses to PCB mixtures is unknown. Safe (1994) compared carcinogenic responses of female rats to 2,3,7,8-TCDD in the diet with responses of female rats of the same strain to Aroclor 1260 in the diet using the TEF approach. TCDD at a TEQ feed concentration of 2,100 ppt induced hepatic adenocarcinomas in 11/50 (22%) rats, whereas a TEQ of only 1,040 ppt from Aroclor 1260 induced adenocarcinomas in 24/47 (51%) rats. For this situation, the TEF approach markedly underestimated the carcinogenic response to Aroclor 1260. A possible explanation is that PCB congeners that are not Ah receptor agonists and are abundant in Aroclor 1260 make significant contributions to the mixture's carcinogenicity. Although this comparison suggests that the TEF approach may underestimate cancer responses to complex PCB mixtures, another study of the tumor promotion activity of a simpler mixture of two CDDs, one CDF, and three PCBs in female rats found that the TEF approach overestimated the observed response by a factor of about 2 (van der Plas et al. 1999). The mixture contained 2,3,7,8-TCDD, 1,2,3,7,8-pentachlorodibenzo-p-dioxin, 2,3,4,7,8-pentachloro-dibenzofuran, 3,3',4,4',5- and 2,3',4,4',4-pentaCB, and 2,3,3',4,4',5-hexaCB at relative levels found in Baltic Sea herring. The rats were initiated with an injection of diethylnitrosoamine, 24 hours after a partial hepatectomy and were administered weekly subcutaneous injections of the mixture for 20 weeks starting 6 weeks after initiation. The volume and volume fraction of glutathione *S*-transferase-positive altered hepatic foci were taken as indicators of tumor promotion activity in this study (van der Plas et al. 1999). Although the composition of this mixture reflected relative concentrations and accounted for >90% of total TEQs in Baltic Sea herring, it did not contain PCBs with multiple *ortho* chlorines, which comprise the predominant bulk of PCB weight in most commercial and environmental mixtures. For example, non-, mono-, and di-*ortho* congeners accounted for <1, 18, and 82% of PCB weight per gram of fat in human milk samples from Italy (Larsen et al. 1994). Another group of rats was similarly treated with the same synthetic mixture plus a di-*ortho* PCB congener (2,2',4,4',5,5'-hexaCB), which is one of the predominant PCB congeners in environmental mixtures and has minimal Ah receptor agonist activity (van der Plas et al. 1999). Mean foci volume and foci volume fraction were increased in rats treated with the supplemented mixture compared with the mixture without the di-*ortho* congener, but the observed responses were still less than that predicted by the TEF approach. Better understanding of the relative contributions of Ah receptor dependent and independent mechanisms to the carcinogenicity of PCB mixtures awaits further research.

3.5.3 Animal-to-Human Extrapolations

As with other organisms, PCB residue levels in humans reflects multiple exposure pathways, and congener-specific elimination. PCB profiles in human serum immediately following exposures reflect the profiles in the exposure sources, however, selective metabolism and excretion begin to alter the congener profile within 4–24 hours (Hansen 1999). Thus, in most cases, the PCB profile in adults represents a steady state body burden which does not match the profile of commercial PCB formulations (Aroclors, etc.). For example, the PCB profile of a composite human milk sample does not resemble the pattern of any commercial PCB formulation (Safe et al. 1985). Furthermore, PCB residue analysis indicates that humans, aquatic mammals, birds, fish, and other biota retain a similar profile of PCB congeners. Borlakoglu and Walker (1989) reported that fisheating sea birds, human fat, American breast milk, and German breast milk have similar PCB congener profiles, which are different from that of Aroclor 1260 or Clophen A60. The hexachlorinated PCBs, 138 (2,2',3,4,4',5') and 153 (2,2',4,4',5,5'), were major congeners present in all samples from this study, while PCB 149 (2,2',3',4',5',6) was only found as a major component of Aroclor 1260 and Clophen A60. PCB 118 (2,3',4,4',5) was a major congener in biological samples and only a minor component of the commercial PCB formulations. McFarland and Clarke (1989) reported that PCBs 118, 138, 153, 156, 170, 180, and 187 were PCBs retained at a high relative abundance in porpoise, carp, duck, oligochaete, seston, shrimp, and human fat and milk. PCB 153 was the most abundant congener present in porpoise, carp, duck, oligochaete, and human fat and milk. In contrast to PCB residues present in the above populations with normal background exposures, humans retained PCB 74 as the most abundant PCB in human fat and serum following a case of occupational exposure (Stellman et al. 1998; Wolff et al. 1982a, 1982b). Thus, selective high level exposures, such as an occupational exposure, may result in an altered profile of retained PCB congeners, relative to that observed in cases of normal background exposure. However, the above studies generally find similar PCB congener profiles in different tissues and species, indicating that the biological fate of PCB congeners is qualitatively similar in various animal species.

Significant interspecies differences in the quantitative metabolism of PCBs contributes directly to the species differences in the relative persistence (biological half-life) of PCB congeners. For example, PCB 153 is often the most prevalent PCB detected in humans, due to exposure and the slow rate of biotransformation of this congener. 3-Hydroxy-2,4,5,2',4',5'-hexaCB was identified as the major metabolite of PCB 153 formed by human CYP2B6 (Ariyoshi et al. 1995). CYP2B6 is constitutively expressed in humans, but only accounts for a maximum of 1–2% of the total CYPs in human liver. Approximately 75% of the subjects examined had no detectable level of CYP2B6 protein by

immunoblotting (Mimura et al. 1993). This may be the reason why no metabolite of PCB 153 was detected in an earlier *in vitro* study using human liver microsomes (Schnellmann et al. 1983). 3-Hydroxy-PCB 153 is also the major metabolite in the feces and bile of monkeys treated with PCB 153 (Norback et al. 1981). The CYP2B isoform represents about 5% of the total P-450 in monkey liver, and this may account for the approximately 5-fold more rapid elimination of PCB 153 from monkeys and the resulting shorter half-life in monkeys, relative to humans (Mes et al. 1995a, 1995b, 1995c; Ohmori et al. 1992). The dog has a unique ability to more rapidly metabolize and eliminate PCB 153 through the CYP2B11- mediated 3-hydroxylation of PCB 153 via a 2,3-arene oxide intermediate (Ariyoshi et al. 1992; Duignan et al. 1987; Sipes et al. 1982). Thus, the high potential for accumulation and persistence of PCB 153 in humans is due to the very low levels of CYP2B6 and low catalytic activity for 3-hydroxylation of this congener.

PCB congeners that are structurally similar to 2,3,7,8-TCDD exhibit Ah receptor-mediated responses. These congeners appear to be the most potent for some PCB-induced effects. Therefore, it would seem reasonable to assume that, at least for these specific toxic effects, differences in susceptibility among animal species could be explained by differences in receptor levels in target tissues or by differences in the affinity of binding of the specific congeners. Information on this subject is mainly derived from studies with 2,3,7,8-TCDD. Data summarized by Okey et al. (1994) indicate that differences in receptor level or receptor affinity cannot explain marked differences in susceptibility to halogenated aromatic hydrocarbon toxicity across species. It is possible that differences in sensitivity among species may be determined by some event or events occurring after the initial binding of the ligand to the receptor.

The Ah receptor has been identified in many human tissues and human cell lines (Okey et al. 1994). Several differences between human and animal Ah receptor protein have been described. Perhaps the most important difference is that the human Ah receptor has a lower affinity for 2,3,7,8-TCDD than the Ah receptor from rats or from responsive strains of mice. This information, although limited, leads to the conclusion that the biochemical and toxicological responses (those that exhibit a threshold) to dioxin-like aromatic hydrocarbons in humans would require higher doses or exposures than in animal species possessing a receptor of higher affinity (Okey et al. 1994).

3.6 ENDOCRINE DISRUPTION

Recently, attention has focused on the potential hazardous effects of certain chemicals on the endocrine system because of the ability of these chemicals to mimic or block endogenous hormones, or otherwise interfere with the normal function of the endocrine system. Chemicals with this type of activity are most commonly referred to as endocrine disruptors. Some scientists believe that chemicals with the ability to disrupt the endocrine system are a potential threat to the health of humans, aquatic animals, and wildlife. Others believe that endocrine disrupting chemicals do not pose a significant health risk, particularly in light of the fact that hormone mimics exist in the natural environment. Examples of natural hormone mimics are the isoflavinoid phytoestrogens (Adlercreutz 1995; Livingston 1978; Mayr et al. 1992). These compounds are derived from plants and are similar in structure and action as endogenous estrogen. While there is some concern over the public health significance of endocrine disrupting chemicals, it is agreed that the potential exists for these compounds to affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development, and/or behavior (EPA 1997). As a result, endocrine disruptors may play a role in the disruption of sexual function, immune suppression, and neurobehavioral function. Endocrine disruption is also thought to be involved in the induction of breast, testicular, and prostate cancers, as well as endometriosis (Berger 1994; Giwercman et al. 1993; Hoel et al. 1992).

In recent years, concern has been raised that many industrial chemicals, PCBs among them, are endocrine-active compounds capable of having widespread effects on humans and wildlife (Crisp et al. 1998; Daston et al. 1997; Safe et al. 1997). (Effects on wildlife are summarized in Section 3.3.2). Particular attention has been paid to the possibility of these compounds mimicking or antagonizing the action of estrogen. Estrogen influences the growth, differentiation, and functioning of many target tissues, including female and male reproductive systems, such as mammary gland, uterus, vagina, ovary, testes, epididymis, and prostate. In addition, there is evidence that some of these environmentally-persistent chemicals alter the thyroid hormone system, which is very a important system in normal structural and functional development of sexual organs and the brain.

Several studies in humans have examined possible associations between body burdens of PCBs and other organochlorines and the incidence of alterations in tissues and systems. Evaluations of blood samples from women who aborted, miscarried, or delivered prematurely showed positive associations between these effects and concentrations of PCBs (Bercovici et al. 1983; Leoni et al. 1989; Wassermann et al. 1980, 1982). However, other chlorinated chemicals were also increased, and the specific contribution of

PCBs, if any, could not be determined. Similar findings were reported in a more recent study of the general population (Gerhard et al. 1998). Another general population study found no association between endometriosis and concentrations of PCBs in the blood (Lebel et al. 1998).

Breast Cancer. The issue of breast cancer has received special attention following reports of high levels of organochlorine compounds in breast cancer patients. However, the hypothesis that environmental exposure to PCBs can cause breast cancer in humans is controversial (Safe and Zacharewski 1997; Wolff and Toniolo 1995). Breast adipose levels of total PCBs or individual congeners were increased in women with breast cancer in some (Dewailly et al. 1994; Falck et al. 1992; Guttes et al. 1998; Wasserman et al. 1976) but not all studies (Aronson et al. 2000; Liljegren et al. 1998; Mussalo-Rauhamaa et al. 1990; Unger et al. 1984), but methodological limitations such as small numbers of subjects and/or inadequate control for known breast cancer risk factors could have contributed to the inconsistent findings. Two of these studies included analyses that suggested increased risks of breast cancer associated with increased tissue levels of some congeners in subgroups of women that were postmenopausal or had estrogen receptor-positive tumors (Aronson et al. 2000; Liljegren et al. 1998). Other environmental exposure studies used serum PCB concentrations as the marker of exposure with blood samples taken after the diagnosis of breast cancer (Moysich et al. 1998, 1999; Wolff et al. 1993; Zheng et al. 2000), or prospectively collected prior to diagnosis (Dorgan et al. 1999; Helzlsouer et al. 1999; Høyer et al. 1998; Hunter et al. 1997; Krieger et al. 1994; Wolff et al. 2000). None of the serum studies found significantly different mean blood levels of PCBs in breast cancer cases and controls. Additionally, there were no significant associations between risk of breast cancer and serum PCBs in most of these studies, although some data suggest that risk may be increased in some subgroups of postmenopausal women (Moysich et al. 1998, 1999). Many of the better designed studies were prospective, and none found that PCBs were associated with the occurrence of breast cancer (Dorgan et al. 1999; Helzlsouer et al. 1999; Høyer et al. 1998; Hunter et al. 1997; Krieger et al. 1994; Wolff et al. 2000). However, the prospective studies are limited by one biomarker of exposure in the distant past, which would not reflect differences over time in exposure, absorption, enzyme induction, or other factors influencing body burden such as breast-feeding. It is still possible that the PCB measurements were too abridged to detect abnormally high proportions of the more labile congeners, which appear to have greater estrogenic activities (Hansen 1998, 1999). Mortality from breast cancer was not increased in studies of workers who were occupationally exposed to relatively high levels of PCBs (Brown 1987b; Brown and Jones 1981; Kimbrough et al. 1999a), providing an additional indication that lower level environmental exposures to PCBs are unlikely to contribute significantly to the disease. Overall, the evidence for an association between breast cancer and PCBs remains inconclusive and needs further study.

Estrogenic and Anti-Estrogenic Activity. In early studies of experimental animals, research was focused on the effects of chemicals administered orally or by parenteral routes. In recent years, most of the research has focused on elucidating the mechanisms of action involved using test systems *in vitro* which, although not without limitations, are easier to manipulate and can be developed into biomarker assays for (anti)estrogenic activity. In general, results from *in vivo* and *in vitro* studies indicate that PCBs have much lower estrogenic potency than the endogenous hormone, 17β-estradiol. Subcutaneous administration of 8 mg of Aroclors 1221, 1232, 1242, or 1248 increased uterine weight and glycogen content in rats, but similar exposure to Aroclors 1254, 1260, 1262, or 1268 did not produce this estrogenic effect (Bitman and Cecil 1970). More recent studies have provided further evidence that PCB mixtures can produce estrogenic responses (albeit weak) and that PCB congeners with multiple *ortho* chlorines (or their hydroxylated metabolites) may be at least partly responsible for these responses. Intraperitoneal doses of Aroclor 1242 (8 mg/rat on day 20 or 0.08 or 0.32 mg/rat on days 20 and 21) significantly increased uterine wet weight in immature female rats to about 40% of the increase produced by 0.001 mg of 17β-estradiol (Jansen et al. 1993). Similar increases in uterine wet weight were produced by exposure to di-*ortho* congeners or hydroxylated derivatives (0.640 mg PCB 52 or 0.250 mg of 2,4,6–trichloro-4'-hydroxy-biphenyl on days 20 and 21), but not by exposure to 0.160 mg of the coplanar congener PCB 77. In another study, the tetra-*ortho* congener, PCB 47, displayed similarly weak estrogenic responses in an *in vitro* human breast cancer cell assay and an *in vivo* immature female rat assay (Arcaro et al. 1999). This congener did not competitively bind *in vitro* to recombinant human estrogen receptors α and β, but a hydroxylated metabolite, 2,2',6,6'-tetrachloro-4'-hydroxy-biphenyl, competitively bound to estrogen receptor α and produced proliferative responses in the breast cancer assay at concentrations about 10-fold lower than effective concentrations of the parent molecule (Arcaro et al. 1999). Evaluation of the offspring from rats given a PCB congener mixture simulating the congener content of human milk from 50 days prior to mating until birth showed significantly increased relative uterine weight in immature females on PND 21 (Hany et al. 1999b).

Anti-estrogenic properties of PCBs also have been examined in numerous studies. Combined exposure of immature rats to 0.32 mg Aroclor 1242 and 0.001 mg 17β-estradiol produced a response similar to estradiol alone, indicating no obvious anti-estrogenic activity, but combined exposure to 0.001 mg estradiol and 0.160 mg of PCB 77 markedly diminished the estradiol-induced increase in uterine wet weight (Jansen et al. 1993). Anti-estrogenic effects similar to those from PCB 77 were observed in rodent uterine tissue (Astroff and Safe 1990) and human breast cancer cells (Krishnan and Safe 1993) by other congeners with no or single *ortho* chlorines (e.g., 3,3',4,4',5-pentaCB, 2',3,3',4,4',5-hexaCB), but commercial PCB mixtures were not anti-estrogenic in the breast cancer cell assay. Whereas the data

collected by Krishnan and Safe (1993) suggest that anti-estrogenic activities of PCBs may be related to Ah receptor binding affinity, anti-estrogenic activities of hydroxylated PCB congeners with multiple *ortho* chlorines have been observed in several assay systems (Connor et al. 1997; Moore et al. 1997; Safe et al. 1998b).

Structure-activity relationships for estrogenic activities of PCB congeners or their metabolites are less clear. Some hydroxylated PCBs (2,4,6-trichloro-4'-hydroxy-biphenyl and 2,3,4,5-tetrachloro-4'-hydroxy-biphenyl) have been demonstrated to competitively bind to mouse estrogen receptor preparations and to increase uterine weight and glycogen in immature mice (Korach et al. 1988). In other estrogenic assays, PCB 104, 2,4,4',6-tetrachloro-4-hydroxy-biphenyl, and 2,4,6-trichloro-4'-hydroxy-biphenyl were equally effective in stimulating proliferation of human breast cancer cells, but only 2,4,6-trichloro-4'-hydroxy-biphenyl caused significant induction of vitellogenin in cultured brown trout hepatocytes (Andersson et al. 1999). A structure-activity study of eight hydroxylated PCBs in a series of *in vivo* and *in vitro* estrogenic assays found that structure-activity relationships were complex and differed from one assay to the next (Connor et al. 1997; Safe et al. 1998b). For example, all but one of the compounds displayed competitive binding to rat and mouse cytosolic estrogen receptors (affinities ranged from about 10^{-3} to 10^{-5} of 17β-estradiol's affinity), but there was no evidence of estrogenic activities (wet weight, peroxidase activity, progesterone receptor level) in the uteri of immature rats and mice exposed to three consecutive daily doses of the individual hydroxylated PCB congeners at levels of 25, 50, or 100 mg/kg. In contrast, four of the hydroxylated congeners produced estrogenic effects in cultured human breast cells and HeLa cells (Connor et al. 1997; Safe et al. 1998). These results suggest that PCB-induced estrogenic activities are weak compared to the endogenous hormone, 17β-estradiol. Further, the wide variability of responses observed across types of PCBs and assays indicates: (1) the involvement of multiple mechanisms, (2) anti-estrogenic activities appear strongly associated with PCBs that are Ah receptor agonists, and (3) hydroxylated metabolites of PCBs seem to be at least partly responsible for physiological responses to PCBs that may involve changes in estrogen receptor-dependent physiological processes.

The results of some studies summarized above suggest that PCBs can produce estrogenic and anti-estrogenic responses by interfering with the binding of natural ligands to their receptors. The type of response varied between assays and was dependent of the concentration of the test material. Reviews of published data suggest that the amount of naturally occurring estrogens ingested daily through a normal diet is far greater than the daily intake of estrogenic organochlorine chemicals (Safe 1995). Moreover, results from many assays indicate that estrogenic organochlorines have a potency of 0.000001 times that of 17β-estradiol, compared to 0.001–0.0001 times for naturally-occurring estrogenic substances. In

addition, many naturally-occurring estrogenic substances, such as bioflavonoids, are also antiestrogenic at some concentrations. Dietary levels of anti-estrogen equivalents (industrial or natural) are significantly higher than the estrogen equivalents of organochlorine chemicals (Safe 1995).

Reproductive Effects. Sager and Girard (1994) have provided evidence of reproductive effects of PCBs that may be related to PCB-induced endocrine disruption. After giving birth, adult female rats were exposed to 0, 8, 32, or 64 mg Aroclor 1254/kg/day by gavage on lactation days 1, 3, 5, 7, and 9. Young, mature, and older adult female offspring were examined at 2–4.5, 5–8, and 8.5–13 months of age, respectively, and mated to untreated males at 112, 200, and 350 days of age, respectively. Effects included a dose-related reduction in preweaning weight gain that was statistically significant at $32 mg/kg/day, delayed puberty as indicated by late vaginal opening and first estrus at $32 mg/kg/day; reduced mating rate (sperm-positive females) in mature offspring at $8 mg/kg/day; reduced implantation rate and mean number of embryos in young and mature offspring at 64 mg/kg/day; reduced uterine weight during proestrus in young, mature, and older offspring at $8 mg/kg/day; and reduced uterine response to exogenous 17β-estradiol in ovariectomized mature offspring at $8 mg/kg/day. Average estrus cycle length was not significantly different in any of the groups, although cycle patterns were altered in low- and high-dose young offspring and in mid-dose mature rats. Pregnancy and ovulation rates, reproductive aging, and ovarian weights were not affected by exposure Aroclor 1254.

Fertility was markedly reduced in male offspring of Holtzman rats that were exposed via lactation to Aroclor 1254 (Sager 1983; Sager et al. 1987, 1991). The maternal rats were treated with 8, 16, 32, or 64 mg/kg doses by gavage on lactation days 1, 3, 5, 7, and 9, and male offspring were mated with untreated females 130–150 days postweaning (Sager 1983; Sager et al. 1987). Significant decreases in numbers of implants and embryos were observed at $8 mg/kg/day (21 and 29% lower than controls, respectively), and there was either a significant decrease or a decline in number and percent of normal fertilized eggs and eggs at the two- to four-cell blastocyte stages at $16 mg/kg/day. The reduction in male fertility appears to be due to impaired ability of sperm to fertilize eggs because sperm production, morphology, and motility were not affected and plasma FSH and testosterone concentrations were not reduced (Sager et al. 1987, 1991). Seminal vesicle and ventral prostate weights were decreased at $16 mg/kg/day.

Fertility was not impaired in male offspring of Sprague-Dawley rats that were administered 0 or 30 mg/kg/day doses of Aroclor 1221, 1242, or 1260 by gavage on days 12–20 of gestation (Gellert and Wilson 1979). There were no exposure-related changes in the percentage of male offspring (F1) siring

progeny when they were mated with unexposed females at approximately 6 months of age, or in the sex ratio of the F2 offspring from this mating. Measurements of absolute testes and ventral prostate weights in the F1 males (relative weights were not determined) showed no changes except for increased testes weight in the Aroclor 1260 group. Conflicting results regarding fertility in the aforementioned studies may be related to the difference in exposure periods. In the experiments by Sager and others, exposure was postnatal via breast milk, whereas in the Gellert and Wilson (1979) study, the male rats were exposed *in utero*.

