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Polychlorinated biphenyls, Polybrominated biphenyls, Polychlorinated dibenzo-p-dioxins and Polychlorinated dibenzofurans

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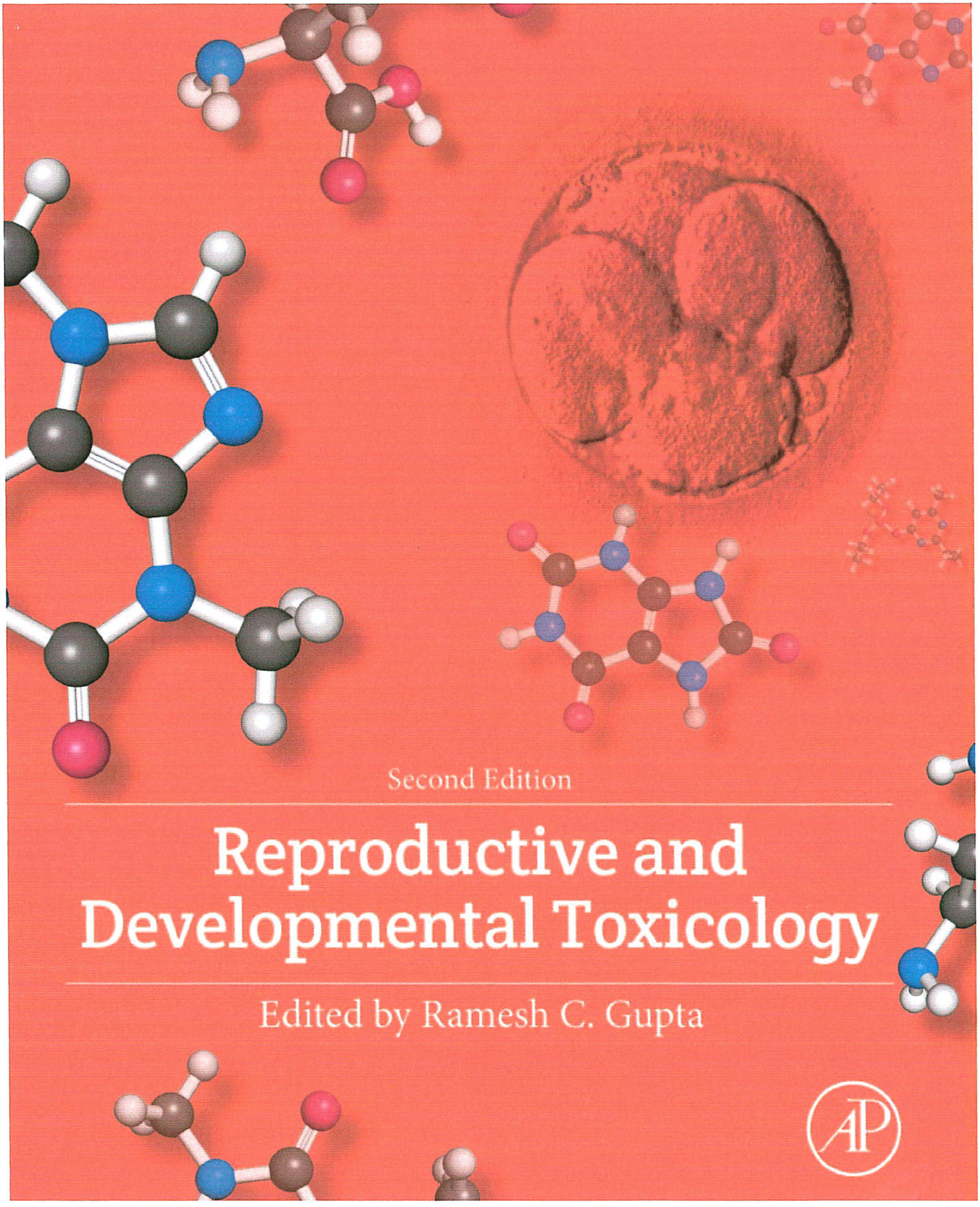

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CHAPTER

39

Polychlorinated Biphenyls, Polybrominated Biphenyls, Polychlorinated Dibenzo-*p*-dioxins, and Polychlorinated Dibenzofurans

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OUTLINE

INTRODUCTION

Polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs) belong to a group of organic compounds that are well known for their contamination in the global environment, bioaccumulate and biomagnify in the food chain