Studies that examined reproductive end points in women found indications that exposure to PCBs may be associated with menstrual disturbances. Mendola et al. (1997) reported that consumption of Lake Ontario sportfish was associated with shorter menstrual cycles in women from the New York State Angler cohort. This is a preliminary finding that needs to be interpreted cautiously because of limitations in the data analysis, particularly the lack of information on confounders such as stress, use of contraceptives, body mass index, and physical exercise. The decreases in menstrual length were small and were considered not likely to be clinically relevant. At the highest exposure levels, the decrease was approximately 0.5 days for women who reported regular cycles and 1 day for all women who reported cycle length information. The effect did not appear to be mediated through irregular cycles since the fish consumption-based exposure levels were similar for women who reported regular or irregular cycles. Menstrual cycle changes (altered intervals, duration, and flow) have also been observed in women exposed to higher doses of PCBs during the *Yusho* poisoning incident (Kusuda 1971). The human populations in which menstrual changes have been observed differ with respect to the sources of PCBs and exposures to other chemicals that may affect susceptibility to menstrual disturbances. Although the studies are insufficient for determining which specific chemical(s) may be responsible for the observed alterations, the available data support a possible association between PCBs and menstrual disturbances.

In a study of 89 women (87% German) with repeated ($2) miscarriages, Gerhard et al. (1998) found that blood concentrations of PCBs were higher than the reference level in 22% of the cases. The effect cannot be specifically attributed to PCBs because blood levels of other organochlorine compounds (pentachlorophenol, DDE, β- and γ-hexachlorocyclohexanes, HCB) were higher than reference ranges in 7–15% of the cases. No significant differences in PCB levels were found between women with early or late miscarriages (after #12 or >12 weeks of gestation) and primary or secondary miscarriages (had never delivered or had delivered at least one baby). Women with a history of at least four miscarriages (n=25) had significantly elevated blood levels of PCBs, although other organochlorine compounds (γ-hexachlorocyclohexane and HCB) were also increased. Hormonal disorders were identified as the

cause of repeated miscarriages in 31% of the women, including hyperprolactinemia in 9%, hyperandrogenemia in 7%, and luteal insufficiency in 14% of the cases. Correlations were found between increasing PCB concentrations and some hormonal parameters (e.g., increasing FSH and LH, decreasing TSH) and immunological parameters (e.g., increasing IgM, monocytes, and NK cells, decreasing interleukin 2 receptor-positive cells), but none of the associations were specific for PCBs. There were no significant associations between PCB concentrations and further conceptions or the outcome of further pregnancies.

Thyroid Effects. Concern that the thyroid hormone system may be important in the mechanism of toxicity of PCBs stems from mainly two important types of observations (Brouwer et al. 1998b; Porterfield and Hendry 1998): (1) extensively corroborated findings in experimental animals that exposure to PCBs *in utero* and/or during early development (e.g., through breast milk) can deplete levels of circulating thyroid hormone in the fetus or neonate, which may give rise to effectively a hypothyroid state during development (Collins and Capen 1980c; Cooke et al. 1996; Corey et al. 1996; Darnerud et al. 1996a; Goldey et al. 1995; Juarez de Ku et al. 1994; Li et al. 1998; Morse et al. 1996c; Rice 1999a; Provost et al. 1999; Schuur et al. 1998a; Seo and Meserve 1995; Zoeller et al. 2000); and (2) the recognition of the importance of thyroid hormones in normal development of the brain and sexual organs.

Studies in animals have shown that, depending of dose and duration of exposure, PCBs can disrupt the production and disposition of thyroid hormones at a variety of levels. The major findings include (1) histological changes in the thyroid gland indicative of both stimulation of the gland (e.g., similar to that induced by TSH or a hypothyroid state) and a disruption of the processing of follicular colloid needed for normal production and secretion thyroid hormone (Chu et al. 1994, 1995, 1996a, 1996b, 1998b; Collins and Capen 1980a; Collins et al. 1977; Hansen et al. 1995; Tryphonas et al. 1986b); (2) depression of serum T_4 and T_3 levels, which may effectively create a hypothyroid state (Byrne et al. 1987; Collins and Capen 1980c; Cooke et al. 1996; Corey et al. 1996; Darnerud et al. 1996a; Desaulniers et al. 1997; Goldey et al. 1995; Gray et al. 1993; Hansen et al. 1995; Hood et al. 1999; Juarez de Ku et al. 1994; Kasza et al. 1978; Li et al. 1998; Morse et al. 1996c; Rice et al. 1988, 1999a; Provost et al. 1999; Schuur et al. 1998a; Seo and Meserve 1995; Van Birgelen et al. 1995; Zoeller et al. 2000); (3) increased rates of elimination of T_4 and T_3 from serum (Goldey and Crofton 1998); (4) increased activities of T_4-UDP-GT in liver (Chu et al. 1995; Desaulniers et al. 1997; Morse et al. 1996c; Schuur et al. 1998a; Van Birgelen et al. 1995), which is an important metabolic elimination pathway for T_4 and T_3; (5) decreased activity of iodothyronine sulfotransferases in liver which are also important in the metabolic elimination of iodothyronines (Schuur et al. 1998a, 1998b, 1999); (6) decreased activity of iodothyronine

deiodinases including brain Type-2 deiodinase, which provide the major pathways for the production of the active thyroid hormone, T_3 (Morse et al. 1996; Schuur et al. 1998a); and (7) decreased binding of T_4 to transthyretin, an important transport protein for both T_4 and T_3 (Cheek et al. 1999; Darnerud et al. 1996a).

The above observations suggest that PCBs can disrupt the production of thyroid hormones (in the thyroid and in peripheral tissues), can interfere with their transport to peripheral tissues, and can accelerate the metabolic clearance of thyroid hormones. The most convincing evidence that PCBs can exert toxicity by disrupting thyroid hormone system derives from two studies in rats. In one study, neurobehavioral deficits in pups that experienced exposures to Aroclor 1254 *in utero* and during nursing were significantly attenuated by subcutaneous injections of T_4 that increased serum T_4 and T_3 concentrations which were otherwise depressed in the PCB-exposed animals (Goldey and Crofton 1998). While this study examined relatively high doses of Aroclor 1254 ($1 mg/kg/day), it nevertheless demonstrated neurodevelopmental effects that are directly relevant to observations made in epidemiological studies and to neurological sequelae of fetal hypothyroidism, including motor disturbances and hearing.

In the second study, increased testis weight and sperm production in rats that were administered Aroclor 1254 on postnatal days 1–25 were attenuated by injections of T_4 on postnatal days 1–25, which also prevented the depression in serum T_4 concentrations (Cooke et al. 1996). Here again, although produced by relatively large doses of Aroclor 1254 ($1.6 mg/kg/day, subcutaneous), similar effects can be produced by other hypothyroid-inducing agents, including PTU. Cooke and coworkers have proposed that the increased testis weight and sperm production is the result of PCBs extending the proliferative period of Sertoli cells, thus increasing their number. A prolonged period of cell division in turn results in a greater total number of germ cells per testis, creating an enlarged testis that produces more sperm than normal (see Chapin et al. 1996 for review). Neonatal hypothyroidism in humans also is known to be associated with disruption of the normal sexual maturation process (Longcope 2000).

In summary, PCBs can affect a wide variety of endocrine systems by directly affecting the components of the endocrine system such as hormones, metabolic enzymes, carrier proteins, receptors, endocrine glands, and feedback regulation systems. Effects on these components can lead to alterations in neurodevelopment, reproduction, and in induction of endocrine-sensitive tumors.

3.7 CHILDREN'S SUSCEPTIBILITY

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans, when all biological systems will have fully developed. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Relevant animal and *in vitro* models are also discussed.

Children are not small adults. They differ from adults in their exposures and may differ in their susceptibility to hazardous chemicals. Children's unique physiology and behavior can influence the extent of their exposure. Exposures of children are discussed in Section 6.6 Exposures of Children.

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects, and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of their bodies as extracellular water and their brains and livers are proportionately larger (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek 1966; Widdowson and Dickerson 1964). The infant also has an immature blood-brain barrier (Adinolfi 1985; Johanson 1980) and probably an immature blood-testis barrier (Setchell and Waites 1975). Many xenobiotic metabolizing enzymes have distinctive developmental patterns. At various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults, and sometimes unique enzymes may exist at particular developmental stages (Komori et al. 1990; Leeder and Kearns 1997; NRC 1993; Vieira et al. 1996). Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in newborns who all have a low glomerular filtration rate and have not developed efficient

tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer remaining lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

Certain characteristics of the developing human may increase exposure or susceptibility, whereas others may decrease susceptibility to the same chemical. For example, although infants breathe more air per kilogram of body weight than adults breathe, this difference might be somewhat counterbalanced by their alveoli being less developed, which results in a disproportionately smaller surface area for alveolar absorption (NRC 1993).

Children are exposed to PCBs in the same manner as the general population, primarily via consumption of contaminated foods, particularly meat, fish, and poultry (Gunderson 1988). Exposure also may occur by transfer of PCBs that have accumulated in women's bodies to the fetus across the placenta. Because PCBs are lipophilic substances, they can accumulate in breast milk and be transferred to nursing infants. Transfer across the placenta, although it may be limited in absolute amounts, is of great concern because of the effects of PCBs on sensitive immature tissues, organs, and systems, with potentially serious long-lasting consequences. Transfer of PCBs via breast milk can be considerable and, like prenatal exposure, has the potential to contribute to altered development.

Although embryos, fetuses, and nursing infants may be exposed to relatively high amounts of PCBs during sensitive periods of development, it is not known if the susceptibility of children to the health effects of PCBs differs from that of adults. Younger children may be particularly vulnerable to PCBs because, compared to adults, they are growing more rapidly and generally have lower and distinct profiles of biotransforamtion enzymes, as well as much smaller fat depots for sequestering the lipophilic PCBs.

The best documented effect of exposure to high concentrations of PCBs in adults is the induction of skin alterations, in particular, chloracne. This has been observed in individuals occupationally exposed to PCBs (Bertazzi et al. 1987; Fischbein et al. 1979, 1982; Maroni et al. 1981a, 1981b; Meigs et al. 1954) and in *Yusho* and *Yu-Cheng* victims, who consumed rice oil contaminated with high concentrations of PCBs and other dioxin-like chemicals (Hsu et al. 1994; Masuda 1994). Children born to *Yusho* and *Yu-Cheng* victims also exhibited acneform eruptions. It is reasonable to assume that children exposed to high amounts of PCBs, particularly dioxin-like congeners, also will develop dermal alterations as occurs in adults.

Numerous studies have investigated the effects of exposure to PCBs in newborn and young children. The main studies can be divided into those of women who consumed high amounts of contaminated fish, primarily from the Great Lakes (Fein et al. 1984a, 1984b; Jacobson and Jacobson 1996a, 1996b, 1997; Jacobson et al. 1984a, 1984b, 1985, 1990a, 1990b, 1992; Lonky et al. 1996, Stewart et al. 1999, 2000a), women from the general population with no known high exposure to PCBs (Gladen et al. 1988; Huisman et al. 1995a, 1995b; Koopman-Esseboom et al. 1994b, 1996; Lanting et al. 1998c; Patandin et al. 1999; Rogan and Gladen 1991, 1992; Rogan et al. 1986a, 1986b, 1987), and women who ingested rice oil accidentally contaminated with high amounts of PCBs and structurally-related compounds (Chen et al. 1992, 1994; Guo et al. 1995; Hsu et al. 1994; Lai et al. 1994; Masuda 1994). The main focus of these studies has been the evaluation of neurobehavioral end points, but information on other end points such as anthropometric measures at birth and growth rate (Dar et al. 1992; Fein et al. 1984b, Jacobson et al. 1990a, 1990b; Lan et al. 1987; Lonky et al. 1996; Patandin et al. 1998; Rogan 1989; Rylander et al. 1995, 1998b; Smith 1984; Taylor et al. 1984, 1989; Vartiainen et al. 1998), immune status (Chao et al. 1997; Dewailly et al. 2000; Weisglas-Kuperus et al. 1995; Yu et al. 1998), and thyroid status (Koopman-Esseboom et al. 1994a) is also available.

Surrogate measures of exposure that have been used in human studies include PCB measurements of maternal blood, breast milk, and cord blood. Cord blood is the most direct marker of fetal exposure, but because of its relatively low fat content, it requires sensitive analytical methods for accurate PCB analysis; analysis of breast milk does not present this difficulty. Analytical techniques have improved enormously in recent years, such that cord blood analysis of PCBs is now more accurate and reliable, but still of concern due to the low concentration of fat in cord blood.

There is evidence that PCBs play a role in neurobehavioral alterations observed in newborn and young children from women with PCB burdens near background levels, but the possibility cannot be ruled out that other lipophilic compounds may contribute to the observed effects, particularly in the studies of consumption of Great Lakes fish contaminated with other chemicals such as CDDs, DDE, and mercury. Newborns from women who ate high amounts of contaminated Lake Michigan fish had a greater number of abnormal reflexes and more motor immaturity than low-fisheaters (Jacobson et al. 1984a). Similar observations were made by Rogan et al. (1986b) in the North Carolina study of children born to women with no known high PCB exposure and in the Oswego study of children from women with high Lake Ontario fish consumption (Lonky et al. 1996). By measuring individual PCB congeners in cord blood of Lake Ontario fisheaters, Stewart et al. (2000b) observed a significant association between highly chlorinated PCBs and poorer Habituation and Autonomic scores of the NBAS for the newborns, but there

was no significant association with abnormal reflexes. No significant association was seen for the lightly and moderately chlorinated PCBs, DDE, lead, mercury, and hexachlorobenzene with any of the neurological scores for newborns of the Lake Ontario fisheaters. In the study of Dutch children, prenatal exposure to four predominant PCBs (PCB levels in maternal or cord plasma) was not associated with either reflex or postural cluster scores of a neurological examination (Huisman et al. 1995a), but there was a significant association between increasing levels of planar PCB TEQ in breast milk and increases in percentage with hypotonia but not percentage with abnormal reflexes. There is limited support from animal studies for the findings of hypotonia and hyporeflexia in newborn humans exposed to PCBs. Overmann et al. (1987) reported that newborn rats from dams exposed to 1.3 mg Aroclor 1254/kg/day showed slower development of air righting ability (an index of neuromuscular maturation) on 1 of 4 testing days and also were slower than controls in a negative geotaxis test on 2 out 4 days of testing.

Assessment of infants from the various cohorts with the Bayley Scales of Infant Development has revealed some additional consistency among the studies. This group of tests yields a mental development index (MDI) and a PDI score, both of which are scaled like a standard IQ test. In the North Carolina cohort, prenatal exposure to PCBs (assessed by PCBs in maternal milk at birth, 1.8 ppm) was associated with a significant decrease in PDI scores at the ages of 6 and 12 months (Gladen et al. 1988), but the association lost statistical significance at the ages of 18 and 24 months (Rogan and Gladen 1991). No significant association was observed between postnatal exposure to PCBs (PCBs in milk factored by duration of breast feeding) and PDI scores between 6 and 24 months of age. Neither prenatal nor postnatal exposure to PCBs showed a significant association with MDI scores. The latter is consistent with a lack of significant association between prenatal or postnatal exposure and MDI scores at 7 or 18 months of age also observed in the Dutch children (Koopman-Esseboom et al. 1996). *Yu-Cheng* children also had lower PDI and MDI scores when tested between the ages of 6 months and 2 years old (Lai et al. 1994). Alterations in memory functions were reported in children from the Michigan cohort at 7 months of age (Jacobson et al. 1985) and at 4 years of age (Jacobson et al. 1990a, 1990b, 1992), but not in other cohorts studied. In both instances, memory deficits were associated with prenatal exposure to PCBs, as measured by PCBs in cord blood. Decreased performance in memory tests has been reported following perinatal exposure to commercial PCB mixtures in rats (Corey et al. 1996; Lilienthal and Winneke 1991) and in monkeys (Levin et al. 1988; Schantz et al. 1989). In addition, decreased performance on a memory task was reported in 60-day-old rats exposed *in utero* to *ortho*-substituted PCB congeners (Schantz et al. 1995).

A brain area of critical importance in the regulation of short-term or representational memory for spatial information is the prefrontal cortex (Brozoski et al. 1979; Goldman et al. 1971). Some studies in animals have found changes in the concentration of neurotransmitters in the frontal cortex following exposure to PCBs. For example, Morse et al. (1996a) reported that rats exposed during gestation days 10–16 to 5 or 25 mg Aroclor 1254/kg/day had a significant increase in the levels of 5-HIAA and in the ratio 5-HIAA/5-hydroxytryptamine in the prefrontal cortex at 90 days of age. These rats also showed a significant decrease in the neuronal marker synaptophysin in the prefrontal cortex, which was interpreted as reactive gliosis (Morse et al. 1996b). A decrease in dopamine in the frontal cortex was also observed in rats exposed to contaminated Great Lakes fish *in utero*, during lactation, and until 88 days of age (Seegal et al. 1998). Exposure of weanling rats to PCB 153 (0.01 mg/kg/day) or PCB 128 (0.005 mg/kg/day) for 90 days also resulted in decreased dopamine levels in the frontal cortex (Chu et al. 1996a; Lecavalier et al. 1997). Changes in neurotransmitter levels have also been observed in other brain areas, but further research is needed before specific neurobehavioral deficits can be correlated with changes in specific neurotransmitters in specific brain areas.

As previously mentioned, neurobehavioral alterations have been observed in rats and monkeys following pre- and/or postnatal exposure to commercial Aroclor mixtures, defined experimental congener mixtures, single PCB congeners, and Great Lakes contaminated fish. Monkeys exposed from birth to age 20 weeks to PCB mixtures of congeneric composition and concentration similar to that found in human breast milk showed learning deficits long after exposure had ceased (Rice 1997, 1998, 1999b; Rice and Hayward 1997, 1999a). This type of study appears to be the most relevant to evaluating risk of PCB exposure by infants since they mimic the exposure scenario for a nursing human infant.

Results from evaluation of anthropometric measurements in newborn and young children have been mixed. Of the studies of women who consumed contaminated fish from the Great Lakes, only one out of four, the Michigan study (Fein et al. 1984b; Jacobson et al. 1990a, 1990b), reported an association between reduced birth weight, head circumference, and gestational age in newborns and with body weight at 4 years with prenatal exposure to PCBs (PCBs in cord blood). In the Oswego cohort (Lake Ontario fish consumption), there was no significant association between prenatal exposure to PCBs, assessed by the same fish consumption measures as in the Michigan study, and birth weight, head circumference, or gestational age (Lonky et al. 1996). In two additional studies of Lake Michigan women (Dar et al. 1992; Smith 1984), fish consumption had a positive effect on birth weight. This finding could be related to the beneficial effects of certain fatty acids in fish (Olsen et al. 1990). In one of these studies (Smith 1984), the concentration of PCBs in breast milk was higher than in breast milk from women from the Michigan

cohort (1.13 vs 0.87 ppm). A study of Swedish wives of Baltic Sea fishermen found an increased risk of low birth weight with increasing maternal blood concentrations of the PCB congener PCB 153 used as a surrogate of PCB exposure during the year of childbirth (Rylander et al. 1998b). In the Dutch cohort, prenatal exposure to PCBs (PCBs in cord blood) was associated with a reduced birth weight, but not with head circumference or height at 10 days of age (Patandin et al. 1998). Prenatal exposure in formula-fed children was associated with reduced growth between birth and 3 months, but no such association was seen in breast fed children, suggesting to the investigators that any detrimental effect observed in newborns due to prenatal exposure to PCBs may have been counteracted by the benefits of breast feeding. No significant association was seen between any measure of exposure to PCBs and growth at the ages of 3–7 months, 7–18 months, or 18–42 months. A study of the general population in Finland found no significant association between birth weight and the concentration of PCBs in breast milk (Vartiainen et al. 1998). In this study, the mean concentration of PCBs in milk (0.4–0.5 ppm) was slightly lower than in the Dutch study (0.62 ppm) (Koopman-Esseboom et al. 1994b). Overall, it seems that if there is an adverse effect of prenatal exposure to PCBs on growth, it is transient, as documented in children from the *Yusho* poisoning episode (Yoshimura and Ikeda 1978).

Studies in rodents, generally with relatively high doses of PCB mixtures or congeners, have shown decreased birth weight and reduced weight gain after birth. This occurred in animals exposed *in utero* and through breast milk, even though their weight at birth was not significantly different than in unexposed controls (Collins and Capen 1980c; Overmann et al. 1987). This suggests that a significant transfer of PCBs occurred via breast feeding. Long-term studies with much lower doses of Aroclors 1016 and 1248 in monkeys also reported decreased birth weight (Allen and Barsotti 1976; Barsotti and Van Miller 1984). Studies with low doses of Aroclor 1254 (0.005–0.08 mg/kg/day) found no significant effects on anthropometric measures at birth or on growth thereafter (Arnold et al. 1995, 1997).

As indicated above, there is information regarding the effects of perinatal exposure to PCBs on immunocompetence in children. In a study of fisheating women from Sheboygan, Wisconsin (Lake Michigan), maternal serum PCB level (mean 5.48–5.76 ppb) was positively and significantly associated with the number of infectious illnesses in the infants ($r=0.33$, $p=0.03$), although breast milk PCB levels (mean 1.13 ppm) had a weak but significantly negative association with infant illnesses (Smith 1984). Possible associations between infectious illnesses and other chemicals in the fish were not investigated.

Susceptibility to infections and immune status were studied in 98 breast-fed and 73 bottle-fed Inuit (Eskimo) infants from Arctic Quebec, Canada (Dewailly et al. 2000). The Inuits have high body burdens

of various organochlorine compounds due to high consumption of marine foods, particularly sea mammal fat. Concentrations of PCBs and other chlorinated pesticides or metabolites were measured in early breast milk fat and used as an index of prenatal exposure to these substances; *p,p'*-DDE showed the highest mean concentration (962 ppb), followed by PCBs (621 ppb; sum of congeners 138, 153, and 180), hexachlorobenzene (107 ppb), dieldrin (30 ppb), and mirex (14 ppb) (Dewailly et al. 1993). The number of infectious disease episodes and status of immunologic parameters (WBCs, total lymphocytes and lymphocyte subsets, serum immunoglobulins) were evaluated during the first year of life. Acute otitis media was the most frequent health problem during the first year of life, with 80.0% of ever breast-fed and 81.3% of bottle-fed infants experiencing at least one episode. Relative risk (RR) analysis by follow-up period and number of episodes showed associations between increasing prenatal exposure to organochlorine compounds and otitis media that were more consistent for hexachlorobenzene and *p,p'*-DDE than PCBs. Because these and other detected organochlorine compounds originated from the same few food items and have concentrations in breast milk that are correlated with each other due to similar properties such as lipid solubility and persistence, the results precluded identification of which compounds could be responsible for the increased susceptibility to otitis media. Immunologic parameters that were significantly lower in the breast-fed babies compared to the bottle-fed group included numbers of WBCs and lymphocytes (CD4 subtype) at 3 months of age, and serum IgA concentrations at 7 and 12 months of age; CD4/CD8 lymphocyte ratios (helper T-cells/cytotoxic T-cells) were also reduced in the breast-fed infants at 7 and 12 months of age, although the change did not reach statistical significance. None of the immune parameters were associated with prenatal organochlorine exposure.

Immunologic effects of pre- and postnatal environmental exposure to PCBs and dioxins were assessed in a subgroup of 55 infants from the Dutch Mother-Child study (Weisglas-Kuperus et al. 1995). No correlation was found between pre- or postnatal exposure to PCBs/dioxin and the number of episodes of rhinitis, bronchitis, tonsillitis, and otitis during the first 18 months of life, or with humoral immunity as evaluated by antibody levels to mumps, measles, and rubella at 18 months of age (infants were immunized at 14 months of age). Determination of monocyte, granulocyte, and lymphocyte counts in cord and venous blood at 3 and 18 months of age showed that a higher prenatal as well as postnatal PCB/dioxin exposure was associated with lower monocyte and granulocyte counts at 3 months of age, and that a higher prenatal exposure was associated with increased total numbers of T-lymphocytes and several T-cell subpopulations ($CD8^+$, $TcR\alpha\beta^+$, and $TcR\gamma\delta^+$) at 18 months of age. There were no significant associations between postnatal PCB/dioxin exposure and T cell markers at 18 months of age. Although there were differences in the leukocyte subpopulation between high and low PCB/dioxin-exposed infants, all values were within the normal range (Weisglas-Kuperus et al. 1995). Follow-up

evaluations at 42 months of age, reported as a study abstract, found that prenatal PCB exposure was associated with increased T cell numbers and lower antibody levels to mumps, measles and rubella (Weisglas-Kuperus 2000). Additionally, PCB body burden at 42 months of age was reported to be associated with a higher prevalence of recurrent middle ear infections and chicken pox and a lower prevalence of allergic reactions.

Children born to mothers from the *Yu-Cheng* poisoning episode had higher prevalence of bronchitis or pneumonia at 6 months of age, respiratory tract infections at 6 years of age, and middle ear infections at 6–14 years of age (Chao et al. 1997; Yu et al. 1998). This group ingested rice oil accidentally contaminated with high concentrations of PCBs and other dioxin-like chemicals.

Results of studies in infant Rhesus monkeys from dams exposed during gestation and lactation to as low as 5 µg Aroclor 1254/kg/day indicated exposure-related reductions in antibody levels to SRBC and mitogen-induced lymphocyte transformation that paralleled the findings in the maternal animals (Arnold et al. 1995). Although assessment of the data is limited by small numbers of infants in the exposed groups, statistical significance was achieved for some end points and evaluation times, including reduced IgM titers at 22–23 and 61–63 weeks of age (following gestational/lactational and/or postweaning dietary exposure) in the infants from dams exposed to 5 µg/kg/day. Infant Rhesus and Cynomolgus monkeys that were orally administered a PCB congener mixture simulating the congener content of human milk at a dose level of 7.5 µg/kg/day for the first 20 weeks of life (i.e., from parturition without *in utero* exposure) had minimal immunological changes. These included uniformly reduced anti-SRBC titers in the treated monkeys compared to controls, although group differences were not statistically significant due to small numbers of animals; and decreased B lymphocyte numbers in the exposed Cynomolgus monkeys compared to controls, but this change was transient since levels returned to normal when monkeys were retested at 1 year of age (Arnold et al. 1999). Anti-SRBC titers were also uniformly reduced in the treated compared to control monkeys, although group differences were not statistically significant due to small numbers of animals. The only other notable immunologic effect was a decrease in B lymphocyte numbers in the exposed Cynomolgus monkeys compared to controls, but this change was transient since levels were similar at 1 year of age. The apparently weaker immunologic response in the infant monkeys exposed to the breast milk congener mixture compared to those exposed *in utero* and lactationally to Aroclor 1254 could be related to the lack of gestational exposure and different congener composition of human and monkey breast milk. These findings provide an indication that monkeys are sensitive to low doses of PCBs whether they are administered as commercial mixtures or as a mixture of congeners representative of those commonly found in breast milk.

The recognition of the importance of thyroid hormones in normal development of the brain, as evident from neurodevelopmental disorders and deficits associated with hypothyroidism, has triggered considerable interest in the thyroid hormone system (Boyages 2000). Hypothyroidism, typified by iodine deficiency (e.g., endemic cretinism), can produce a wide range of neurodevelopmental deficits, including auditory, motor, and intellectual deficits. These outcomes suggest an importance of thyroid hormones in the normal development of the fetal cochlea, basal ganglia, and cerebral cortex, which begin to develop in humans during the second trimester of gestation. This is also the time in which the fetal thyroid gland becomes functional. Key cell migration for brain development occurs prior to the time fetal thyroid produces T_3, and is therefore dependent on maternally produced thyroid hormone.

The results of several studies that examined relationships between indices of PCB exposure and thyroid hormone status in children have been mixed, with negative, positive, or no correlations observed. Evaluation of thyroid status of 105 mother/infant pairs from the Dutch cohort during the first months of life revealed that higher CDD, CDF, and PCB levels in breast milk, expressed as TEQs, correlated significantly with lower plasma levels of maternal total T_3 and total thyroxine, and with higher plasma levels of TSH in the infants in the 2nd week and 3rd month after birth (Koopman-Esseboom et al. 1994a). Infants exposed to higher dioxin TEQ levels also had lower plasma free and total thyroxine in the 2nd week after birth. It should be noted, however, that plasma total T_3, T_4, free T_4, and TSH levels of all mother-infant pairs were in the normal range. Longnecker et al. (2000) assayed umbilical cord sera from 160 children from the North Carolina cohort for total thyroxine, free thyroxine, and TSH. The cord blood had been stored frozen since its collection in 1978–1982. The investigators found that background-level exposure to PCBs had no effects on levels of thyroid-related hormones at birth. Since the exposure levels between the Dutch and the North Carolina cohorts appeared comparable, the difference in the results for TSH across studies is unclear.

A small (correlation coefficient, 0.15), but statistically significant positive correlation was found between total serum PCB and TSH concentrations in cord blood of 170 infants from the general population in Düsseldorf, Germany (Winneke et al. 1998a). Nagayama et al. (1998a) examined the relationship between serum TSH, total T_4, and total T_3 in 1-year-old infants and estimated intake of 2,3,7,8-TCDD TEQ in breast milk during the first year of postnatal life. The mothers had no known high exposure to PCBs. Small, but significant negative correlations were found for serum T_4 and T_3; no relationship was apparent between TEQ intake and infant serum TSH or TBG. The mean total dioxin TEQ intake was 34 ng/kg; however, the co-planar PCB contribution to the estimated TEQ intake, and intakes of other PCBs were not reported. Osius et al. (1999) examined the relationship between whole blood

concentrations of various PCB congeners and serum TSH, free T_4, and free T_3 in children who lived near a hazardous waste incinerator. All values were within expected ranges for children. A significant positive association was found between blood PCB 118 concentration and serum TSH concentration. Significant negative associations were found between serum T_3 and PCBs 138, 153, 180, 183, and 187.

Most studies that examined effects of PCBs on thyroid function in young animals have been conducted in rats that were administered commercial PCB mixtures or single PCB congeners during gestation and/or lactation. A common finding has been a decrease in circulating T_4 in both the dams and the fetus/offspring, whereas T_3 levels may or may not change (Corey et al. 1996; Goldey and Crofton 1998; Goldey et al. 1995; Juarez de Ku et al. 1994; Morse et al. 1996c; Provost et al. 1999; Schuur et al. 1998a; Zoeller et al. 2000). Goldey et al. (1995) reported neurobehavioral deficits in the pups, which were attenuated by subcutaneous injections of T_4 that increased serum T_4 and T_3 concentrations (Goldey and Crofton 1998). Rates of elimination of both hormones from serum were accelerated in the pups that had been exposed to Aroclor 1254, relative to controls. These observations suggest that the observed neurobehavioral deficits may have been attributable to deficits in thyroid hormone. The increased elimination of T_4 and T_3 from serum is consistent with an induction of UDP-GT or other elimination pathways for thyroid hormones (e.g., deiodination of T_4 to T_3). Reduction in T_4 levels in pups also have been induced by maternal administration of the dioxin-like congeners 3,3',4.4',5-pentaPCB (PCB 126) (Rice 1999a) and 3,3',4,4'-tetraPCB (PCB 77) (Darnerud et al. 1996a).

There is no evidence that PCBs are teratogenic in humans, and studies in rodents suggest that teratogenicity may occur, but only at very high doses (Haake et al. 1987; Zhao et al. 1997b). Adverse reproductive effects have been observed in male animals following perinatal exposure to PCBs. Fertility was markedly reduced in male offspring of rats that were lactationally exposed to $8 mg/kg/day Aroclor 1254 (Sager 1983; Sager et al. 1987, 1991). The reduction in male fertility appears to be due to impaired ability of sperm to fertilize eggs because sperm production, morphology, and motility were not affected and plasma FSH and testosterone concentrations were not reduced (Sager et al. 1987, 1991). Fertility was not impaired in the male offspring of rats that were administered 30 mg/kg/day of Aroclor 1221, 1242, or 1260 by gavage during gestation (Gellert and Wilson 1979), but this study did not include postnatal exposure. Results of oral and subcutaneous studies with single congeners have shown that gestational, lactational, or adult exposures can adversely affect morphology and production of sperm and fertility in male rats and mice (Faqi et al. 1998; Huang et al. 1998a; Smits-van Prooije et al. 1993), although congeneric structure-activity relationships are unclear. There were no significant effects on number of implantation sites or litter size in rats that were exposed to 4 mg/kg/day of a PCB congener

mixture simulating the congener content of human milk from 50 days prior to mating until birth (Hany et al. 1999b). Evaluation of the offspring, however, showed significantly increased relative uterine weight in immature females (PND 21) and reduced testes weights and serum testosterone levels in adult males (PND 170). There is increasing evidence that thyroid hormone serum concentrations play a crucial role in testicular development by binding to thyroid hormone receptors expressed in Sertoli cells during a critical window of time neonatally (Cooke et al. 1996).

PCBs have been shown to have both estrogenic and anti-estrogenic properties. These properties of some commercial PCB mixtures, PCB congeners, and hydroxylated derivatives of PCB congeners have been assayed by examining uterine variables in immature or ovariectomized female rodents, cell proliferation or gene expression variables in cultured cells including human breast cancer or HeLa cells, and *in vitro* binding to estrogen receptor preparations (see Andersson et al. 1999; Arcaro et al. 1999; Battershill 1994; Connor et al. 1997; Gierthy et al. 1997; Hansen 1998; Kramer et al. 1997; Krishnan and Safe 1993; Li and Hansen 1997; Moore et al. 1997; Safe 1999; Safe et al. 1998 for reviews). In general, PCB-induced estrogenic activities have been characterized as weak compared to the endogenous hormone, 17β-estradiol, a wide variability of responses has been observed across types of PCBs and assays indicating the involvement of multiple mechanisms, anti-estrogenic activities have been most strongly associated with PCBs that are Ah receptor agonists, and hydroxylated metabolites of PCBs are postulated to be at least partly responsible for physiological responses to PCBs that may involve changes in estrogen receptor-dependent physiological processes. Further details of some of these studies are presented in Section 3.6, Endocrine Disruption.

There is no information regarding possible transgenerational effects of PCBs in humans and limited information is available in animals. Dominant lethal mutations were not induced in male Osborne-Mendel rats following treatment by gavage with a single doses of 625–2500 mg/kg Aroclor 1242, by gavage with five daily doses of 125 or 250 mg/kg Aroclor 1242 or 75–300 mg/kg Aroclor 1254, or in the diet with estimated doses of 1.25 or 5 mg/kg/day Aroclor 1254 for 70 days (Green et al. 1975b). Lack of dominant lethality was indicated by no consistent changes in numbers of implantations and dead implantations per pregnant untreated female. The 70-day duration of the feeding study covered the spermatogenic cycle of the rat.

There is no information regarding the pharmacokinetics of PCBs in children or the nutritional factors that may influence the absorption of PCBs. Both phase I and phase II metabolic enzymes participate in the biotransformation and elimination of PCBs and metabolites. Because the metabolism of PCB congeners

depends not only on the degree of chlorination, but also on the chlorine substitution pattern, many different cytochromes P-450 (CYP enzymes) are involved. Thus, metabolism of PCBs in fetuses, neonates, and children may differ from adults depending on whether a particular cytochrome P-450 (CYP) subfamily is developmentally regulated or not. Phase II enzymes such as glucuronosyltransferases (UGT) and sulfotransferases also are involved in PCB metabolism and both are known to be developmentally regulated (Leeder and Kearns 1997). Because PCBs are lipophilic substances, they are stored in the mother's body and can be transferred to offspring through the placenta, as well as accumulate in breast milk and be transferred to nursing infants. This has been well documented by measurements of PCBs in both umbilical cord blood and breast milk (Fein et al. 1984a; Greizerstein et al. 1999; Jacobson et al. 1984b; Koopman-Esseboom et al. 1994b; Kostyniak et al. 1999; Mes et al. 1993; Rogan et al. 1987; Stewart et al. 1999). Placenta passage of PCBs is further evidenced by findings of significant correlations between maternal and cord serum PCB levels in groups of women and newborn infants (e.g., Jacobson et al. 1984b). Additionally, increased PCB residues were detected in blastocytes (day 6 postcoitum) from female rabbits administered Aroclor 1260 before insemination, but not in cleavage stage embryos (day 1 postcoitum) (Seiler et al. 1994). In pregnant mice fed PCBs through the first 18 days of gestation, the highest levels of serum PCBs were found in 1–2-week-old offspring compared with 18-day fetuses or with older offspring (Masuda et al. 1979). Results such as these have led to the conclusion that suckling may account for higher exposure of young offspring than does placental transfer, although the fetus may be more sensitive. Both prenatal and breast milk exposures have been associated with neurodevelopmental deficits in newborn and young children as discussed above. No PBPK models have been developed specifically for PCBs that could be used to quantitatively predict transfer of PCBs across the placenta or via breast milk.

Since adverse health effects are of concern, particularly for prenatal exposure to PCBs, Lackmann et al. (1999) investigated the influence of maternal age and duration of pregnancy on serum concentrations of PCBs in full-term neonates. Blood samples were taken from 80 full-term German neonates within the first 12 hours of life, before the first oral feeding. The median serum concentration of total PCBs was 0.96 µg/L (<0.30–3.14, range), with PCBs 138, 153, and 180 detected at median levels of 0.34 (<0.10–1.01), 0.42 (<0.10–1.42), and 0.17 (<0.10–0.78) µg/L, respectively. All detectable PCB congeners and total PCBs correlated significantly with the gestational age of the newborns, with 50–140% higher serum levels in children born at 42 weeks of gestation as compared with neonates born in the 38th week. Although the correlation between the PCB congeners and maternal age was not quite statistically significant, higher PCB concentrations were observed with rising age. PCB levels were not correlated with birth weight. As expected, the distribution pattern of the PCB congeners in newborns also

corresponds to that previously observed in adults. Thus, the neonatal body burden of PCBs depends on maternal age and duration of pregnancy, reflecting the increase in body burden with time as well as the continuous transplacental transfer of PCBs from mother to fetus during pregnancy.

Hagmar et al. (1998) measured PCB levels in whole blood, and cord blood from 30 Finnish women. The concentrations of PCBs 118, 138, 153, and 180 in cord blood were generally 2- to 3-fold lower than in the whole blood from the mothers. Positive correlations were observed between PCB concentrations in whole blood and cord blood (r=0.67–0.80). The correlation from this study was better than that reported earlier between PCB levels in maternal and cord serum in the Lake Michigan study (r=0.42; Jacobson et al. 1984b); however, the correlation was consistent with the findings in the Dutch study on delivering mothers (Koopman-Esseboom et al. 1994a, 1994b). Although the concentration of PCB congeners in cord blood is 2- to 4-fold lower than in maternal blood, cord blood represents a significant route for prenatal exposure to PCBs as confirmed by the direct measurements made on the serum of new full-term neonates (Lackmann et al. 1999).

Several human studies have investigated the levels of PCBs in human breast milk, not only because it offers a means to assess body burden, but also because it represents a significant route for maternal excretion and neonatal exposure.

In women not known to have been exposed to high concentrations of PCBs, Masuda et al. (1978) reported a significantly higher PCB level in infants' blood than in maternal blood; PCB levels in cord blood were lower than in maternal blood. These results suggested that larger amounts of PCBs are transferred through milk compared with placental transfer. Based on PCB levels in Canadian women's milk, it was estimated that after the first 14 days of breast-feeding, infants would have ingested 144 µg of PCBs, and their PCB body burden would be 0.32 ppm (Mes et al. 1984). The average PCB concentration in maternal whole blood was 2 ng/g (whole blood), whereas the average concentration in breast milk in 1982 was 26 ng/g (whole milk) (Mes et al. 1984). In 1986, the average PCB concentration in breast milk had declined to 6 ng/g (whole milk) (Mes 1994). Data summarized by Kimbrough (1995) indicate that in some industrialized countries an infant may accumulate 6.8% of its lifetime PCB body burden during a nursing period of 6 months.

Lanting et al. (1998a) measured the levels of PCB congeners 118, 138, 153, and 180 in cord plasma, breast milk, and plasma from 42-month-old children (n=126) living in the Groningen area, The Netherlands. In 42-month-old children who were fully breast-fed for at least 6 weeks, the median total

plasma PCB level was 0.81 µg/L (range: 0.23–2.2), compared to the formula-fed children that had levels of 0.18 µg/L (range: 0.07–1.49). The median total PCB concentration in breast milk was 11.9 µg/L (range: 3.3–28.2), while the levels of PCBs 118, 138, 153, and 180 were below the limit of detection in the formula milk samples. While the plasma level of each of these congeners increased in the breast-fed children between birth (cord blood) and 42-months of age, the formula-fed children exhibited a decrease in the plasma level of each of these congeners over this same time period. The relative abundance of PCB congeners was similar within samples of cord plasma, breast milk, and plasma at 42-months of age, with PCB 153>138>180>118. Based on regression analysis of the above data, Lanting et al. (1998a) proposed a model to estimate total PCBs in the plasma of 42-month-old children. Using this model, each week of additional breast-feeding is estimated to increase the $\Sigma 3PCB_{42mo}$ by 0.28% (SE=0.05%) of the $\Sigma 3PCB_{milk}$. For a mother with a median $\Sigma 3PCB_{milk}$ of 11.9 µg/L, as in this study, this results in an increase the $\Sigma 3PCB_{42mo}$ level of 0.033 µg/L per week of full breast-feeding.

Similar results were observed in 93 formula-fed and 100 breast-fed children at 3.5 years of age in the Rotterdam Area, The Netherlands (Patandin et al. 1997, 1999). $\Sigma 3$PCBs 118, 138, 153, and 180 in plasma of formula-fed children had a median level of 0.21 µg/L (range: 0.08–0.46), compared to the breast-fed group which had a median level of 0.75 µg/L (range: 0.23–5.9). PCB levels in maternal plasma (2.04 µg/L, range: 0.59–7.35) and cord plasma (0.40 µg/L, range: 0.08–2.08) were significantly correlated with the PCB levels at 3.5 years in the breast-fed and formula-fed groups. In the breast-fed group, PCB levels were significantly correlated with the period of breast feeding and milk PCB levels. A higher body weight of the child was significantly associated with lower plasma PCB levels at 3.5 years in both groups, suggesting that growth in body mass is diluting the plasma PCB level. With the assumptions that the half-life for plasma PCBs is 2.8 years in children (Yakushiji et al. 1984), and that dietary intake of PCBs after weaning is negligible, compared to prenatal and lactational exposure, it seems likely that plasma levels of PCBs in infants during breast-feeding are similar to that of their mother's.

Dietary exposure to dioxin-like coplanar PCBs (77, 126, 169) and PCDDs and PCDFs from infancy until adulthood was also estimated in this group of breast-fed and formula-fed children (Patandin et al. 1999). The $\Sigma 3$PCB 77, 126, and 169 in breast milk had a median level of 14.8 pg TEQ/g milk fat (range: 4.4–45.7), while the TEQ due to PCDDs and PCDFs in breast milk was 30.6 pg TEQ/g milk fat (range: 11.1–76.4). Thus, the coplanar PCBs contribute about one third of the total dioxin TEQs in human breast milk. The daily TEQ intake per kg body weight is about 50 times higher in breast-fed infants and 3 times higher in toddlers than in adults. Based on a model that included intake measures, food questionnaires, and national food consumption and contamination data, breast-feeding for 6 months contributed about

12% (boys) or 14% (girls) of the cumulative PCB/dioxin TEQ intake until 25 years of age. In toddlers, dairy products contribute 43% of the PCB-TEQ, meat and meat products contributed 14%, and processed foods 23%. Further information on exposures of children can be found in Section 6.6.

There are no biomarkers of exposure or effect for PCBs that have been validated in children or in adults exposed as children. There are no biomarkers in adults that identify previous childhood exposure. No studies were located regarding interactions of PCBs with other chemicals in children or adults. No information was located regarding pediatric-specific methods for reducing peak absorption following exposure to PCBs, reducing body burden, or interfering with the mechanism of action for toxic effects. In addition, no data were located regarding whether methods for reducing toxic effects in adults might be contraindicated in children.

3.8 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NAS/NRC 1989).

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very limited. A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid(s), or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to [substance x] are discussed in Section 3.8.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by polychlorinated biphenyls are discussed in Section 3.8.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.10 “Populations That Are Unusually Susceptible”.

3.8.1 Biomarkers Used to Identify or Quantify Exposure to Polychlorinated Biphenyls

PCBs are pervasive environmental contaminants that are found in body tissues and fluids of the general population. Because they are lipophilic and generally have half-lives longer than 1 week, PCBs are preferentially stored in adipose tissue and are present in serum, blood plasma, and human milk. Serum, including umbilical cord serum, and adipose tissues are indicators of exposure, but serum or plasma PCB concentrations can be significantly influenced by serum lipid content due to partitioning of PCBs between adipose tissue and serum lipids (Brown and Lawton 1984). Therefore, serum or plasma lipid PCB concentrations are better indicators of body burden than PCB levels uncorrected by lipid content (Brown and Lawton 1984). This was clearly illustrated in a study by Phillips et al. (1989b). These authors showed that the concentration of PCBs in nonfasting serum samples from 20 healthy adult males was 29% higher than in fasting serum samples. Total serum lipids (total cholesterol, free cholesterol, triglycerides, and phospholipids) were 20% higher in the nonfasting group. When the concentration of PCBs was corrected by total serum lipids, the difference between fasting and nonfasting samples was no longer statistically significant. Differences in metabolic profiles among different congeners will also influence the serum concentration at any given time. Variations in procedures and methods of reporting data can make interlaboratory comparison difficult (Jensen 1987). It should also be mentioned that, except for large exposures, blood should be collected quickly (days to weeks after exposure) if elevation

is to be found to document a given exposure. The lack of obvious elevation months to years after exposure does not, of itself, indicate lack of exposure.