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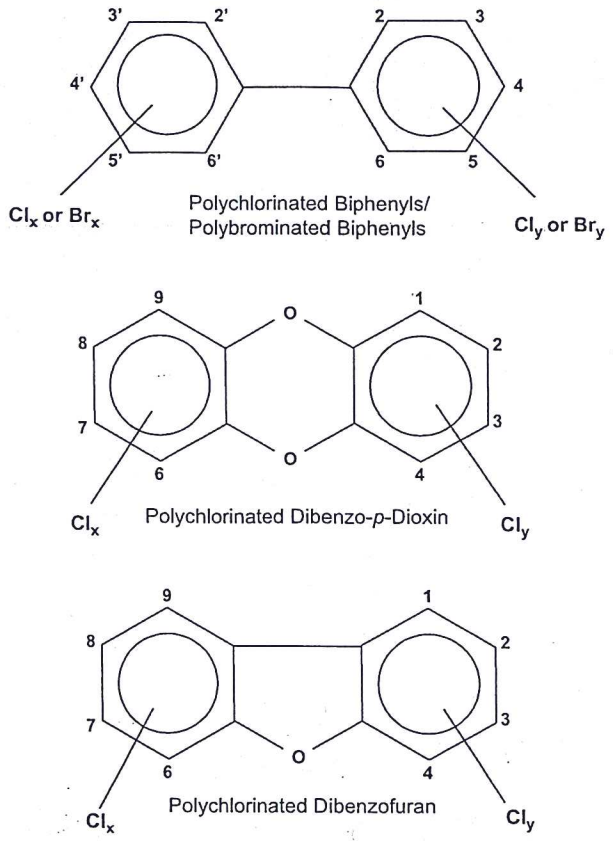

FIGURE 39.1 Core structures of polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs). The numbers indicates position of halogens.

and exert toxic effects in wildlife and humans (Huwe, 2002; Schecter et al., 2006; Kodavanti et al., 2014a,b). PCBs and PBBs were produced commercially for a variety of applications, whereas the PCDDs and PCDFs occur as by-products of industrial and natural processes. PCBs and PBBs are structurally similar and formed by substituting chlorine or bromine, respectively, for hydrogen on the biphenyl molecule that consists of two benzene rings (Fig. 39.1). Theoretically, there are 209 possible PCB and PBB congeners considering the five chlorine- or bromine-binding sites on each ring. Each congener has been assigned a unique number from 1 to 209 in accordance with the rules of the International Union of Pure and Applied Chemistry. Commercial PCB and PBB products were mixtures of congeners that differed with respect to the number and positions of chlorination or bromination. PCDDs are composed of two benzene rings connected by two oxygen atoms and contain four to eight chlorines, for a total of 75 congeners (Fig. 39.1). PCDFs are also composed of two benzene rings. The rings have one oxygen molecule between them and have four chlorine-binding sites available on each ring (Fig. 39.1). There are 135 different PCDF congeners (Huwe, 2002; Kodavanti et al., 2014a,b; Loganathan and Masunaga, 2015).

Certain approximate stereoisomers in this group, often referred to collectively as dioxins and dioxin-like compounds, induce a common suite of effects and have a common mechanism of action mediated by binding of the polyhalogenated aromatic hydrocarbons (PHAHs) ligand to a specific high-affinity cellular protein. This group of chemicals includes 7 PCDD congeners, 10 PCDF congeners, and 12 PCB congeners. Although the PBB congeners analogous to the 12 PCB congeners could also be considered dioxin-like chemicals, the relatively short commercial lifespan and restricted environmental distribution of PBBs generally preclude them from consideration. The prototype for the dioxins is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Toxicity and persistence of the PHAHs are determined by structure, with lateral substitutions on the ring resulting in the highest degree of toxicity. For the PCDDs and PCDFs, congeners with chlorines in the 2, 3, 7, and eighth positions fall into this category. The TCDD-like PCB congeners are the non—*ortho*-substituted and mono-*ortho*-substituted compounds with no chlorines or no more than one chlorine on the 2, 2′, 6, or 6′ position (Schecter et al., 2006; Kodavanti et al., 2014a,b).

The PCDDs, PCDFs, and PCBs are widely distributed in the global environment and highly resistant to environmental degradation and metabolism. As a result, these compounds readily accumulate in the food chain with the greatest tissue concentrations being found in species at the higher trophic levels. Residues have been detected in a variety of biological samples (including human tissues) from several countries (Safe, 1998; Loganathan, 2012; Loganathan et al., 2016a,b). In some situations, the environmental concentrations of these contaminants are high enough to pose a health risk to animals and humans. Because of this risk, efforts should be made to minimize exposure to these environmental contaminants.