Quantitative exposure to PCB mixtures can be estimated if the steady-state body burden and the elimination half-life for the mixture are known. In the simplest model, it is assumes that elimination of PCBs from the body can be described as a first-order process. Elimination half-lives of 6–7 months and 33–34 months were estimated for Aroclor 1242 and 1260, respectively, in two groups of capacitor workers (Steele et al. 1986). In a subsequent study, the same group of investigators (Phillips et al. 1989a) indicate that recalculation of the half-life for Aroclor 1242 yielded a median value of 1.9 years. This was comparable to a half-life of 2.6 years estimated in a different group of workers over a period of 8 years (Phillips et al. 1989a). In individuals exposed to river water contaminated with PCBs, it was estimated that the half-life elimination from blood for the PCB mixtures was . 8–9 months, whereas skin lipid PCBs had half-lives of 5 months (Jan and Tratnik 1988).

Short-term exposure to PCB mixtures that are rapidly eliminated may not result in the achievement of a steady-state blood level, in which case, the elimination half-life determined will be misleading. If a true half-life is substantially longer than the calculated half-life, the steady-state burdens may actually be higher than reported. On the other hand, an underestimate of half-life, given adequate steady-state body burden data, will result in an over-estimation of intake.

PCB congeners with a high degree of chlorination and congeners that lack unsubstituted *meta-para* positions are better candidates for bioaccumulation (see Section 3.4.3, Metabolism). This conclusion is consistent with the finding that congeners with unsubstituted 3,4 positions on at least one of the phenyl rings were found at a lower concentration in the blood and adipose tissue of capacitor manufacturing workers than those with substitutions in the 2,4 or 3,4 positions on both rings (Wolff et al. 1982a). This means that fatty tissues will preferentially accumulate the retained congeners leading to a different congeneric pattern compared with the original PCB source.

Eighty-nine PCB peaks were identified and confirmed in serum and adipose tissue of exposed workers (past and/or present exposure) and nonexposed subjects (Fait et al. 1989). Elimination of PCBs over time was inferred from the fact that the total PCB levels in adipose tissue of previously exposed workers were not significantly different than in nonexposed subjects. Congeneric composition of adipose tissue did not differ between previously exposed and nonexposed individuals indicating that single PCB congeners are not good indicators of previous exposure. However, the concentration of hepta- and octachlorobiphenyls

in the serum of previously exposed workers was significantly higher than in the comparison group and equivalent to the currently exposed group. Differences in serum levels of specific PCB congeners have been observed in individuals exposed to PCB mixtures occupationally, accidentally, or environmentally (Luotamo 1988). Differences in the concentrations of trichlorinated and tetrachlorinated isomers were found in the serum samples of the three groups. Only one pentachlorinated isomer was found in individuals environmentally exposed to PCBs, whereas five other pentachlorinated isomers were found in accidentally exposed individuals. The congeners that best indicated occupational exposure were 2,4,4N-triCB, 2,4,4N,5-tetraCB, 2,3,4,4N-tetraCB, and 2,3N,4,4N-tetraCB (Luotamo et al. 1993). Those that indicated accidental exposure were 2,4,4N-triCB, 2N,3,4-triCB, and 2,3N,4,4N-tetraCB (Luotamo et al. 1993). Thus, it would appear that in some cases isomer-specific monitoring of serum levels of PCB congeners in humans can determine likely exposure sources (Luotamo 1988).

PCB residue data in humans and other animals (see Section 3.4.2, Distribution) suggest that tissue or body burdens of PCBs should be based on individual congeners or groups of congeners and not on profiles of commercial PCB formulations. The simplest approach involves using one congener as a marker of total PCBs in a biological specimen. Levels of 2,2',4,4',5,5'-hexaCB (PCB 153), a very stable and often the most abundant congener, have been shown to correlate with the total amount of PCBs in human breast milk (Johansen et al. 1994) and human plasma, with a correlation coefficient of r=0.99 (Grimvall et al. 1997). PCB 153 was highly correlated (r=0.95) with total PCBs in 460 serum samples from Swedish men and women (Atuma and Aune 1999). PCB 153 was also highly correlated with total PCBs in serum (r=0.99) and follicular fluid (r=0.99) (Pauwels et al. 1999). In addition, PCB 153 levels correlated (r=0.91) with the total PCB-TEQs in human plasma (Grimvall et al. 1997). However, if a more complete profile of congeners is considered, the correlations are lower (Bachour et al. 1998; Hansen 1998, 1999). Total PCBs or PCB 153 as a marker of the total therefore could be a misleading indicator of the differential exposure to other individual or groups of congeners of toxicological significance.

Another important issue related to exposure biomarkers is whether analysis of PCBs in serum and adipose tissue provide comparable information on body burden. Stellman et al. (1998) measured 14 PCB congeners in adipose tissue and serum from 293 women with nonoccupational exposure. The relative patterns of the 14 PCB congeners were similar to those reported in other human studies. Significant positive serum to adipose correlation coefficients were obtained for PCBs 74, 99, 118, 138, 146, 153, 156, 167, 170, 180, 183, and 187, while PCBs 172 and 178 did not reach statistical significance. Thus,

this study supports the conclusion that either serum or adipose tissue PCB levels may serve as useful biomarkers of body burden and/or exposure.

Due to its high fat content, human milk concentrates PCBs, which are then transferred to children through lactation (EPA 1984d; Jacobson et al. 1984b). For example, among 122 mothers' milk samples in Massachusetts screened for PCBs, 4 had total PCB levels ranging from 1,100 to 2,400 ng/g milk fat, which were significantly higher than the group mean of 320 ng/g milk fat (Korrick and Altshul 1998). PCB levels in milk have been positively correlated with consumption of PCB-contaminated fish (EPA 1984d). With the use of high resolution analytical techniques, it has been possible to compare the congeneric composition of PCBs in milk with that of commercial PCB mixtures (Safe et al. 1985b). Gas chromatograms of human milk samples from Michigan did not resemble the pattern of any commercial mixture; however, several PCB congeners possessing common structural features, which rendered them metabolism resistant, were major components of both milk and Aroclor 1260 (Safe et al. 1985b). Conversely, other PCB congeners that are minor components of Aroclor 1260 were major components of the human milk. Yet, a different group of congeners, comprised only 28% of the PCBs present in Aroclor 1260, only composed 0.81% of the human milk PCBs; this latter group was formed by congeners having two adjacent unsubstituted carbons, which facilitates metabolic degradation (Safe 1989a). Burse et al. (1994) showed that PCB chromatograms of human serum matched the pattern of goats fed Aroclors better than Aroclor standards. This led the authors to suggest that some animal species could be useful in delineating the source of the PCB exposure in humans. In breast milk, most of the dioxin-like activity in the milk was due to the high concentrations of (coplaner) PCB congeners (Dewailly et al. 1991). Similar findings were reported for milk from Norwegian mothers (Johansen et al. 1994). In summary, highly chlorinated PCB congeners and congeners that lack unsubstituted *meta-para* positions constitute the most reliable biomarker of long-term exposure because they are metabolism resistant and, therefore, tend to accumulate in tissues. However, the specific PCB congeners or group of congeners to be used as exposure biomarkers will be dependent on the outcomes under study (e.g., immunological effects, reproductive end points, cancer).

Chloracne and other dermal alterations are well known markers of exposure to PCBs and structurally-related halogenated aromatic hydrocarbons (Rice and Cohen 1996). Chloracne and other dermal alterations have been reported in subjects occupationally exposed to PCBs (Bertazzi et al. 1987; Fischbein et al. 1979, 1982; Maroni et al. 1981a, 1981b; Meigs et al. 1954; Ouw et al. 1976, 1982; Smith et al. 1982) and in individuals exposed by accidental ingestion of rice oil contaminated with high concentrations of PCBs, CDFs, and related chemicals during the *Yusho* or *Yu-Cheng* poisoning incidents

(Guo et al. 1999; Kuratsune 1989; Lu and Wu 1985; Rogan 1989). Skin lesions consistent with those observed in exposed adults were also commonly observed in children born to mothers with *Yusho* or *Yu-Cheng* exposure (Funatsu et al. 1971; Gladen et al. 1990; Hsu et al. 1985; Rogan et al. 1988; Taki et al. 1969; Yamaguchi et al. 1971; Yoshimura 1974). No adverse dermal effects have been observed in subjects with high consumption of Great Lakes fish contaminated with PCBs and other environmentally persistent chemicals or in other cohorts from the general population. In general, chloracne appears in individuals with serum PCB levels 10–20 times higher than those of the general population, but there is great variability among individuals. Therefore, chloracne is not a sensitive (or specific) biomarker of PCB exposure.

3.8.2 Biomarkers Used to Characterize Effects Caused by Polychlorinated Biphenyls

Several studies of PCB-exposed workers and general population subjects attempted to correlate serum PCB levels with health indices. Statistically significant correlations of serum PCB levels with serum levels of liver-related enzymes (e.g., AST, ALT) and levels of serum lipids (cholesterol, triglycerides) have been reported in workers occupationally exposed to PCBs (Baker et al. 1980; Emmett et al. 1988a, 1988b; Fischbein 1985; Fischbein et al. 1979; Lawton et al. 1985a, 1985b; Smith et al. 1982). However, associations between serum PCBs and these hepatic effects are inconclusive due to small and inconsistent increases, lack of correction for confounding variables such as alcohol consumption, and other study limitations. Additionally, correlations between serum PCBs and lipids are influenced by partitioning of PCBs between lipids in adipose tissue and serum. This indicates that measurements of serum triglycerides and cholesterol are more useful for correcting serum PCB levels to more accurately reflect body burden than for detecting effects of PCBs. It must also be pointed out that PCB mixtures display different induction profiles, so that individual PCB congeners can be phenobarbital-type, 3-methylcholanthrene-type, or mixed-type mixed-function oxidase (MFO) inducers, or they may be inactive as enzyme inducers. Furthermore, the clinical significance of the alterations in liver-associated enzymes is uncertain, as the increases may be nonspecific and are often in the normal range, and indices of obstructive liver disorders have not been demonstrated even in occupationally exposed groups. The existing evidence in animals suggests that liver enzyme induction is perhaps the most sensitive biomarker of PCB effects, but it is nonspecific (Nims et al. 1992). MFO induction has been demonstrated indirectly in PCB-exposed workers by increased metabolic clearance of antipyrine (Alvares et al. 1977). The caffeine breast test (CBT) appears to be a sensitive method for characterizing exposure/and or effects of certain PCBs and related chemicals (Lambert et al. 1992). In this test, ^{13}C-methyl caffeine is ingested by subjects, and hepatic cytochrome P-4501A2-dependent caffeine 3-N-demethylase activity is monitored by

determining the amount of caffeine exhaled as radiolabeled CO_2. The CBT is not specific for PCBs since PCDFs, PCDDs, and other polyaromatic hydrocarbons also induce cytochrome P-4501A.

Results from a study with a feral mouse species showed that induction of hepatic EROD (CYP1A1-mediated activity) was a sensitive biomarker of effect (and/or exposure) for Aroclor 1254 (Lubet et al. 1992). Based on hepatic levels of the enzyme, the investigators could clearly distinguish between a population of mice living in a PCB-contaminated area and a population of the same species from a nonPCB reference site. Furthermore, the relative levels of the enzyme correlated well with hepatic PCB burdens. When the results based on feral mice were compared to results obtained in female Fischer 344/NCr rats exposed for acute or intermediate durations to Aroclor 1254 or in male $B6C3F_1$ mice exposed acutely to Aroclor 1254 in the laboratory, the order of responsiveness was Fischer 344/NCr > $B6C3F_1$ > feral mice. Results from a study by Nims et al. (1992) showed that increased CYP1A1 activity could be detected directly or indirectly in rats treated with relatively low doses of Aroclor 1254 (0.1 mg/kg/day for 7 days) in the diet. CYP2B1 activity was a much less sensitive indicator of effect (and/or exposure). It should be noted that induction of CYP1A1-mediated activity may also result from exposure to a variety of other environmental contaminants. Recently, a human hepatoma cell line, HepG2, was used to determine the dose response of various Aroclor mixtures as well as several dioxin-like PCB congeners (Anderson et al. 1995). In this assay, the human CYP1A1 gene was engineered such that, when activated by an inducer, produces luciferase instead of P-450. The reaction is then monitored by measuring luminescence and protein content. Of the seven Aroclor mixtures assayed, Aroclor 1260 produced the greatest induction. Aroclor 1016 and 1221 induced the lowest levels; for the remaining Aroclor mixtures, 1232, 1242, 1248, and 1254, induction did not correlate with the percentage of chlorination. The order of inducing potential for the congeners was 3,3',4,4',5-pentaCB >3,3',4,4',5,5'-hexaCB >2,3,4,4',5-pentaCB > 3,3',4,4'-tetraCB >2,3,3',4,4',5-hexaCB. Based on the results of the assays, the authors estimated that except for Aroclors 1016 and 1221, the approximate detection limit in environmental samples for the other Aroclors would be in the 2–4 µg/g range; for the congeners the detection limit was in the range 0.01–1 µg/g. For the purpose of comparison, for 2,3,7,8-TCDD the detection limit would have been 0.00005 µg/g.

Correlations between serum PCB levels and hypertension or various hepatic indices (e.g., serum enzymes and lipids) in people who were environmentally exposed to PCBs are also generally unclear due to confounding variables (Kreiss et al. 1981; Stehr-Green et al. 1986a, 1986b; Steinberg et al. 1986). These exposures involved consuming contaminated fish or living or working near an electrical manufacturing plant.

3.9 INTERACTIONS WITH OTHER CHEMICALS

As discussed in Section 3.5.2 (Mechanisms of Toxicity), PCBs represent a group of 209 structurally related chemicals with several subgroups displaying biological actions involving different potential mechanisms. Some biological activities of PCBs involve initial Ah-receptor mediated mechanisms (e.g., induction of hepatic CYP1A oxygenases and Phase II enzymes such as UDP glucuronyl transferases, epoxide hydrolases, or glutathione transferase, body wasting, thymic atrophy, and porphyria), other activities involve Ah-receptor independent mechanisms (e.g., induction of CYP2B and CYP3A oxygenases, induction of changes in brain dopamine levels, and disruption of Ca+2 homeostasis), and other biological activities of PCBs may involve both Ah-receptor dependent and independent mechanisms (e.g., liver hypertrophy, disruption of steroid hormone homeostasis or thyroid hormone homeostasis, disruption of immune functions, and induction and promotion of liver cancer). Because of this diversity in biological activities, there is a large potential for opportunities for PCB mixtures to alter the toxicity of other chemicals or other chemicals to alter the toxicity of PCBs.

Interactions Due to PCB Induction of Hepatic Enzymes

One type of interaction that received considerable early research attention involves PCB-induced changes in hepatic profiles of Phase I and II enzymes, leading to altered metabolism of other xenobiotic agents, and subsequent alteration of their toxicity. For example, observed effects of PCB pretreatment on toxicity of other chemicals in animals include increased metabolism and excretion of pentobarbital and decreased pentobarbital sleeping times (Villeneuve et al. 1972), increased genotoxicity of numerous carcinogens (e.g., benzo[a]pyrene) *in vitro* (Hayes 1987; Hutton et al. 1979), increased duodenal ulcerogenicity of acrylonitrile (Szabo et al. 1983), and increased renal toxicity of trichloroethylene (Kluwe et al. 1979). The capacity of PCB mixtures to induce cytochrome P-450 has resulted in increased toxicity of other chemicals whose toxicity depends on metabolic activation. For example, pretreating animals with PCB mixtures resulted in increased hepatotoxicity due to halothane, vinylidene fluoride, diethylnitrosamine, trichloroethylene, carbon tetrachloride, 1,1,2-trichloroethane, tetrachloroethylene, and 2,2,2-trifluoroethylvinyl ether (Conolly et al. 1979; Gans and Pintauro 1986; Kluwe et al. 1979; Moslen et al. 1977; Murphy et al. 1979; Sipes et al. 1987).

Interactions Between PCB Congeners, PCBs and CDDs, and PCBs, CDDs, and CDFs

Research in the 1970s and 1980s focusing on mechanistic similarities between PCBs, CDDs, and CDFs led to the development and use of a TEF approach to evaluating health hazards from complex environmental mixtures of these halogenated aromatic hydrocarbons. The approach relies on an assumption that components in these complex mixtures jointly act in an additive manner through a common Ah-receptor initial mechanism. Concern about this assumption has led to several investigations of possible interactions between specific PCB congeners, between some PCB congeners or PCB mixtures and 2,3,7,8-TCDD, and among PCBs, CDDs, and CDFs. Evidence for additivity and nonadditive interactions (e.g., potentiation or antagonism) has been found depending on the PCB congeners involved, end point examined, and dose levels of examined agents.

Interactions Between PCB Congeners. Observations of nonadditive interactions between specific PCB congeners include: 2,2',4,4',5,5'-hexaCB (10–50 mg/kg) antagonism of embryo malformations, edema, and liver lesions in chickens exposed to 2 μg/kg 3,3',4,4',5-pentaCB (Zhao et al. 1997a); 2,2',4,4',5,5'-hexaCB (271 mg/kg) antagonism of cleft palate formation in mice exposed to 0.78–1.04 mg/kg 3,3',4,4',5-pentaCB (Zhao et al. 1997b); 2,2',4,4',5,5'-hexaCB (18–72 mg/kg) antagonism of impairment of immune response in mice exposed to 6–12 μg/kg 3,3',4,4',5-pentaCB (Harper et al. 1995; Zhao et al. 1997b); synergism between 20 weekly subcutaneous doses of 5 mg/kg 2,2',4,4',5,5'-hexaCB and 1–10 μg/kg 3,3',4,4',5-pentaCB in promoting formation of γ-glutamyl transpeptidase-positive hepatic foci in partially hepatectomized rats initiated with 30 mg/kg nitrosodiethylamine (Bager et al. 1995); strong antagonism by 2,2',5,5'-tetraCB (10 or 25 μM) or 2,2',3,3',4,4'-hexaCB (12.5 or 25 μM) of luciferase expression induced by 3,3',4,4'-tetraCB (10 nM) in cultured recombinant Hepa1c1cc7 mouse hepatoma cell lines, but not in guinea pig GPC16 colon adenocarcinoma cells or human HepG2 hepatoma cells (Aarts et al. 1995); weak antagonism between 20 weekly subcutaneous doses of 0.13–6.6 μg/kg 3,3',4,4',5-pentaCB and 66–3,302 μg/kg 2,3,3',4,4'-pentaCB in promoting formation of γ-glutamyl transpeptidase-positive hepatic foci in partially hepatectomized rats initiated with 30 mg/kg nitrosodiethylamine (Haag-Grönlund et al. 1998; Johansson et al. 1999); and weak antagonism between 0.13–6.6-μg/kg doses of 3,3',4,4',5-pentaCB and 220–11,003-μg/kg doses of 2,2',4,4',5,5'-hexaCB in promoting formation of γ-glutamyl transpeptidase-positive hepatic foci, in changing concentrations of plasma retinol and liver retinoids, in increasing relative liver weight, and in inducing liver CYP2B1/2 activities (Haag-Grönlund et al. 1998; Johansson et al. 1999).

Interactions Between PCBs and CDDs. Acute parenteral administration of several commercial PCB mixtures (Aroclors 1242, 1248, 1254, and 1260) and a synthetic mixture of PCB congeners reflective of PCBs detected in human milk antagonized 2,3,7,8-TCDD-induced impairment of the immune response to SRBC in mice at PCB:TCDD dose ratios >1000:1 (Bannister et al. 1987; Davis and Safe 1989). Aroclor 1232 had no effect on TCDD-induced immunotoxicity in these studies (Davis and Safe 1989). Aroclor doses that antagonized the acute immunotoxicity of single doses of 0.0012 or 0.0036 mg/kg 2,3,7,8-TCDD ranged from about 1 to 50 mg/kg/day (Bannister et al. 1987; Davis and Safe 1989). Among seven individual PCB congeners examined for their ability to influence this immunotoxic action of single intraperitoneal doses of 0.0012 µg/kg 2,3,7,8-TCDD in mice (six hexachlorobiphenyls and one pentachlorobiphenyl with different chlorine substitution patterns), three were antagonistic (the 2,3,3',4,5,5'-, 2,3,3',4,5'- and 2,2',4,4',5,5'-congeners), and 4 showed no influence (the 2,3,3',4,4',5' -, 2,3',4,4',5',6-, 2,2',4,4',5,6'-, and 2,2',4,4',6,6'-congeners) (Biegel et al. 1989b; Davis and Safe 1990; Smialowicz et al. 1997). In these studies, doses of individual PCB congeners ranged from about 1 to 100–300 mg/kg.

Oral co-exposure of pregnant mice to 244 mg/kg Aroclor 1254 and 0.020 mg/kg 2,3,7,8-TCDD, at an Aroclor:TCDD dose ratio of 12,200:1, completely antagonized TCDD-induced cleft palate formation in offspring (Haake et al. 1987). The complexity of interactions between PCBs and TCDD-induced developmental toxicity is illustrated by observations that, among one tetrachlorobiphenyl and two hexachlorobiphenyl congeners examined, one (the 2,3,3',4,4',5-congener) potentiated TCDD-induced cleft palate formation (Birnbaum et al. 1985) and the other two (the 2,2',4,4'- and 2,2',4,4',5,5'-congeners) antagonized TCDD's actions (Biegel et al. 1989a, 1989b; Birnbaum et al. 1985; Morrissey et al. 1992). Antagonism of TCDD-induced cleft palate formation in mouse offspring by 2,2',4,4'-tetraCB and 2,2',4,4',5,5'-hexaCB showed complex (i.e., inverted U-shape) relationships with dose (Morrissey et al. 1992). For example, no antagonistic effect (against a TCDD dose of 0.0015 µg/kg) was produced by 10–20 mg/kg doses of 2,2',4,4',5,5'-hexaCB, but antagonism increased with increasing 2,2',4,4',5,5'-hexaCB dose to a maximum (500 mg/kg), and then declined to no antagonism at 1,000 mg/kg 2,2',4,4',5,5'-hexaCB (Morrissey et al. 1992). 2,2',4,4',5,5'- HexaCB also antagonized TCDD-induced hydronephrosis in mouse offspring showing a similar inverted U-shape relationship with dose (Biegel et al. 1989b; Morrissey et al. 1992). In contrast, combined exposure of pregnant mice to 2,3,3',4,4',5-hexaCB and 2,3,4,7,8-pentachlorodibenzofuran appeared to additively produce hydronephrosis and cleft palate in the offspring (Birnbaum et al. 1987).

Several 13-week oral exposure studies have examined possible binary interactions between three PCB congeners (at several dietary concentrations delivering daily doses ranging from about 0.1 to 10 mg/kg/day) and 2,3,7,8-TCDD (at several dietary concentrations delivering daily doses ranging from about 0.00003 to 0.3 mg/kg/day) in influencing several end points in rats (van Birgelen et al. 1992, 1994a, 1994b, 1996a; van der Kolk et al. 1992). The PCB:TCDD concentration ratios administered in these studies were selected to reflect relative concentrations in samples of human milk and fat. 2,2',4,4',5,5'-HexaCB and 2,3,7,8-TCDD showed joint additive action in decreasing thyroid hormone levels at 4 weeks, but synergistic action at 13 weeks (van Birgelen et al. 1992), whereas 2,3,3',4,4',5-hexaCB (van Birgelen et al. 1994a) and 3,3',4,4',5-pentaCB (van Birgelen et al. 1994b) showed less-than-additive joint action with 2,3,7,8-TCDD in decreasing thyroid hormone levels. 2,2',4,4',5-Hexachlorobiphenyl did not influence TCDD-induced effects on body weight and thymus weight, and additively increased relative liver weight with TCDD (van der Kolk et al. 1992), whereas 2,3,3',4,4',5-hexaCB and 3,3',4,4',5-pentaCB showed less-than-additive joint action with TCDD on these end points. 2,2',4,4',5,5'-hexaCB and 2,3,7,8-TCDD showed a distinct synergism in increasing hepatic porphyrin levels, but 2,3,3',4,4',5-hexaCB and 3,3',4,4',5-pentaCB showed no such synergism with 2,3,7,8-TCDD (van Birgelen et al. 1996a). All three of these congeners individually decreased hepatic levels of retinol and retinylpalmitate. In combination with TCDD, less-than-additive joint actions were noted, but TCDD doses used in these studies produced a near maximal response in decreasing retinoid levels (van Birgelen et al. 1992, 1994a, 1994b).

Interactions Among PCBs, CDDs, and CDFs. Liver tumor promotion activity was examined in partially hepatectomized rats exposed to a mixture containing 68 ppm 2,3,7,8-TCDD, 223 ppm 1,2,3,7,8-pentachloro-*p*-dioxin, 1,151 ppm 2,3,4,7,8-pentachlorodibenzofuran, 4,130 ppm 3,3',4,4',5-pentaCB, 866,604 ppm 2,3',4,4',5-pentaCB, and 127,824 ppm 2,3,3',4,4',5-hexaCB and compared with predicted tumor promotion activity using TEFs based on tumor promotion activity of the individual components compared to TCDD activity (Van der Plas et al. 1999). The mixture composition was reflective of relative concentrations, and accounted for about 90% of total TCDD TEQs, found in samples of Baltic Sea fish. Observed tumor promotion activity of the mixture was about one-half of predicted activity. Another mixture, containing, in addition to the above components, 20,000 g 2,2',4,4',5,5'-hexaCB per g of 2,3,7,8-TCDD, showed a tumor promotion activity that was also less than that predicted by the TEF approach. A possible explanation of the differences between the observed and TEF predicted values is that the components may have interacted in a less-than-additive manner (e.g., less potent PCBs may antagonize tumor promotion by the more potent 2,3,7,8-TCDD), but equally as plausible is the possibility that the TEFs are inaccurate and overestimate tumor promotion potencies (van

der Plas et al. 1999). In related studies, 2,2',4,4',5,5'-hexaCB antagonized TCDD promotion of malignant transformations of carcinogen-initiated mouse fibroblasts (Wolfe 1998), whereas 3,3',4,4',5-pentaCB added to promotion of fibroblast transformation in the presence of 2,3,7,8-TCDD (Wolfe 1998) and to promotion of liver tumors in rats with co-exposure to 2,3,7,8-TCDD (Hemming et al. 1985).

The possibility that interactions among PCBs, CDDs, and CDFs may influence reproductive end points (blockage of ovulation, reduction of ovarian weight gain, and changes in preovulatory hormone levels) was examined in gonadotropin-primed immature female rats given single oral doses of 0.057–0.457 mg/kg 3,3',4,4',5-pentaCB (a PCB with known Ah receptor agonist activity) or 0.010–0.160 mg/kg 2,3,4,7,8-pentachlorodibenzofuran alone or together in combination with 2,3,7,8-TCDD, 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin, and 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin, all of which were shown to be effective blockers of ovulation in this assay (Gao et al. 1999, 2000). The mixture was administered at TCDD-TEQ doses ranging from 0.0038 to 0.0303 mg/kg. Parallel dose-response relationships for inhibition of ovulation were found for the individual agents and for the equipotent mixture. This finding is consistent with the hypothesis that the agents in the tested mixture are likely to block ovulation by additive joint action in a similar mechanism and supports the use of the TEF approach for this type of endocrine disruption. Another PCB congener, 2,2',4,4'-tetraCB (which has no detectable Ah receptor agonist activity), was inactive at the dose examined in this assay (41.9 mg/kg). The effect of its presence in a mixture with effective components, however, was not studied (Gao et al. 2000).

Interactions Between PCBs and Methylmercury

PCBs and methylmercury represent a combination of agents of public health concern that are potential neurotoxicants found in the complex mixture of biopersistent toxicants in contaminated fish from the U.S. Great Lakes and the Baltic Sea. Changes in neurological function or development from PCBs and methylmercury have been proposed to at least partly involve disruption of calcium homeostatic mechanisms in neural cells leading to changes in neurotransmitter release (e.g., dopamine) or cell damage. Exposure of rat striatal tissue for 4 hours with methylmercury alone at concentrations ranging from 1 to 40 µM or a 1:1 mixture of Aroclors 1254 and 1260 at concentrations ranging from 10 to 200 ppm resulted in a significant, dose-dependent depletion of tissue dopamine levels (Bemis and Seegal 1999). Bemis and Seegal (1999) noted that these concentrations were, in general, higher than those measured in samples of Great Lakes fish (0.84–1.9 ppm PCBs and 0.34 ppm mercury). Combined *in vitro* exposure of rat striatal tissue to methylmercury and this 1:1 mixture of Aroclors 1254 and 1260

(10–200 ppm) synergistically depleted tissue levels of dopamine (Bemis and Seegal 1999). For example, combined exposure to 4 µM methylmercury and 200 ppm PCBs, or 10 µM methylmercury and 20, 40, 100, or 200 ppm PCBs, showed a significant statistical interaction (in an analysis of variance) indicative of synergistic effects on depleting tissue dopamine levels. These results suggest a possible synergism in affecting neurological dysfunction and development, but no *in vivo* demonstration of such a synergism is available.

A study of combined oral exposure of pregnant female mice to methylmercury (0.4 or 4 mg/kg/day) and Kanechlor 500 (about 940 mg/kg/day) from gestation day 15 to day 21 after delivery found no evidence for obvious synergistic effects on righting and swimming ability, hindlimb support, general open field activity, and learning ability in offspring evaluated at several postnatal periods or on reproductive performance in the F0 and F1 generations (Tanimura et al. 1980). Methylmercury, at 4 (but not 0.4) mg Hg/kg/day, potentiated PCB-induced decreased postnatal survival in mice (Tanimura et al. 1980). Survival of male offspring in all Kanechlor groups showed a marked decline, compared with controls, at about 5 weeks after birth; at 10 weeks after birth, male offspring survival percentages were about 60, 60, and 40% for the groups with Kanechlor plus 0, 0.4, and 4 mg Hg/kg, compared with >90% in the control and methylmercury alone groups. Autopsies revealed no obvious or specific cause of death. Survival data for female offspring were reported to have been similar.

In a study of minks, reproductive end points, serum thyroid hormone levels (T3 and T4), and histology of brain, kidney, adrenals, pituitary, and thyroid were evaluated in groups of adult ranch-bred minks fed a commercial mink food supplemented with 0 or 1 ppm Aroclor 1254, 1 ppm Hg as methylmercury, 1 ppm Aroclor 1254 + 1 ppm methylmercury, or 0.5 ppm Aroclor 1254 + 0.5 ppm methylmercury for 8 months that spanned one breeding period (December 1984 through June 1985) (Wren et al. 1987a, 1987b). Exposed groups contained 12 females and 4 males; control groups had 15 females and 5 males. Food intake and body weight data were not reported, but estimates of 0.2 mg/kg/day Aroclor 1254 and 0.2 mg Hg/kg/day are derived for the 1-ppm treatment based on a food intake of 150 g/day and body weight of 0.9 kg for minks (Aulerich et al. 1987). During the third month of exposure, eight females and one male in the 1 ppm methylmercury group, and three females in the 1 ppm Aroclor + 1 ppm methylmercury group, died, displaying convulsions, tremors, and lethargy. The mortality was attributed to a combination of cold stress and methylmercury poisoning, and surviving minks were fed diets containing 1 ppm methylmercury every other day for the remainder of the study. No exposure-related effects were found on the thyroid, pituitary, adrenal glands, or serum T_4 or T_3 levels in adult minks that survived the 8-month exposure period. Fertility of adult male minks, percentage of females whelped, or number of offspring

born per female were not significantly affected by any of the treatments. The average number of offspring per female at weaning (5 weeks after birth) was significantly ($p< 0.05$) lower in the 1 ppm Aroclor + 1 ppm methylmercury group (2.1 offspring/female) than in the control (4.5), 1 ppm Aroclor (5.0), 1 ppm methylmercury (4.0), or 0.5 ppm Aroclor + 0.5 ppm methylmercury groups (3.6), indicating that postnatal offspring mortalities were increased by combined exposure to the high levels of methylmercury and Aroclor 1254.

Wren et al. (1987b) concluded that these observations showed a synergistic effect of Aroclor 1254 and methylmercury on decreased postnatal survival of mink offspring. An alternative interpretation of the results is that combined exposure induced postnatal mortality at concentrations of the individual agents (1 ppm) that did not induce postnatal mortality, but it is not possible to discern if they acted together in a less-than-additive, additive, or greater-than-additive manner without including treatments involving 2 ppm concentrations of the individual agents alone. A clear demonstration of synergistic action would have involved increased postnatal mortality produced by the 0.5 ppm Aroclor + 0.5 ppm methylmercury treatment; however, postnatal mortality was not changed, compared with control, by this treatment.

Intermediate-duration exposures of quail to methylmercury or Aroclor 1260 in the diet led to accumulation of porphyrins in liver; hepatic porphyrin levels in quail exposed to both agents simultaneously were similar to levels predicted based on additivity of response (Leonzio 1996b). Combined exposure of rats or quail to commercial PCB mixtures and methylmercury appears to counteract PCB induction of hepatic CYP enzymes (Leonzio et al. 1996a; Takabatake et al. 1980), but the toxicological significance of this interaction is unclear.

Interactions Between PCBs and *p,p'*-DDE

Results from animal (and some human) studies identify several sensitive shared targets of PCBs and *p,p'*-DDE oral toxicity including the liver (hepatomegaly, degenerative histological effects, and liver cancer), immune system (suppression of cell-mediated immunological responses), neurological development (altered neurobehavior in offspring exposed *in utero* or during nursing periods), and altered reproductive function or development. A limited amount of *in vitro* and *in vivo* data regarding possible interactions between PCBs and *p,p'*-DDE are available as reviewed below.

Incubation of an estrogen receptor preparation from alligator oviducts with a mixture containing 18 µM *p,p'*-DDE, 2.6 µM *p,p'*-DDD, 0.63 µM dieldrin, 0.53 µM Aroclor 1242, 0.25 µM *trans* nonachlor,

0.16 µM *cis* nonachlor, 0.22 µM chlordane, and 0.2 µM toxaphene inhibited the binding of tritium-labeled 17β-estradiol to estrogen receptors by 57% (Vonier et al. 1996). The individual agents, at the concentrations used in this mixture, did not inhibit the *in vitro* binding of 17β-estradiol to the estrogen receptors, with the exception that 2.6 µM *p,p'*-DDD inhibited binding by 20%. Vonier et al. (1996) concluded that combinations of these chemicals decreased estradiol binding "in a greater-than-additive manner." Design limitations of this study, however, preclude drawing definitive conclusions whether the mode of joint toxic action among these chemicals in this screening assay was less-than-additive, additive, or greater-than-additive.

Combined dietary exposure of mallards to 40 ppm *p,p'*-DDE and Aroclor 1254 did not alter DDE-induced egg shell thinning, but appeared to decrease the number of intact eggs that were produced compared with values for control groups or groups exposed to either agent alone (Risebrough and Anderson 1975). Dietary exposure of groups of mallards (4 drakes and 10 hens) to 40 ppm *p,p'*-DDE or 40 ppm *p,p'*-DDE + 40 ppm Aroclor 1254 for 5 months caused 17 and 19%, respectively, reduction in mean egg shell thickness compared with control groups (Risebrough and Anderson 1975). Exposure to 40 ppm Aroclor 1254 alone did not affect egg shell thickness. Combined exposure reduced total egg production over the study period by about 35% compared with controls. Egg production in the first 7 weeks was similar in all groups, but markedly dropped thereafter in the DDE+Aroclor 1254 group. About 25% of the decline in egg production in the combined exposure group was attributed to egg eating. Further information or studies regarding this apparent synergism between *p,p*'-DDE and Aroclor 1254 were not located. Additional research may help to determine if a similar synergism may occur between *p,p'*-DDE and PCBs in affecting reproductive function in mammals.

Interactions Between PCBs and Other Chemicals

An initial report (Arnold et al. 1996) that binary mixtures of hydroxylated PCBs and weakly estrogenic pesticides (dieldrin, endosulfan, toxaphene, and chlordane) resulted in synergistic increases in estrogen receptor binding and reporter gene-expression in transfection-facilitated yeast and endometrial carcinoma-derived cell cultures was subsequently withdrawn by the investigators due to the inability to reproduce the results (McLachlan 1997). A subsequent examination of possible synergy among binary mixtures of two hydroxylated PCBs (2,4,6-trichloro-4'-biphenylol and 2,3,4,5-tetrachloro-4'-biphenylol) and two pesticides (endosulfan and dieldrin) in two *in vitro* estrogenic activity assays (competitive estrogen receptor binding and induction of multicellular nodules in human cancer-derived MCF-7 cells) found no evidence for synergy between these hydroxylated PCBs, between these pesticides, or between

2,4,6-trichloro-4'-biphenylol and physiologically relevant concentrations of 17β-estradiol (Arcaro et al. 1998). Likewise, no evidence for obvious synergy was found between tributyltin (50 nM) and 3,3',4,4',5-pentaCB (100 nM) or Aroclor 1016 (50 ppm) in inhibiting human natural killer cell *in vitro* lytic actions against leukemia cells (Whalen et al. 1998) or among methylmercury (0.1–2 µg/mL), a CDD/CDF mixture of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin, 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin, 2,3,7,8-tetrachlorodibenzofuran, and 1,2,3,7,8-pentachlorodibenzofuran (1–15 pg/mL), and three commercial PCB mixtures, Aroclor 1242, 1254, and 1260 (0.01–0.5 µg/mL) in altering several *in vitro* activities (mixed leukocyte reaction, natural killer cell activity, and phagocytic activities) of rat leukocytes (Omara et al. 1998).

Simultaneous exposure of rats to Aroclor 1254 or 1260 and chemicals of environmental concern such as the pesticides mirex, photomirex, and/or kepone in the diet resulted in increased severity of the liver lesions attributed to exposure to chlorinated biphenyls alone (Chu et al. 1980). Induction of hepatic AHH activity by Aroclor 1254 in the diet of lactating rats was increased in an additive manner by simultaneous dietary exposure to polybrominated biphenyls such as Firemaster BP-6 (McCormack et al. 1979).

The induction of liver carcinogenesis by Aroclor 1254 in C57BL/10ScSn mice is markedly increased by iron (Madra et al. 1995). A single dose of iron dextran in mice fed Aroclor resulted in a significant increase in octoploid nuclei within 2 weeks; it persisted for 6 months and resulted in massive hepatic porphyria. In another study (Madra et al. 1996), the iron-enhanced toxicity appears to be due to the induction of P-450 1A1 isoforms in the nuclear membrane as well as in the microsomes.

Pretreatment of rats with Aroclor 1254 protected against hepatotoxicity due to inhalation of 1,1-dichloroethylene, suggested that MFO induction by PCBs may be responsible for detoxification of 1,1-dichloroethylene (Reynolds et al. 1975). This detoxification might occur if the epoxide of 1,1-dichloroethylene isomerizes rapidly to an aldehyde before reacting with tissues.

Increased dietary ascorbic acid may protect against some of the toxic effects of PCBs, such as altered enzyme activity and liver histopathology, perhaps by inhibiting lipid peroxidation (Chakraborty et al. 1978; Kato et al. 1981a). The exact mechanism is not known.

Co-administration of cadmium and Aroclor 1248 resulted in a significant increase in growth retardation and plasma cholesterol, compared to controls or rats fed a diet containing cadmium or Aroclor 1248 alone. The effects were found to be additive (Suzuki 1980).

Pretreatment of rats with Aroclor 1254 markedly accelerated the biotransformation and bioactivation of the industrial chemical 2,6-dinitrotoluene (Chadwick et al. 1993). This resulted in an increased formation and excretion of mutagenic metabolites in the urine. Also, Aroclor 1254 potentiated the formation of 2,6-dinitrotoluene-derived DNA adducts in the liver.

PCBs can interact with structurally diverse carcinogens in various ways. Oral studies have shown that Aroclor 1254 and other PCBs with similar percentages of chlorine by weight (e.g., Kanechlor 500, Clophen A50) promote the development of liver preneoplastic foci, liver tumors, and lung tumors in rats or mice that have been treated with other carcinogens as initiators, including nitrosamines and 2-acetylaminofluorene (Anderson et al. 1986; Deml and Oesterle 1987; Kimura et al. 1976; Oesterle and Deml 1983, 1984; Pereira et al. 1982; Preston et al. 1981; Tatematsu et al. 1979). PCBs also can enhance or inhibit the activity of other hepatocarcinogens when simultaneously administered orally (Ito et al. 1973; Kimura et al. 1976; Makiura et al. 1974). There is no conclusive evidence that Aroclor is a skin tumor promoter when repeatedly applied to the skin of mice that were initiated with DMBA or MNNG (Berry et al. 1978, 1979; Poland et al. 1983), but a single dermal application of Aroclor 1254 to mice showed weak initiator activity when promoted with TPA (DiGiovanni et al. 1977). Pretreatment with a single dermal dose of Aroclor 1254 inhibited skin tumor initiation by DMBA in mice (Berry et al. 1979). Intraperitoneal injection of Aroclor 1254 to mice on Gd 19 protected the offspring from lung tumors, but increased the incidence of liver tumors, following injection of N-nitrosodimethylamine on postnatal day 4 or 14 (Anderson et al. 1983). The genotoxicity of numerous carcinogens is potentiated *in vitro* by PCBs, but this does not indicate that PCBs should be regarded universally as tumor promoters because of the protective role of PCBs against carcinogenicity of many genotoxic carcinogens *in vivo* (Hayes 1987).

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to PCBs than will most persons exposed to the same level of PCBs in the environment. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters result in reduced detoxification or excretion of PCBs, or compromised function of organs affected by PCBs. Populations who are at greater risk due to their unusually high exposure to PCBs are discussed in Section 5.7, Populations With Potentially High Exposures.

The potential susceptibility of embryos, neonates, and children are discussed in detail in Section 3.7, Children's Susceptibility.

Other subpopulations that are potentially more susceptible to PCBs include those with incompletely developed glucuronide conjugation mechanisms (Calabrese and Sorenson 1977; Lester and Schmid 1964), such as those with Gilbert's Syndrome. Gilbert's Syndrome is a relatively common and benign congenital liver disorder that is characterized by mild, fluctuating increase in serum bilirubin, and is estimated to occur in 3–7% of the adult population (American Liver Foundation 2000). Persons with hepatic infections may have decreased glucuronide synthesis, making them more sensitive because of their decreased capacity to detoxify and excrete PCBs (Calabrese and Sorenson 1977). Those with compromised liver function, such as in the case of liver cirrhosis or hepatitis B, could also be considered more susceptible to PCB toxicity. PCBs, via induction of ALA synthetase, might be capable of precipitating an attack of porphyria in patients with acute intermittent porphyria.

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to PCBs. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to PCBs. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice. No texts were found that provided specific information about treatment following exposures to PCBs.

3.11.1 Reducing Peak Absorption Following Exposure

Human exposure to PCBs can occur by inhalation, ingestion, or by dermal contact. PCBs are readily absorbed through the gastrointestinal tract, respiratory system, and skin. Data from animal studies suggest that the rate of absorption following oral exposure is greater than that following inhalation or dermal exposures. Specific information regarding the prevention or reduction of toxicological effects following acute exposure to PCBs was not located in the literature. General recommendations for reducing absorption of PCBs following acute exposure include removal of contaminated food, water, air, and/or clothing from the exposed individual. Multiple washes of contaminated skin with soap and water immediately following dermal exposure to PCBs have also been recommended (HSDB 1995). Washing is most effective immediately following exposure, since PCBs are readily absorbed through the skin.

Trichlorobenzene and mineral oil have been found useful in decontaminating exposed areas of skin in rats (Wester et al. 1990). However, using hydrocarbon-based solvents to cleanse PCB-contaminated skin could carry the risk of increasing the dermal absorption of those fat-soluble compounds in humans.

Many of the clinical symptoms that result from PCB exposure, such as chloracne, are delayed in onset. Therefore, ingestion of PCBs is normally not recognized until long after the time when inducing emesis might be beneficial. In addition, emesis may result in aspiration of the lipid materials into the lungs, possibly causing lipoid pneumonitis. The value of administering activated charcoal to decrease the absorption of PCBs is unknown, but is frequently recommended as a slurry, either aqueous or mixed with a saline cathartic or sorbitol (HSDB 1995). Repetitive administration of activated charcoal might be useful in preventing reabsorption of metabolites. In rats, rice bran fiber decreased absorption of PCBs in the intestinal tract and had a stimulatory effect on fecal excretion of PCBs (Takenaka and Tarahashi 1991). It is unclear that rice bran would be of benefit in PCB-poisoned humans.

3.11.2 Reducing Body Burden

There are no known treatment methods for reducing body burden of PCBs. It should be noted that significant amounts of PCBs can be eliminated through lactation (see Section 3.7, Children's Susceptibility), indicating that breast feeding can reduce maternal body burden of PCBs. However, in most cases, the benefits of breast feeding outweigh any possible PCB risks to the mother from the body burden or to the child from exposure via the milk.

Limiting or preventing further exposures appears to be the most practical method for reducing body burden of PCBs. For the general population, especially subgroups that consume diets high in contaminated fish (e.g., sport fisherman), this can be achieved through public health advisories on fish consumption. A listing of fish advisories for PCBs is provided in Chapter 8 (Table 8-1).

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

No specific information was located regarding clinical methods of interfering with PCB mechanisms of toxic action. Some of the toxic effects, such as immunological effects, body weight loss, enzyme induction and porphyria, appear to be mediated by a common initial mechanism involving the Ah cytosolic receptor (Poland et al. 1976; Safe 1984, 1990), as discussed in Section 3.5.2 (Mechanisms of Toxicity). The responsiveness of a particular organ to PCB congeners may depend on the presence of

functional Ah receptors. PCB binding to the Ah receptor is followed by a series of events that lead to the accumulation of occupied nuclear receptor complexes and enhanced CYP1A gene expression. Although speculative, it is possible that interference with this mechanism may lead to a more specific treatment for reducing some of the toxic effects of PCB congeners that exert this mechanism of toxic action. Future research on Ah receptor antagonists may provide new insight for clinical treatment of PCB Ah receptor-mediated toxicity, but at present, only symptomatic and supporting therapy is available for PCB-exposed humans.

Toxic effects of PCBs may also involve Ah-receptor independent mechanisms, or both Ah-receptor dependent and independent mechanisms (see Section 3.5.2). For example, PCBs can be metabolized to reactive arene oxide intermediates that may alkylate critical cellular macromolecules and result in injury (Gardner et al. 1973; Safe 1990). Clinical intervention to interfere with Ah-receptor independent mechanisms have not been developed.

In summary, presently no specific treatments are available for patients with acute or long-term exposure to PCBs. Additional research is necessary to develop specific methods to mitigate PCB toxicity.

3.12 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of polychlorinated biphenyls is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of polychlorinated biphenyls.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

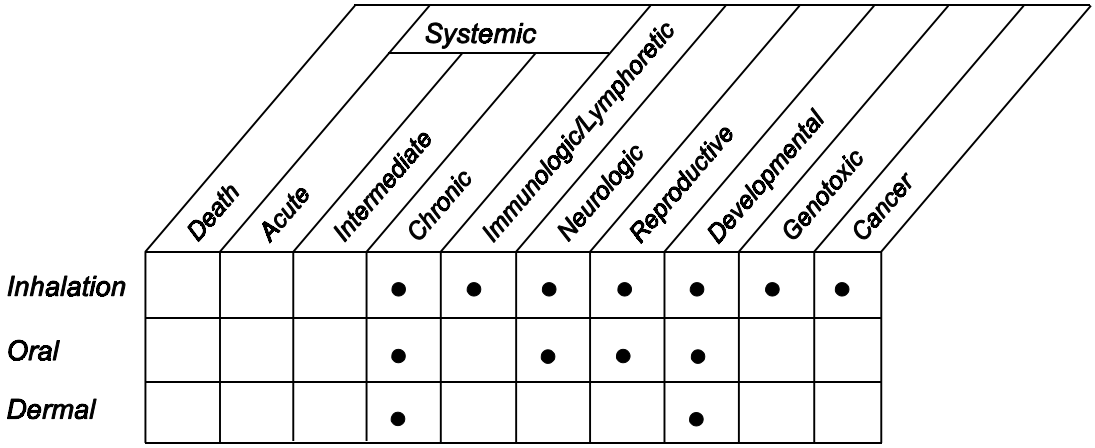
3.12.1 Existing Information on Health Effects of Polychlorinated Biphenyls

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to polychlorinated biphenyls are summarized in Figure 3-5. The purpose of this figure is to illustrate the existing information concerning the health effects of polychlorinated biphenyls. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be interpreted as a "data need". A data need, as defined in ATSDR's *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (ATSDR 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

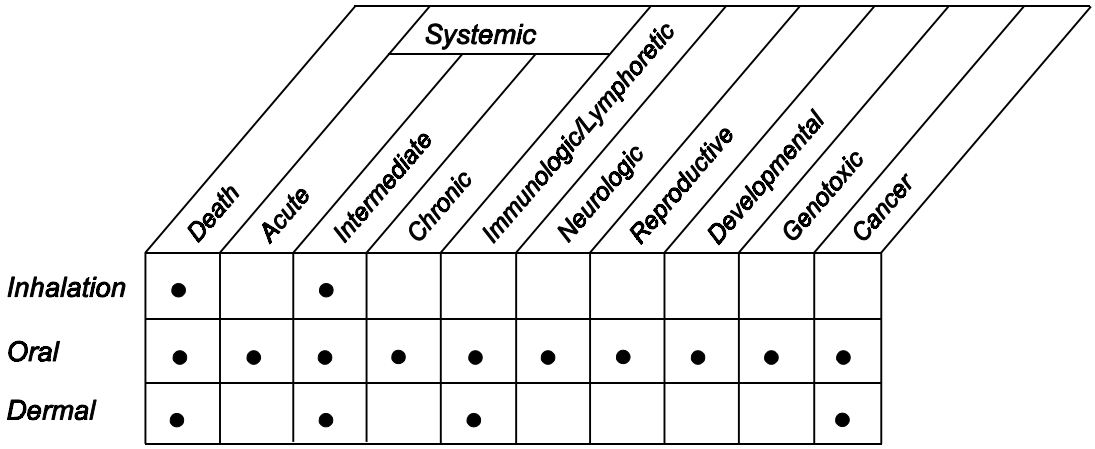
This section identifies information needs regarding PCB mixtures which, if met, would contribute to a more precise association between levels of exposure at hazardous waste sites and adverse health effects. Most of the information evaluated in this report has been obtained from studies in which commercial PCB

mixtures were used, and this is the most practical basis for hazard evaluation. People are environmentally exposed to PCB mixtures of different congeneric composition than commercial PCB mixtures. Although the toxicity of environmental PCB mixtures consequently may be increased or decreased compared to commercial mixtures, there are insufficient mixture toxicity data on which to assess hazards and directly base minimal risk levels for environmental PCBs. One approach that has been widely considered for estimating the risk from environmental exposure to PCBs is the TEF method. As discussed in Section 3.5.2, the TEF approach can be used to estimate the potency of PCB mixtures by comparing the relative toxicity of individual PCB congeners to that of 2,3,7,8-TCDD, which is the most toxic and extensively studied of these structurally-related halogenated aromatic hydrocarbons. Although TEFs are used to some extent to guide public health decisions because of the limited toxicological data for complex environmental mixtures and many of their components, the approach has received limited validation and has a number of limitations related to assumptions that the components jointly act in an additive manner through a common Ah-receptor mechanism of toxicity. In particular, the TEF approach does not account for evidence that non-Ah-receptor-binding congeners are major components in PCB-containing environmental mixtures that may contribute to induction of health effects. Due to evidence of non-additive interactions between specific PCB congeners and between some PCB congeners and 2,3,7,8-TCDD (see Section 3.9), as well as increasing evidence that PCB-induced effects may involve

Figure 3-5. Existing Information on Health Effects of Polychlorinated Biphenyls



Human



Animal

● *Existing Studies*

Ah-receptor-dependent mechanisms, Ah-receptor-independent mechanisms, or both Ah-receptor-dependent and Ah-receptor-independent mechanisms (see Section 3.5.2), the accuracy of the TEF approach is questionable. Due to the current lack of any alternative validated congener-based risk assessment methodology, and considering the likelihoods that (1) multiple mechanisms are involved in PCB-induced health effects, (2) different PCB congeners may produce effects by different mechanisms, and (3) humans are exposed to complex mixtures of interacting PCBs with differing biological activities, it appears reasonable to use commercial mixtures as a surrogate for environmental mixtures in assessing health risks from exposure to environmental mixtures of PCBs. Because toxicity data on commercial PCB mixtures are likely to provide a better approximation of the toxicity of environmental mixtures than existing methods based on unmixed congeners, since a congener based approach would poorly reflect the net contribution of components to the toxicity of a mixture, toxicity data on commercial mixtures are the most appropriate basis for deriving minimal risk levels for environmental mixtures of PCBs. Consequently, although additional congener studies are necessary to further elucidate the significance and mechanisms of neurological, immunological and other effects of concern, studies of commercial PCBs and other relevant mixtures of congeners (e.g., the mixture simulating the congener composition of breast milk used in the intermediate MRL study) are most relevant to human health risk assessment.

Information on the human health effects of PCBs containing low levels of CDF contaminants is primarily available from occupational exposure studies of industries in which PCBs are no longer used. Information on effects in children exposed to PCBs during gestation and/or lactation also is available, particularly regarding neurodevelopmental effects. These studies examined the effects in children born to women with no known high exposure to PCBs as well as children from women who consumed, for a long time, fish contaminated with PCBs and other persistent chemicals, especially from areas surrounding the Great Lakes. The relative contribution of the inhalation and dermal routes in the occupational exposures is unknown, but existing information on health effects in exposed workers is included with inhalation exposure in Figure 3-5. Health effects information is available on humans who were exposed to heated PCBs during the *Yusho* and *Yu-Cheng* incidents, but, as discussed in Section 3.1, CDFs are considered to be the main causal agent due to relatively high levels of these contaminants. The other human data are generally limited by insufficient exposure information and other factors, but seem to be consistent with effects observed in animals. Information on health effects in animals is extensive and available for all effect categories, but is almost completely limited to oral exposure studies. This appears to reflect experimental practicality and concern for what is thought to be the most prevalent and likely route of environmental exposure.

3.12.2 Identification of Data Needs

Acute-Duration Exposure. The hepatotoxicity of PCBs in rats is reasonably well characterized for acute-duration oral exposure (Carter 1984, 1985; Carter and Koo 1984; Kato and Yoshida 1980; Kling et al. 1978; Price et al. 1988), but it is unclear if the liver is the most sensitive target organ for acute exposure. Other targets appear to include the kidneys, stomach, and thyroid (Bruckner et al. 1973; Hansen et al. 1976; Kimbrough et al. 1972; Price et al. 1988), but insufficient information exists to determine if effects in these or other tissues occur at lower doses or are more critical than effects in the liver. Acute oral studies in other species are needed to determine the most sensitive target and species for acute exposure basis and the possible basis for an acute oral MRL. Studies with monkeys would be informative because intermediate- and chronic-duration studies indicate that this species is more sensitive than the rat and that developmental, endocrinological, and immunological effects are particularly sensitive end points.

Information on toxic effects of acute-duration exposure to PCBs by routes other than oral are limited to LD_{50} values for dermal exposure (Fishbein 1974; Puhvel et al. 1982), but these data may not be reliable due to possible delayed lethality. PCBs are well absorbed after exposure by all routes, and distribution to and retention by adipose tissue has been observed in humans after inhalation, oral, and/or dermal exposure (Brown and Lawton 1984; Fait et al. 1989; Jensen 1987). Mobilization of PCBs from adipose tissue to target organs is likely to be similar regardless of the route of exposure. Additional acute dermal studies are relevant because the skin is a route of concern for exposure at or near hazardous waste sites, particularly due to possibilities for brief contact. Acute inhalation toxicity studies may be relevant due to the potential for inhalation exposure from electrical appliances in buildings and downwind from PCB disposal facilities and incinerators.

Intermediate-Duration Exposure. The preponderance of toxicity data for PCBs is available from animals exposed to PCBs in the diet in intermediate-duration studies. Studies have been performed with various species, but the rat, monkey, and mink have been tested most extensively, and the monkey and mink consistently appear to be the most sensitive. The liver, skin, and stomach are unequivocal targets, but existing studies do not identify NOAELs for effects in these organs in monkeys and minks (Allen 1975; Allen and Norback 1973, 1976; Allen et al. 1973, 1974a; Andrews 1989; Barsotti et al. 1976; Becker et al. 1979; Bell 1983; Bleavins et al. 1980; Bruckner et al. 1973, 1974, 1977; Goldstein et al. 1974; Hansen et al. 1976; Hornshaw et al. 1986; Kimbrough and Linder 1974; Kimbrough et al. 1972; Kling et al. 1978; Koller 1977). Anemia consistently occurs in monkeys at doses similar to those

producing other effects, but a NOAEL and the relative importance of this effect is not known (Allen 1975; Allen and Norback 1976; Allen et al. 1973, 1974a). There is evidence suggesting that effects occur in the thyroid and adrenal glands of rats at doses lower than those producing effects in other tissues in monkeys and minks (Bruckner et al. 1973, 1974; Byrne et al. 1987, 1988; Collins and Capen 1980b, 1980c; Collins et al. 1977; Kasza et al. 1978; Kato et al. 1982a; Tryphonas et al. 1986a; Wassermann et al. 1973), but these doses are in proximity to those producing developmental toxicity in monkeys. A series of intermediate-duration studies in infant monkeys found neurodevelopmental effects of a low dose (7.5 µg/kg/day) of a congener mixture that simulated the congener composition of human breast milk (Rice 1997, 1998, 1999b; Rice and Hayward 1997, 1999a). The single dose level tested was a LOAEL that was used as the basis for the intermediate MRL. Additional intermediate-duration oral studies are needed to better characterize the neurodevelopmental effects of similarly low doses of PCB mixtures as well as the LOAEL region for immunological, endocrinological, and other sensitive end points. Additional studies could also corroborate evidence indicating PCB-related changes in bone structure in growing rats (Andrew 1989) and on PCB-related endocrine disruption.

Some information is available on effects of PCBs in animals by inhalation (one study with rats, mice, rabbits, and guinea pigs, and a second study with rats) (Casey et al. 1999; Treon et al. 1956) or dermal exposure (two studies with rabbits, one study with mice) (Puhvel et al. 1982; Vos and Beems 1971; Vos and Notenboom-Ram 1972) for intermediate durations. Although limited by various study inadequacies including insufficient numbers of animals, dose levels, end points and NOAEL data, this information is essentially consistent with the oral data in indicating that the liver, kidneys, thyroid and skin are main targets of toxicity. The limitations of these studies and lack of intermediate-duration inhalation studies in other species known from oral studies to be more sensitive than rats precludes the derivation of an MRL for this route and duration. Well-designed intermediate-duration studies in the more sensitive species, particularly monkeys, are needed to determine thresholds for other targets. Additional investigation could help determine whether the respiratory system effects observed in workers exposed by inhalation (Emmett et al. 1988a, 1988b; Fischbein et al. 1979; Lawton et al. 1986; Smith et al. 1982; Warshaw et al. 1979) were truly PCB effects or were caused by other contaminants. Toxicity studies by the inhalation route would be relevant due to the potential for environmental exposure by this route, particularly in the vicinity of waste sites, but may not be practical due to low volatility. Additional intermediate-duration dermal studies, especially in sensitive species, are relevant because the skin is a route of concern for exposure at or near hazardous waste sites.

Chronic-Duration Exposure and Cancer. Some epidemiological studies of PCB-exposed workers, which involve inhalation and dermal exposure, have provided evidences that PCBs were associated with adverse health effects, including hepatic and dermal changes (Alvares et al. 1977; Baker et al. 1980; Bertazzi et al. 1987; Chase et al. 1982; Colombi et al. 1982; Emmett 1985; Emmett et al. 1988a, 1988b; Fischbein 1985; Fischbein et al. 1979, 1982, 1985; Kimbrough et al. 1999a, 1999b; Lawton et al. 1985a, 1985b, 1986; Maroni et al. 1981a, 1981b; Meigs et al. 1954; Ouw et al. 1976, 1982; Smith et al. 1982; Warshaw et al. 1979). Reported effects on the respiratory system and gastrointestinal tract in these workers are suggestive. Hypertension in a population that consumed fish containing PCBs and DDT or other environmentally-exposed populations cannot be attributed conclusively to PCBs (Kreiss 1985; Kreiss et al. 1981; Massachusetts Department of Public Health 1987; Stehr-Green et al. 1986a). As discussed in following subsections, there is growing evidence that immunologic, reproductive, and thyroid effects are effects of concern in PCB-exposed populations. Relatively few toxicity studies of animals with chronic oral exposure to PCBs have been performed (Allen and Norback 1976; Arnold et al. 1993a, 1993b, 1995; General Electric Co. 1997a, 1997b; Kimbrough et al. 1975; Loo et al. 1989; Mayes et al. 1998; NCI 1978; Phillips et al. 1972; Tryphonas et al. 1986a, 1986b, 1989, 1991b), and chronic inhalation and dermal toxicity studies with animals, which could support or refute the findings of occupational studies, are lacking. Although limited in quantity, the available chronic animal oral toxicity data essentially corroborate the results of intermediate-duration studies with respect to effects in the liver, skin, stomach, blood, and thyroid, but provide no information on renal effects. Additional studies could help explain a lack of adrenal effects in monkeys exposed chronically (Loo et al. 1989) and changes in this organ in rats exposed in intermediate-duration studies. Additional studies would be necessary to determine the most sensitive animal target organ and species for chronic exposure and verify that immunologic effects are the most appropriate basis for the MRL. Additional human studies could help verify and elucidate suggestive effects, including whether gastrointestinal symptoms in workers are secondary to liver toxicity and the possible association between PCBs and hypertension. Additional evaluations of the thyroid would be particularly informative because intermediate-duration animal studies indicating that the thyroid may be a particularly sensitive target of toxicity in monkeys has limitations (Tryphonas et al. 1986b). Other chronic-duration exposure studies targeting the potential of specific PCB congeners to act as endocrine disruptors would be useful.

There is sufficient evidence that commercial PCB mixtures containing 60% chlorine by weight are carcinogenic in rats (General Electric Co. 1997a, 1997b; Kimbrough et al. 1975; Mayes et al. 1998; Norback and Weltman 1985; Schaeffer et al. 1984). Aroclor 1254 and other lower chlorinated commercial PCB mixtures have a lower carcinogenic potential than the 60% chlorine mixtures (General

Electric Co. 1997a, 1997b; Ito et al. 1973; Kimbrough and Linder 1974; Kimbrough et al. 1972; Mayes et al. 1998; Morgan et al. 1981; NCI 1978; Schaeffer et al. 1984; Ward 1985). Although the evidence that PCBs are carcinogenic in rats is conclusive, additional studies could provide information on interspecies differences. Further studies with PCB congeners aimed at elucidating the mechanism of promotion and the possible role of intercellular communication in tumor promoting activity (Hemming et al. 1992) would be valuable.

Human studies provide suggestive evidence that PCBs are carcinogenic. The carcinogenicity of PCBs in humans has been investigated in retrospective cohort mortality studies, which investigated cancer in exposed workers, and in case-control studies of environmental exposure that examined associations between serum or adipose tissue levels of PCBs and occurrence of cancer. Some of the mortality studies suggest that occupational exposures to PCBs were associated with cancer at several sites, particularly the liver, biliary tract, intestines, and skin (melanoma) (Bahn et al. 1976, 1977; Bertazzi et al. 1987; Brown and Jones 1981; Brown 1987b; Gustavsson and Hogstedt 1997; Gustavsson et al. 1986; Hardell et al. 1996; Hsieh et al. 1996; Kimbrough et al. 1999a, 1999b; Kuratsune et al. 1987; Loomis et al. 1997; Nicholson and Landrigan 1994; Rothman et al. 1997; Shalat et al. 1989; Sinks et al. 1992; Tironi et al. 1996). There is no clear association between occupational exposures to PCBs and cancer in other tissues, including the brain, hematopoietic, and lymphatic (e.g., non-Hodgkin's lymphoma). The hypothesis that environmental exposure to PCBs can cause breast cancer in humans is controversial and needs to be further studied. A number of case-control studies have investigated possible associations between breast cancer and concentrations of PCBs in breast tissue or blood in the general population. Breast adipose levels of total PCBs or individual congeners were increased in women with breast cancer in some but not all studies (Aronson et al. 2000; Dewailly et al. 1994; Falck et al. 1992; Guttes et al. 1998; Liljegren et al. 1998; Mussalo-Rauhamaa et al. 1990; Unger et al. 1984; Wasserman et al. 1976). Other environmental exposure studies used serum PCB concentrations as the marker of exposure with blood samples taken after the diagnosis of breast cancer (Moysich et al. 1998, 1999; Wolff et al. 1993; Zheng et al. 2000), or prospectively collected prior to diagnosis (Dorgan et al. 1999; Helzlsouer et al. 1999; Høyer et al. 1998; Hunter et al. 1997; Krieger et al. 1994; Wolff et al. 2000). None of the serum studies found significantly different mean blood levels of PCBs in breast cancer cases and controls. There also were no significant associations between risk of breast cancer and serum PCBs in most of these studies, although some data suggest that risk may be increased in some subgroups of postmenopausal women (Moysich et al. 1998, 1999). Many of the better designed studies were prospective, and none of the prospective studies found that PCBs were associated with the occurrence of breast cancer (Dorgan et al. 1999; Helzlsouer et al. 1999; Høyer et al. 1998; Hunter et al. 1997; Krieger et al. 1994; Wolff et al. 2000). Additional studies,

including follow-up of existing cohorts, are needed to better characterize the relationship between PCBs and cancer in humans.

Genotoxicity. An increased percentage of chromosomal aberrations was reported in a study in which workers were exposed to PCBs for >10 years (Kalina et al. 1991). However, there was simultaneous exposure to benzene, which is known to cause genotoxic effects in humans. A different study reported a slight increase in the incidence of sister chromatid exchanges in 12 men exposed to PCBs following a fire in an electric station (Melino et al. 1992). It is quite possible, however, that toxic chlorinated dioxins and/or furans were generated during the fire. Studies with Aroclor 1254 in human lymphocytes *in vitro* gave conflicting results; Hoopingarner et al. (1972) found no evidence of chromosomal damage at a concentration of 100 µg/mL, whereas Sargent et al. (1989) observed chromosomal damage at a concentration of 1.1 µg/mL. Aroclors 1242 and 1254 were not genotoxic in rats and mice when administered orally in acute- and intermediate-duration studies (Garthoff et al. 1977; Green et al. 1975a, 1975b; Robbiano and Pino 1981); however, longer term studies were not located. Furthermore, other PCB mixtures have not been tested. Studies by the inhalation and dermal routes would help develop dose-response relationships for these routes. Available pharmacokinetic data do not suggest route-specific target organs. Aroclor 1254 was not mutagenic in *Salmonella* (Bruce and Heddle 1979; Heddle and Bruce 1977; Schoeny et al. 1979). Studies with other mixtures, and using other prokaryotes, would provide information regarding differences in potencies of different mixtures and in the sensitivities of different organisms. Cytogenetic analysis of human populations exposed to PCBs in occupational settings or exposed by consumption of food contaminated with PCBs would provide an opportunity to assess the genotoxic potential of these compounds in humans. However, the generally negative results of *in vitro* and *in vivo* animal studies indicate that commercial PCB mixtures are not likely to pose a genotoxic threat to humans.

Reproductive Toxicity. Limited information is available on reproductive effects of PCBs in humans. In women, there was no apparent effect of occupational exposure to various Aroclor mixtures on mean number of pregnancies (Taylor et al. 1989). Due to study limitations and lack of information on gravidity in other studies, the effect of PCBs on human conception is unclear. Studies that examined reproductive end points in women whose diets contained Great Lakes fish found suggestive evidence that consumption of the fish may be associated with a slightly shorter menstrual cycle length (Mendola et al. 1997), but not with increased risk for spontaneous fetal death (Mendola et al. 1995a). Studies of one cohort of Great Lakes fisheaters indicated that women were more likely to have positive associations with conception delay than their exposed husbands (Buck et al. 1997, 1999, 2000), although contrary results were found in

another cohort which found an association between conception delay and Great Lakes fish consumption in exposed men, but not in their wives (Courval et al. 1999). The strength of the human evidence that consumption of Great Lakes fish may or may not be associated with adverse effects on conception and other reproductive abilities is weak given the small magnitude of effects when they have been detected and study limitations as discussed in Section 3.2.5.2. Additional long-term prospective or longitudinal epidemiology studies are needed are needed to follow these PCB-exposed populations for reproductive end points as well as to assess the clinical relevance of the effects.

Oral studies with animals provide conclusive evidence for reproductive toxicity of PCBs in females of various species and some evidence for effects in male rats. Effects that have been induced in female animals include estrus changes and reduced implantation rate in adult rats and/or their offspring, decreased conception in mice, partial or total reproductive inhibition in minks, and menstrual alterations and decreased fertility in monkeys (Allen et al. 1974a; Arnold et al. 1990, 1993a, 1993b, 1995; Aulerich and Ringer 1977; Backlin and Bergman 1995; Backlin et al. 1997, 1998a, 1998b; Barsotti et al. 1976; Brezner et al. 1984; Jones et al. 1997; Kihlstrom et al. 1992; Sager and Girard 1994; Welsch 1985). Monkeys (Rhesus) and minks are the most sensitive species tested, although reproductive effects were not induced at doses quite as low as those inducing the critical neurobehavioral, immunological, and dermal/ocular effects used to derive the intermediate and chronic MRLs. In male animals, short-term exposure to high oral doses of Aroclor 1254 induced no changes in the weight or histology of the testes or accessory glands in adult rats (Dikshith et al. 1975; Sanders et al. 1974), although seminal vesicle weights and caudal epididymal weights and sperm counts were reduced in rats that were exposed for several months as weanlings (Gray et al. 1993). No studies in male mice or rats evaluated reproductive capability. There is limited evidence of hypoactivity of the seminiferous tubules in monkeys that were chronically exposed to a dose of Aroclor 1248 that also caused clinical signs of toxicity (Allen and Norback 1976). In contrast to the limited evidence for reproductive effects in male adult animals, fertility was markedly reduced in male offspring of rats that were lactationally exposed to relatively high doses of Aroclor 1254 (Sager 1983; Sager et al. 1987, 1991), and results of oral and subcutaneous studies with single congeners have also shown that gestational and neonatal exposures can adversely affect morphology and production of sperm and fertility in male rats and mice (Faqi et al. 1998; Huang et al. 1998a; Smits-van Prooije et al. 1993). As discussed in Section 3.5.2, effects on male reproductive organs appear to involve postnatal developmentally-specific vulnerable periods of responsiveness. Additional animal studies could help characterize effects on fertility in exposed adults, interspecies and sex differences in sensitivity, and define the NOAEL region for reproductive effects.

Developmental Toxicity. There is mounting evidence that perinatal exposure to PCBs induces adverse developmental effects in humans, specifically, but not limited to, neurobehavioral alterations in newborn and children exposed during gestation and/or via breast milk. This has been seen in children born to mothers exposed to PCBs by consumption of contaminated fish from the Great Lakes (Fein et al. 1984a, 1984b; Jacobson et al. 1984a, 1990a, 1990b, 1992; Lonky et al.1996; Stewart et al. 1999, 2000b) and in children from women with no known high-exposure to PCBs in North Carolina (Gladen et al. 1988; Rogan and Gladen 1991; Rogan et al. 1986a, 1986b, 1987), The Netherlands (Huisman et al. 1995a, 1996b; Koopman-Esseboom et al. 1996; Lanting et al. 1998a; Patandin et al. 1999), and Germany (Winneke et al. 1998b). In the various cohorts studied, some common findings of neurodevelopmental effects have been reported, other affected end points have not been the same in all studies. This is not unexpected given the different degrees of control for confounders and the different measures of exposure used. Moreover, apparent inconsistencies between studies may reflect not only limitations in study design, but also problems inherent in detecting neurobehavioral deficits at exposure levels near the threshold for effects. Effects associated with PCB exposure included abnormal reflexes and more motor immaturity in newborns (Jacobson et al. 1984a; Lonky et al. 1996; Rogan et al. 1986b), altered PDI scores at 1–2 years of age (Gladen et al. 1988; Koopman-Esseboom et al. 1996), and alterations in memory functions at 7 months of age (Jacobson et al. 1985) and at 4 years of age (Jacobson et al. 1990a, 1990b, 1992) and in cognitive abilities at 42 months using the Kaufman Assessment Battery for Children (Patandin et al. 1999). It must be kept in mind, however, that in all of these studies, there is a possibility that other lipophilic compounds may have contributed to the observed effects, particularly in the studies on consumption of Great Lakes fish contaminated with other chemicals such as CDDs, DDE, and mercury. It is expected that children from these prospective studies continue to be monitored in order to assess the impact of these subtle neurobehavioral alterations as they grow older and their potential implications at a population level. It is also important to address the issue of continuity of effect over time. This means establishing whether the children who showed impaired performance in some tests at 18 and 24 months are the same who performed poorly in tests given as neonates. This would help interpretation of the biological significance of the exposure.

Improvements in analytical methods for measuring PCBs should greatly facilitate analysis of PCB congeners in cord blood, the most accurate surrogate of exposure during gestation. This should allow researchers to establish more precise potential associations between specific PCB congeners and health outcomes, as done for example by Stewart et al. (2000b) in their study of Lake Ontario fisheaters. It is also important that researchers provide as much information as possible regarding not only the analytical

methods used for measuring PCBs, but also the methods for measuring lipids so that comparisons between studies can be made.

Studies in animals support the findings in humans. Studies in rodents have provided valuable information, but monkeys, whether exposed during gestation and/or during infancy, have proved to be much more very sensitive to PCBs and some structurally-related chemicals. Investigators should continue efforts to develop an operant test battery that measures a variety of functions that can be validated for use in rodents, monkeys, and humans and that can be applied in epidemiological studies. Studies with single congeners are valuable in that they provide information on possible mechanisms of action, but people, specifically nursing infants, are exposed to a mixture of PCB congeners in the milk. Therefore, further studies that administer PCB mixtures of congeneric composition similar to that of human breast milk represent the most relevant approach to mimicking real life exposure to infants. Varying the congener composition of the reconstituted milk sample may help associate specific PCB congeners with specific neurodevelopmental outcomes.

Some studies in humans have suggested that gestational exposure to PCBs and other chemicals can affect the thyroid hormone system in infants (Koopman-Esseboom et al. 1994a; Nagayama et al. 1998a; Winneke et al. 1998a). These observations have been extensively corroborated in experimental animals (Collins and Capen 1980c; Corey et al. 1996; Goldey et al. 1995; Juarez de Ku et al. 1994; Li et al. 1998; Morse et al. 1996b; Seo and Meserve 1995). Yet, further information is needed comparing thyroid hormone levels in the brain and histological changes of exposed animals during crucial periods of nerve tract development and neuronal differentiation. Normal thyroid status is also crucial for the normal development and functioning of reproductive organs, and further research in this area is also needed.

Perinatal exposure to PCBs also has been associated with alterations in immunocompetence in children (Dewailly et al. 2000; Smith 1984; Weisglas-Kuperus 2000; Weisglas-Kuperus et al. 1995). These children should continue to be observed for any indication of reduced immunocompetence which may potentially lead to increased incidence of illnesses. The findings of immune alterations following PCB exposure are consistent with observations in animals. Immunological alterations have been reported in adult monkeys and their offspring after long-term exposure to commercial PCB mixtures at doses as low as 0.005 mg/kg/day (Arnold et al. 1995; Tryphonas et al. 1989, 1991a, 1991b).

Immunotoxicity. There are indications of altered immune status in adult and infant human populations who were orally exposed to mixtures of PCBs and other chemicals. Information on immunological effects of PCBs in humans is mainly available from studies of people exposed occupationally (Chase et al. 1982; Emmett et al. 1988a, 1988b; Lawton et al. 1985a; Maroni et al. 1981b; Smith et al. 1982), by consumption of contaminated fish and other marine foods (Dewailly et al. 2000; Smith et al. 1984), by consumption of contaminated rice oil in the *Yusho* and *Yu-Cheng* poisoning incidents (Chang et al. 1981, 1982a, 1982b; Chao et al. 1997; Kuratsune 1989; Lu and Wu 1985; Nakanishi et al. 1985; Rogan 1989; Shigematsu et al. 1971; Yu et al. 1998), and via general environmental exposures in a Dutch population (Weisglas-Kuperus et al. 1995). The occupational studies provide little information for assessing immunotoxicity because evaluations were essentially limited to inconclusive routine clinical measurements of WBC counts and serum proteins with no investigations of functional immune parameters. The most conclusive findings were in the *Yusho* and *Yu-Cheng* populations who experienced the highest levels of PCB exposure and least complex exposure mixture. Interpretation of the data from the other human studies is complicated by responses that were generally subtle and exposures that included a number of persistent toxic substances in addition to PCBs that are also potentially immunotoxic. Overall, there appears to be a consistency of effects among the human studies suggesting sensitivity of the immune system to PCBs and these other chemicals, particularly in infants exposed *in utero* and/or via breast feeding. For example, susceptibility to respiratory tract infections was increased in *Yusho/Yu-Cheng* adults and their children, and there was an association between infectious illnesses and PCBs in the children of mothers who consumed Lake Michigan or Sheboygan River fish. Children born to *Yu-Cheng* mothers also had an increased prevalence of middle ear infections, and the incidence of acute otitis media was increased in Inuit infants of mothers whose diets were based on marine mammal fat. Serum IgA and/or IgM antibody levels were decreased in the *Yusho* and *Yu-Cheng* populations as well as in the Inuit children. Monocyte counts were reduced in *Yu-Cheng* patients and the infants of the Dutch mother-child study, and changes in T lymphocyte subsets were found in the *Yu-Cheng*, Inuit child, and Dutch child populations. However, due to the mixed chemical nature of the exposures and generally insufficient information on possible exposure-response relationships, the human studies provide only limited evidence that exposed adults and infants exposed *in utero* or via breast feeding may have compromised their immune system rendering them unable to overcome infection. Additional studies are needed to better characterize the immunologic potential of PCBs in exposed humans, particularly by incorporating an immunological component in the early design of epidemiologic studies, as well as establishing a broad database of normal values for clinical immunology end points to which experimental results can be compared. The hypothesis that a possible

relationship between PCBs and non-Hodgkin's lymphoma could be related to the immunosuppressive effects of PCBs is another area requiring further research.

The immunotoxicity of PCBs in animals has been documented in various species that were orally exposed via commercial mixtures, mixtures of congeners analogous to human breast milk, Great Lakes fish, or single congeners. Studies in rats, mice, guinea pigs, and rabbits showed that intermediate-duration exposures to relatively high doses of commercial PCB mixtures caused morphological and functional alterations in the immune system. Effects observed in these species included thymic and splenic atrophy, reduced antibody responses to SRBC and other foreign antigens, increased susceptibility to infection by viruses and other microbes, reduced skin reactivity to tuberculin, and increased proliferation of splenic lymphocytes in response to mitogenic stimulation (Allen and Abrahamson 1973; Bonnyns and Bastomsky 1976; Exon et al. 1985; Imanishi et al. 1980; Koller 1977; Loose et al. 1977, 1978a, 1978b, 1979; Smialowicz et al. 1989; Street and Sharma 1975; Talcott and Koller 1983; Talcott et al. 1985; Vos and Van Driel-Grootenhuis 1972). Immunological assessments of rats and mice that were fed diets containing low doses of PCBs and other chemicals in Great Lakes fish were generally mixed, although some alterations were found that are similar to those observed in the studies of commercial PCB mixtures (Cleland et al. 1989; Tryphonas et al. 1998a, 1998b).

Oral studies of Aroclor mixtures in monkeys confirm the findings of immunotoxicity in the other species and further indicate that the immune system of monkeys is particularly sensitive to PCBs. Immunological effects of PCBs in monkeys include decreased antibody responses to SRBC, increased susceptibility to bacterial infections, altered lymphocyte T-cell subsets, decreased lymphoproliferative responsed to mitogens, and histopathological changes in the thymus, spleen, and lymph nodes (Abrahamson and Allen 1973; Allen and Barsotti 1976; Allen et al. 1980; Barsotti et al. 1976; Thomas and Hinsdill 1978; Truelove et al. 1982; Tryphonas et al. 1986a, 1989, 1991a, 1991b). The parameters most consistently affected in monkeys are reduced IgM and IgG antibody responses to SRBC, which were induced at chronic oral doses as low as 0.005 mg/kg/day (Tryphonas et al. 1989, 1991a, 1991b). Results of studies in gestationally- and lactationally-exposed infant monkeys are consistent with the data in adult animals showing immunosuppressive effects of PCBs (Aroclor 1254) at doses as low as 0.005 mg/kg/day, with reductions in IgM and IgG antibody levels to SRBC and mitogen-induced lymphocyte transformation that generally paralleled the findings in maternal animals (Arnold et al. 1995). The 0.005 mg/kg/day LOAEL for immunological effects in monkeys was used as the basis of the chronic oral MRL for PCBs. Also, minimal immunological alterations were induced in infant monkeys that were orally exposed to a similar dose (0.0075 mg/kg/day) of a PCB congener mixture simulating the congener content of human milk for

the first 20 weeks of life (Arnold et al. 1999). Additional immunological studies in animals are needed to verify that the immune system is the most sensitive target of PCBs and the most appropriate basis for chronic MRL derivation, as well as better characterize dose-response relationships in sensitive species following intermediate-duration exposure.

Neurotoxicity. As previously mentioned under Developmental Toxicity, one of the main focus of research on PCBs has been the evaluation of a possible association between exposure to PCBs during gestation and/or lactation and neurobehavioral alterations in newborn and young children. Thus far, there is no evidence that PCBs at the levels found in the environment are neurotoxic to adults. There is no conclusive evidence that workers who were exposed to commercial PCB mixtures for long periods and had high PCB body burdens developed neurological deficits (Emmett et al. 1988a; Fischbein et al. 1979; Smith al. 1982). However, sensory and motor nerve alterations were observed in *Yusho* and *Yu-Cheng* patients who ingested rice oil contaminated with high amounts of PCBs, CDFs, and other structurally-related chemicals (Chia and Chu 1984, 1985; Kuratsune 1989; Rogan 1989). Evaluation of an adult population on a visual-motor coordination test and a hand steadiness test revealed no significant effect from exposure to PCB/DDE through long-term consumption of Lake Michigan fish (Schantz et al. 1999). The results from cognitive assessment of this cohort are expected to be available in the near future.

The mechanism(s) of neurotoxicity of PCBs is not entirely clear, but evidence accumulated in recent years suggests that multiple mechanisms may be involved including alterations in levels of neurotransmitters in various brain areas, of calcium homeostasis (Kodavanti et al. 1993), inositol phosphates (Shafer et al. 1996), protein kinase C (Kodavanti et al. 1995), ryanodine receptor binding (Wong and Pessah 1996), and neutrophil activation (Ganey et al. 1993). Continued research in these areas is necessary to establish correlations between biochemical, morphological, and functional alterations in the brain of PCB-exposed animals, as well as to determine possible preferential accumulation of PCB congeners in specific brain areas that could be associated with specific neurobehavioral effects. Establishing relationships between *in vitro* and *in vivo* effects is important for the development of appropriate *in vitro* preparations in which putative neurotoxicant PCBs can be easily tested.

Epidemiological and Human Dosimetry Studies. Consumption of contaminated food (particularly diets high in fish from contaminated waters) and inhalation of indoor air in buildings that have electrical parts that contain PCBs are the main sources of exposure for the general population. PCBs can pass across the placenta and also can accumulate in breast milk such that breast-fed infants and

unborn children are at risk of being exposed to PCBs (DeKoning and Karmaus 2000; Fein et al. 1984a, 1984b; Huisman et al. 1995a, 1996b; Jacobson et al. 1984a, 1990a, 1990b; Rogan et al. 1986a, 1986b, 1987). Although PCBs are no longer manufactured in the United States, PCB-containing transformers and capacitors remain in use. Thus, occupational exposure may occur in workers during accidents or repair of electrical equipment containing PCBs. Present and future occupational exposure to PCBs may also occur from residual PCBs in workplaces, from disposal of PCBs and/or contaminated equipment, or during cleanup of hazardous waste sites. A number of studies have examined possible associations between health effects and exposure to PCBs, particularly in adults occupationally exposed to PCBs (Alvares et al. 1977; Baker et al. 1980; Bertazzi et al. 1987; Chase et al. 1982; Colombi et al. 1982; Emmett 1985; Emmett et al. 1988a, 1988b; Fischbein 1985; Fischbein et al. 1979, 1982, 1985; Kimbrough et al. 1999a; Lawton et al. 1985a, 1985b, 1986; Maroni et al. 1981a, 1981b; Meigs et al. 1954; Ouw et al. 1976, 1982; Smith et al. 1982; Taylor et al. 1989; Warshaw et al. 1979), adults and/or their children following maternal consumption of contaminated fish from the Great Lakes and other waters (Buck et al. 1997, 1999, 2000; Courval et al. 1999; Dewailly et al. 2000; Fein et al. 1984a, 1984b; Jacobson et al. 1984a, 1990a, 1990b, 1992; Kreiss 1985; Kreiss et al. 1981; Lonky et al. 1996; Mendola et al. 1995a, 1997; Smith 1984; Stewart et al. 1999, 2000b), and in children from women in North Carolina (Gladen et al. 1988; Rogan and Gladen 1991; Rogan et al. 1986a, 1986b, 1987), the Netherlands (Huisman et al. 1995a, 1995b; Koopman-Esseboom et al. 1994a, 1996; Lanting et al. 1998; Patandin et al. 1999; Weisglas-Kuperus 2000; Weisglas-Kuperus et al. 1995), and Germany (Winneke et al. 1998b) with no known high-exposure to PCBs. Chloracne and other skin changes, and various hepatic alterations including increased serum levels of liver enzymes and lipids have been associated with occupational exposures. There are also reports of respiratory, gastrointestinal, hematological, skeletal, developmental, and neurological effects in exposed workers, but the evidence is not strong enough to conclusively establish cause-effect relationships. The epidemiologic studies of the contaminated fisheating populations and people exposed via the general environment raise concern for reproductive effects in adults and neurodevelopmental and immunological alterations in children of exposed parents, as discussed above in the Reproductive Toxicity, Developmental Toxicity, Neurotoxicity, and Immunotoxicity data need subsections. Additional well conducted epidemiological investigations, particularly follow-up studies and transgenerational studies of high risk populations, are needed to better characterize the potential for PCBs to induce these effects. These studies should also address limitations that constrain some of the existing human studies, such as unmeasured PCB exposure concentrations, lack of controls for confounding co-exposures, and lack of comparative population data.

Concern that even low levels of PCBs transferred to the fetus across the placenta and that greater amounts might be transferred to nursing infants via breast milk has triggered many of the epidemiological studies. The results from some of these studies suggest that perinatal exposure to PCBs may induce subtle long-lasting neurological damage in children (Fein et al. 1984a, 1984b; Gladen et al. 1988; Huisman et al. 1995a, 1996b; Jacobson et al. 1984a, 1990a, 1990b, 1992; Koopman-Esseboom et al. 1996; Lanting et al. 1998a; Lonky et al. 1996; Patandin et al. 1999; Rogan and Gladen 1991; Rogan et al. 1986a, 1986b, 1987; Stewart et al. 1999, 2000b). Suggestive evidence for immunological (Dewailly et al. 2000; Smith 1984; Weisglas-Kuperus et al. 1995; Weisglas-Kuperus 2000) and thyroid effects (Koopman-Esseboom et al. 1994a; Nagayama et al. 1998a; Winneke et al. 1998a) in children also has been presented. Many of these are prospective studies that have followed-up the children for many years and are expected to continue to do so in order to ascertain the duration and real life significance of these subtle alterations.

Biomarkers of Exposure and Effect.

Exposure. PCBs are stored at highest concentrations in adipose tissue and are present in serum and human milk. Several studies have shown that serum and adipose PCB levels are biomarkers of exposure (Brown and Lawton 1984; EPA 1984d; Fait et al. 1989; Jacobson et al. 1984b; Jan and Tratnik 1988; Luotamo 1988; Safe et al. 1985b; Schecter et al. 1994; Steele et al. 1986; Wolff et al. 1982a). It has been proposed that measurement of PCB levels in both serum and adipose tissue may be more predictive of body burden than each value separately, although either serum or adipose tissue PCB levels may serve as useful biomarkers of body burden and/or exposure (Stellman et al. 1998). Further studies on the predictive value of levels of PCBs (particularly congeners) in serum and adipose tissue in individuals exposed to PCBs for short, intermediate, and chronic durations would provide valuable information that could lead to early detection of PCB exposure.

PCB residue data in humans and other animals (see Section 3.4.2, Distribution) suggest that tissue or body burdens of PCBs should be based on individual congeners or groups of congeners and not based on profiles of commercial PCB formulations. The simplest approach involves using one congener as a marker of total PCBs in a biological specimen. For example, levels of 2,2',4,4',5,5'-hexaCB (PCB 153), a very stable and often the most abundant congener, have been shown to correlate well with the total amount of PCBs in human breast milk, plasma, or follicular fluid (Atuma and Aune 1999; Grimvall et al. 1997; Johansen et al. 1994; Pauwels et al. 1999). However, if a more complete profile of congeners is considered, the correlations are lower (Bachour et al. 1998; Hansen 1998, 1999). Use of PCB 153 or congener groups as a marker of the total therefore could be a misleading indicator of the differential

exposure to other individual or groups of congeners of toxicological significance. Further studies are needed to assess the feasibility of using individual congeners or groups of congeners as a marker for total exposure to PCBs.

Effect. There are no specific biomarkers of effects for PCBs. Numerous studies have attempted to correlate serum PCB levels with liver-associated enzymes in PCB-exposed workers and general population subjects; however, no conclusive association has been found (Baker et al. 1980; Emmett et al. 1988a, 1988b; Fischbein 1985; Fischbein et al. 1979; Kreiss et al. 1981; Lawton et al. 1985a, 1985b; Smith et al. 1982; Stehr-Green et al. 1986a, 1986b; Steinberg et al. 1986). Further studies to identify specific biomarkers of effects of PCBs would facilitate medical surveillance leading to early detection of potentially adverse health and possible treatment. Congener-specific analysis combined with TEF calculations may be useful for characterizing dioxin-like health effects. However, as discussed in the introduction to Section 3.11.1, use of congener-specific analyses is not yet practical for most laboratories, and the TEF approach for PCBs is still under development and limited to dioxin-like congeners' effects only.

Absorption, Distribution, Metabolism, and Excretion. There are no quantitative data regarding absorption in humans by the inhalation or dermal route, but data from occupationally exposed individuals suggest that PCBs are well absorbed by these routes (Wolff 1985). Only one study was located that provided quantitative oral absorption data in a volunteer (Buhler et al. 1988). Schlummer et al. (1998) used a mass balance approach to assess the gastrointestinal absorption of specific PCB congeners from food in seven individuals, 24–81 years of age, with different contaminant body burdens. Together, the data support the passive diffusion model for gastrointestinal absorption, where the concentration of the contaminant in the blood is the major factor determining absorption. PCB congeners showing nearly complete net absorption had very low or nondetectable levels in the serum lipids. For other congeners, there was a trend for decreasing net absorption and/or increasing net excretion with increasing congener concentration in serum lipids. Similar congener specific, mass balance human studies are needed to confirm and extend these findings. The animal data indicate that PCBs are efficiently absorbed by the oral route (Albro and Fishbein 1972; Hansen 1999), but most of the information is derived from excretion data. Inhalation and dermal absorption data are limited. No studies were located in which a range of doses of PCB mixtures of different chlorine contents were administered by the inhalation, oral, and dermal routes, and for various exposure periods.

As with absorption, distribution data in humans are limited to serum, milk, and/or tissue PCB residue data from occupationally and environmentally exposed subjects, and suggest that PCBs distribute preferentially to tissues with high fat content regardless of the route of exposure (Brown and Lawton 1984; Fait et al. 1989; Jensen 1987). As with other organisms, PCB residue levels in humans reflects multiple exposure pathways, and congener-specific elimination. PCB profiles in human serum immediately following exposures reflect the profiles in the exposure sources; however, selective metabolism and excretion begin to alter the congener profile within 4–24 hours (Hansen 1999). In large population based studies, it is often necessary to summarize large quantities of congener-specific data from many individuals in order to highlight mean and range data for each PCB congener. While this approach is necessary to summarize data for publication, congener profiles for individuals are often never reported. Thus, it may not be possible to identify a few individuals that may have had an unusual profile or elevated congener level due to a recent PCB exposure. Human studies reporting congener specific PCB residue data should consider citing electronic databases, which could contain the complete data set for each subject in the study.

Moysich et al. (1999) recently evaluated proposed frameworks for grouping PCBs, including a more simple approach based on relative abundance and degree of chlorination. McFarland and Clarke (1989) proposed an approach to grouping PCB congeners based on their potential risk to the environment and human health. Another framework for grouping PCB was proposed by Wolff et al. (1997), based on the biological activities of the congeners and their presence in house dust and humans. Numerous factors, such as the analytical methods used by various labs for sample preparation and analysis, the type of human sample (milk, serum, plasma, adipose), sample size, year sample was collected, subject age, and exposure history are all critical to the detection and quantification of a specific congener in a given sample. These factors need to be considered when adopting an optimal framework for grouping PCB congeners to assess exposure and relative risk .

Recently, Dewailly et al. (1999) measured the concentration of 14 PCB congeners in subcutaneous fat, omental fat, brain, and liver from autopsy tissue samples collected from Greenlanders between 1992 and 1994. PCB concentrations (lipid basis) were similar in omental fat and subcutaneous abdominal fat, while the hepatic concentrations were generally about 20% lower than fat. PCB levels in brain (lipid basis) were about 10–20% of the levels found in subcutaneous fat. The lower concentration in brain cannot be explained by the presence of the blood-brain barrier because PCBs are highly lipophilic and are therefore expected to freely diffuse across this barrier. The difference in accumulation may be due to the nature of more polar brain lipids, which are mainly phospholipids. PCBs may partition to a greater extent

into the triglycerides found in adipose tissue. Further investigation is needed to understand factors regulating the tissue specific distribution of PCB congeners, particularly to critical target organs such as the brain.

Studies in animals that could provide a basis for assessing the comparative distribution of PCBs when administered by the inhalation, oral, and dermal routes of exposure were not located. A recent study in ferrets by Apfelbach et al. (1998) reported for the first time that the olfactory system may be a potentially significant portal for the entry of airborne PCBs. The olfactory bulbs of the exposed ferrets had the highest total PCB concentration (642 ng/g lipids), while the liver, adipose tissue, and brain had levels of 202, 303, and 170 ng/g lipids, respectively. The data suggest that inhaled PCBs pass into the dentrites of olfactory sensory neurons and are transported via olfactory axons directly to the bulbs where they accumulate. While the olfactory system appears to be a significant site for the disposition of airborne PCBs, further studies are needed to confirm this observation and assess whether greater disposition in the brain is associated with inhalation exposure.

Data derived from oral administration of PCBs to animals indicate that PCBs distribute first to liver and muscle, and are subsequently translocated to adipose tissue and skin for storage (Allen et al. 1974b; Curley et al. 1971; Hashimoto et al. 1976; Klasson-Wehler et al. 1989a). Studies regarding distribution through the placenta after inhalation and dermal exposures were not available.

Other than isolated studies with human microsomes (Schnellmann et al. 1983), data regarding biotrans-formation of PCBs in humans are limited to information about occupationally exposed individuals, whose PCB intake is assumed to derive mainly from inhalation and dermal exposure (Fait et al. 1989; Jensen and Sundström 1974; Wolff et al. 1982b). The use of human cell systems in culture might be considered a useful alternative to studying the metabolic fate of PCBs, but limited expression of a complete profile of enzymes reduces their value. The metabolism of PCBs after oral administration in experimental animals has been extensively studied (Safe 1989a). Although information regarding metabolism following inhalation or dermal exposure is lacking, there is no reason to believe that other pathways would operate after exposure by these routes.

State of the art PCB exposure assessment utilizing human serum, milk, and/or tissues should not only include congener specific PCB analysis, but analyze persistent PCB metabolites. Since certain hydroxylated and methylsulfonyl ($MeSO_2$) PCB metabolites are present in some cases at levels higher

than their respective parent compounds, it is necessary to further investigate the potential biological and/or toxicological activities of these persistent metabolites.

Studies regarding urinary or fecal excretion of PCBs in humans were not located; however, elimination of PCBs through maternal milk is well documented (Masuda et al. 1978; Mes et al. 1984). Fecal excretion is the main route of elimination of PCBs in animals after oral exposure (Lutz and Dedrick 1987). Although data regarding excretion in animals after inhalation exposure were not located, there is no reason to suspect different patterns of excretion. Dermal data suggest that excretion of certain PCBs may follow a two-phase elimination process, as described for oral exposure, but this information is derived from a single study (Wester et al. 1990).

Comparative Toxicokinetics. The existing evidence suggests that qualitative differences in the toxicokinetic disposition of PCBs exist among humans and the numerous animals species studied and also among animal species (Lutz and Dedrick 1987; Safe 1989a; Sipes and Schnellmann 1987). However, these differences appear to be highly dependent on the specific congener or mixture studied. Further pharmacokinetic modeling studies with additional groups of PCB congeners would be valuable to determine the validity of extrapolating data. In addition, studies with human cell systems *in vitro* could help estimate metabolic rate constants for use in pharmacokinetic models. In general, all species absorb PCBs efficiently and accumulate PCBs in tissues rich in fat. Once absorbed, PCBs distribute in a biphasic manner in all species examined (Lutz and Dedrick 1987). Identification of metabolites in humans and animals suggests that all species examined share some common biochemical reactions. Experimental data in animals indicate that fecal elimination is the main route of excretion (Bleavins et al. 1984; Klasson-Wehler et al. 1989a, 1989b; Mühlebach and Bickel 1981), but no human information was located in the existing literature. Analysis of the excreta of humans exposed in the workplace and near hazardous waste sites would provide information regarding biotransformation and elimination kinetics in humans. In addition, similar target organs have been identified across animal species. Monkeys and minks are the most sensitive species tested regarding dioxin-like effects, but pharmacokinetic data in minks are scant. Although the toxicological data in humans (Emmett et al. 1988a; Fischbein et al. 1979) are limited, adverse cutaneous reactions documented in humans are also seen in monkeys (Arnold 1993a, 1995), although at much lower doses. This and other effects seen in monkeys, not observed in populations occupationally or environmentally exposed to PCBs, have led some to suggest that monkeys may not represent a suitable animal model (James et al. 1993; Kimbrough 1995). However, the clinicopathologic picture in monkeys is more like humans than any other species. As these studies suggest, the monkey is more sensitive than humans on a dose basis. Attention must be paid to differences

between adults and immature animals of all species. The more rapidly growing immature animals generally have lower and distinct profiles of biotransformation enzymes as well as much smaller peripheral fat depots for sequestering the lipophilic PCBs.

Methods for Reducing Toxic Effects. The mechanism by which PCBs enter the blood stream in humans is not known; consequently, there are no established methods for reducing absorption. In experimental animals, however, administration of rice bran fiber reduced gastrointestinal absorption (Takenaka and Tarahashi 1991). Identification of additional substances that could prevent or delay absorption and that do not represent a toxic risk themselves would be valuable. There are no established methods for reducing body burden in humans, but a few reports have indicated that fasting may be effective (Imamura and Tung 1984). Studies examining the effect of fasting in animals exposed to PCBs would provide useful information that can be used to better characterize the effectiveness of this approach. The metabolism of PCBs leads to the formation of highly reactive and potentially toxic derivatives. Thus, additional studies examining the feasibility of favoring metabolic pathways leading to the formation of nontoxic metabolites would be valuable. The mechanisms of toxic actions of PCBs are not completely understood, and no methods exist to block the toxic response due to exposure to PCBs. Further studies aimed at elucidating the mechanisms of action of PCBs would help in developing possible methods for reducing toxic effects.

Children's Susceptibility. Data needs relating to developmental effects are discussed more extensively above in the Developmental Toxicity subsection. Prenatal exposure to PCBs has been associated with neurodevelopmental effects. However, many exposures to PCBs involve mixtures including other chemicals. Studies employing specific congeners of PCBs are needed to establish the association between exposure, neurotransmitter and T_4 levels in the brain, and neurobehavioral effects. Experiments aimed at defining critical windows of PCB action in the developing organism would provide valuable information, especially for pregnant women who may wish to alter their dietary habits during pregnancy. In addition, studies to define the relationship of prenatal exposure to specific PCB congeners, its activity as an endocrine disruptor, and effects on sexual differentiation are needed. Continued monitoring of children from the Dutch, Lake Michigan, Lake Ontario prospective studies is expected with particular emphasis on evaluation of immune competence, thyroid function, and cognitive abilities. Development of PBPK models that could be used to predict PCB body burdens in neonates and infants as a function of maternal body burden would be useful. Studies of the pharmacokinetics of PCBs in the fetus (i.e., assessment of placental permeability for individual congeners), infant, and child would be useful in further defining the potential for toxic effects of these compounds. Areas of focus include the

effect of the developing and changing metabolic capacities of the fetus, neonate, and child on the production of toxic metabolites and/or detoxification of the parent compound and metabolites.

Child health data needs relating to exposure are discussed in 6.8.1 Identification of Data Needs: Exposures of Children.

3.12.3 Ongoing Studies

Table 3-15 lists ongoing studies on the health effects of PCBs identified from the Federal Research in Progress database (FEDRIP 2000). Ongoing studies from the ATSDR Great Lakes Human Health Effects Research Program are listed in Table 3-16.

Table 3-15. Ongoing Studies on the Health Effects of PCBs

Investigator	Affiliation	Title	Sponsor
Aulerich, R	Michigan State University East Lansing, MI	Fur animal studies (mink)	USDA or cooperating state institutions
Berkowitz, Gertrud S	Mount Sinai School of Medicine of CUNY, New York, NY	Exposure of indoor pesticides and PCBs—Effects on growth and neurodevelopment	NIEHS
Bernstein, Leslie	Univ of Southern Calif Los Angeles	Organochlorine residue levels and risk of breast cancer	NIEHS
Bradfield, Christopher A	University of Wisconsin Madison	Transgenic models of dioxin action	NIEHS
Burchiel, Scott W	University of New Mexico	Effects of immunotoxic xenobiotics on human peripheral blood lymphocytes	NCRR
Bursian, S	Michigan State University	The fate and biological effects of xenobiotics in animals	USDA or cooperating state institutions
Carpenter, David O	State University of New York at Albany	Mechanisms responsible for cognitive impairment caused by exposure to PCBs	NIEHS
Charles, MJ	University of California Davis	Exploration of linkages among organochlorines, oxidative DNA damage, and breast cancer	USDA or cooperating state institutions
Chou, K	Michigan State University	Control mechanisms of male reproduction and sperm fertilizing ability	USDA or cooperating state institutions
Cohn, Barbara A	Public Health Institute, Berkeley, CA	Prenatal organochlorine exposure and human reproduction	NIEHS
Dean, Charles E	Colorado State University	Promotion of hepatic neoplasia by PCB mixtures	NIEHS
Dorgan, J	NCI, NIH	Prediagnostic breast cancer serum bank	Division of Cancer Prevention and Control
Dukelow, W Richard	Michigan State University	Toxic chemical influences on *in vivo* and *in vitro* reproduction	NIEHS
Finlay, Mary F	Benedict College, Columbia, SC	Pilot study—Toxicological effects of PCB during spermatogenesis	NCRR

Table 3-15. Ongoing Studies on the Health Effects of PCBs *(continued)*

Investigator	Affiliation	Title	Sponsor
Gammon, Marilie D	Columbia University	Breast cancer and the environment on Long Island	NCI
Ganey, PE	Michigan State University	Mechanisms and consequences of neutrophil activation by hazardous chemicals	USDA or cooperating state institutions
Gierthy, John F	State Univ of New York at Albany	PCB estrogenicity in human breast cancer cells	NIEHS
Glauert, Howard P	University of Kentucky	Mechanisms of hepatic tumor promotion by PCBs	NIEHS
Gore, Andrea	Mount Sinai School of Medicine of CUNY	Neuroendocrine mechanisms of environmental toxicity during early development	NIEHS
Grandjean, Phillippe	Boston University	Serum PCB as a risk indicator for breast cancer in women	NIEHS
Greeley, George H, Jr	University of Texas Medical Branch	Dioxin action in the alimentary canal	NIEHS
Hansen, LG	University of IL Urbana	Identification of PCB congeners associated with fish consumption	USDA or cooperating state institutions
Harris, Craig	Michigan State University	PCB effects on uterine wall	NIEHS
Hassoun, Ezdihar A	University of Toledo, Toledo, OH	TCDD induced oxidative stress in the tissues	NIEHS
Hejtmancik, Milton	Battelle Memorial Institute, Columbus, OH	Evaluation of dioxin toxic equivalency factors	NIEHS
Hennig, Bernhard	University of Kentucky	Superfund chemicals and endothelial cell dysfunction	NIEHS
Henny, Charles J	Forest and Rangeland Ecosystem Science Center Corvallis, OR	Environmental endocrine disruptors: effects and possible mechanism(s) in young male river otters	USGS Biological Resources Division
Hertz-Picciotto, Irva	University of North Carolina Chapel Hill	Fetal PCB exposure, thyroid function, and neurodevelopment	NIEHS
Hooper, Michael	University of Washington, Seattle, WA	Wildlife biomarker applications to remediation decision making	NIEHS

Table 3-15. Ongoing Studies on the Health Effects of PCBs *(continued)*

Investigator	Affiliation	Title	Sponsor
Hunter, David J	Brigham and Women's Hospital, Boston, MA	Environmental risk factors and breast cancer	NIEHS
Jefcoate, Colin R	University of Wisconsin Madison	Organochlorine compounds and human breast cytochrome P-450	NIEHS
Keefe, Thomas J	Colorado State University	Historical prospective — organochlorines/breast cancer	NCI
Klaassen, Curtis D	University of Kansas Medical Center	Environmental hormones— effects on thyroid function	NIEHS
Klebanoff, MA	NICHD, NIH	Fetal, neonatal and childhood effects of *in utero* exposure to PCBs and DDE	National Institute of Child Health and Human Development
Korrick, Susan A	Brigham and Women's Hospital, Boston, MA	Polychlorinated biphenyls and infertility	NIEHS
Korrish, Susan	Harvard University	*In utero* PCB and metal exposure and infant development	NIEHS
Laessig, Susan A	Marine Biological Laboratory, Woods Hole, MA	*Ortho*-substituted PCB on calcium homeostasis in aplysia bag cell neurons	NCRR
Longnecker, MP	NIEHS, NIH	Human health effects of exposure to organochlorine compounds	NIEHS
Matte, Thomas	Mount Sinai School of Medicine of CUNY	Prenatal PCB exposure and neurodevelopmental outcomes in adolescence and adulthood	NIEHS
Mc Coy, George L	Benedict College, Columbia, SC	Pilot study—Toxic and estrogenic actions of PCB in reproduction	NCRR
Ozonoff, David M	Boston University	Superfund basic research center at Boston University	NIEHS
Peterson, Richard E	University of Wisconsin Madison	Ah receptor independent central nervouse system/reproductive effects of PCBs	NIEHS
Quimby, Fred W	Cornell University	Model for assessment of immunotoxicity of environmental pollutants	NIEHS

Table 3-15. Ongoing Studies on the Health Effects of PCBs *(continued)*

Investigator	Affiliation	Title	Sponsor
Rattner, Barnett A	Patuxent Wildlife Research Center Laurel, MD	Effects of organochlorine contaminants on reproductive success of black-crowned night-herons (*Nycticorax nycticorax*) nesting in Baltimore Harbor, Maryland	USGS Biological Resources Division
Robertson, Larry W	University of Kentucky	Activation of PCB's to genotoxins *in vivo*	NIEHS
Rogan, WJ	NIEHS, NIH	Human exposure to halogenated aromatic compounds	NIEHS
Roth, Robert A	Michigan State University	Mechanisms and consequences of neutrophil activation by hazardous chemicals	NIEHS
Safe, SH	Texas A&M University	Endocrine toxicology studies	USDA or cooperating state institutions
Safe, Stephen H	Texas A&M University	Toxic halogenated aromatics	NIEHS
Santiago-Rivera, Azara L	State University of New York at Albany	Biopsychosocial well being among Akwesasne residents	NIEHS
Schantz, SL	University of Illinois Urbana	Developmental effects of fish-borne toxicants in rats	USDA or cooperating state institutions
Schantz, SL	University of Illinois Urbana	Developmental effects of combined PCB and MEHG exposure	USDA or cooperating state institutions
Schell, Lawrence M	State University of New York at Albany	PCBs and well being of Mohawk children and youth—growth, development, and cognition	NIEHS
Schwartz, Stephen M	Fred Hutchinson Cancer Research Center, Seattle, WA	Phytoestrogens, organochlorines, and fibroid risk	NIEHS
Seegal, Richard F	Wadsworth Center, Albany, NY	Developmental effects of fish borne toxicants in the rat	NIEHS
Shiverick, Kathleen T	University of Florida, Gainesville	Placental/uterine effects of chlorinated hydrocarbons	NIEHS

Table 3-15. Ongoing Studies on the Health Effects of PCBs *(continued)*

Investigator	Affiliation	Title	Sponsor
Spink, David C	State University of New York at Albany	Alterations in estrogen metabolism caused by exposure to PCBs	NIEHS
Stellman, Steven D	American Health Foundation, Valhalla, NY	Epidemiology of breast cancer	NCI
Thomas, Peter M	University of Texas Austin	Mechanisms of reproductive neuroendocrine toxicity	NIEHS
Trosko, James E	Michigan State University	Evaluation of Superfund chemicals as epigenetic toxicants	NIEHS
Weston, Ainsley	Mount Sinai School of Medicine of CUNY	Effects of PCB-containing river sediments on carcinogen metabolism	NIEHS
Wolff, Mary S	Mount Sinai School of Medicine of CUNY	Inner city toxicants and neurodevelopmental impairment	NIEHS
Zacharewski, T	Michigan State University	Identification and assessment of endocrine disruptors	USDA or cooperating state institutions
Zhu, Bao T	University of South Carolina at Columbia	Effects of cigarette smoking or PCBs on human estradiol	NCI
Zoeller, RT	University of Massachusetts	PCB and thyroid hormone action in developing cochlea	NIEHS

Source: FEDRIP (2000), USDA Current Research Information System (2000), USGS-BRD Science Information System (2000)

NCI = National Cancer Institute; NCRR = National Center for Research Resources; NICHD = National Institute of Child Health and Human Development; NIEHS = National Institute of Environmental Health Sciences; NIH = National Institutes of Health; USDA = U.S. Department of Agriculture; USGS = U.S. Geological Survey

Postal abbreviations used

Table 3-16. Ongoing Studies on the Human Health Effects of PCBs Sponsored by ATSDR

Investigator	Affiliation	Title
Anderson, HA	Wisconsin Department of Health and Social Services Madison, Wisconsin	Consortium for the health assessment of Great Lakes sport fish consumption
Darvill, T	State University of New York at Oswego Oswego, New York	Behavioral effects of consumption of Lake Ontario fish: Two methodological approaches
Dellinger, J	University of Wisconsin at Milwaukee Milwaukee, Wisconsin	Ojibwa Health Study II: Epidemiology, laboratory toxicology, and outreach
Fitzgerald, E	New York State Department of Health Albany, New York	Neurologic effects of environmental exposure to PCBs along the upper Hudson River
Karmus, W.	Michigan State University East Lansing, Michigan	Assessing effects in human reproductive health for PCB exposure via consumption of Great Lakes fish
Schantz, SL	University of Illinois at Urbana-Champaign Urbana, Illinois	Human health effects of PCB exposure from contaminated fish
Vena, J	State University of New York at Buffalo Buffalo, New York	The New York Angler Study: Exposure characterization and reproductive and developmental health
Waller, DP	University of Illinois at Chicago Chicago, Illinois	Great Lakes fish as a source of maternal and fetal exposure to chlorinate hydrocarbons

ATSDR = Agency for Toxic Substances and Disease Registry