HISTORICAL BACKGROUND

Sources

PCBs were first synthesized by Schmidt and Schultz in 1881. Commercial production of PCBs for a variety of uses began in the United States in 1929 and continued until 1977 (Kimbrough, 1995; Headrick et al., 1999). PCBs were used in closed systems such as electrical transformers, capacitors, and heat transfer and hydraulic systems. For a period of time, PCBs also had a large number of open-ended applications including paints, polymers, and adhesives; as lubricants, plasticizers,

fire retardants, and immersion oils; as vehicles for pesticide application; and as agents for the suspension of pigments in carbonless copy paper (Headrick et al., 1999; Loganathan and Lam, 2012). The PCB products in the United States had the trade name Aroclor followed by four digits that identified the particular mixture. The first two digits referred to the 12 carbon atoms of the biphenyl molecule and the last two digits referred to the percent of chlorine in the mixture, by weight. Aroclors 1221, 1232, 1242, 1254, 1260, and 1268 were the commercial PCB products that were containing 21%, 32%, 42%, 54%, 60%, and 68% chlorine by weight, respectively. Similar commercial PCB mixtures were produced by other manufacturers worldwide including the Clophens (Germany), Phenoclors and Pyralenes (France), Fenclors (Italy), and Kanechlors (Japan) (Kimbrough, 1987, 1995; Safe, 1994; Kodavanti et al., 2014a,b).

The physical and chemical properties of PCBs, such as high stability, inertness, and dielectric properties, which were advantageous for many industrial purposes, led to the indiscriminate use of PCBs in large quantities (Loganathan, 2012; Kodavanti et al., 2014a,b). For example, the estimated cumulative production of PCBs in the United States between 1930 and 1975 was 700,000 tons. About 1.2 million tons were estimated to have been produced worldwide. Domestic sales of PCBs in the United States during this time period totaled 627,000 tons (Kimbrough, 1987, 1995; Tanabe, 1988). As a result of widespread use, PCBs were identified in environmental media and biota. Because of their widespread environmental contamination in the 1970s, PCB production decreased and eventually ceased (Loganathan and Kannan, 1994). Although PCBs are no longer commercially produced and used because of their persistence, PCBs are still found in the environment and biological tissues. About 31% (370,000 tons) of all the PCBs produced is present in the global environment. It is estimated that 780,000 tons are still available in older electric equipment and other products, deposited in landfills or in storage (Tanabe, 1988).

PBBs were manufactured for use as flame retardants in industrial and consumer products (Damstra et al., 1982; Kodavanti et al., 2014a,b). It is estimated that approximately 13 million pounds were produced in the United States from 1970 to 1976 and used for incorporation into plastic products that included business machine housings, radios, televisions, thermostats, electric shavers, hand tools, and various automotive parts (DiCarlo et al., 1978; Headrick et al., 1999). Three commercial PBB products were manufactured in the United States: hexabromobiphenyl, octabromobiphenyl, and decabromobiphenyl (DiCarlo et al., 1978; Hardy, 2000).

Hexabromobiphenyl was the predominant product with approximately 11.8 million pounds being produced. More than 98% of the hexabromobiphenyl was produced as FireMaster BP-6 with the remainder being produced as FireMaster FF-1 (Hesse and Powers, 1978) after addition of an anticaking agent to FireMaster BP-6. Production of PBBs ceased in 1974 (DiCarlo et al., 1978).

PCDDs and PCDFs are by-products that are formed during the synthesis of certain industrial halogenated aromatic chemicals, as by-products from other commercial processes, and as by-products of combustion (Safe, 1990; Kodavanti et al., 2001). Some of the important industrial sources of PCDDs and PCDFs have included their formation as by-products in the production of PCBs; chlorinated phenols and chlorinated phenol-derived chemicals; hexachlorobenzene; technical hexachlorocyclohexanes; and chlorides of iron, aluminum, and copper. PCDDs and PCDFs have also been identified in effluents, wastes, and pulp samples from the pulp and paper industry and in finished paper products. Emissions from municipal and hazardous waste incinerators and home heating systems that use wood and coal, diesel engines, forest and grass fires, and agricultural and backyard burning contain PCDDs and PCDFs (Safe, 1990; Huwe, 2002; Loganathan and Masunaga, 2015). In addition, these compounds might come from naturally formed PCDD/Fs, which have been detected in deep soils and clays from the southern United States and Germany (Safe, 1990; Huwe, 2002).

Environmental Fate

The release of PCBs into the environment primarily has been the result of leaks, spills, and improper disposal. As stated earlier, it is estimated that approximately 370,000 tons of PCBs are present in the global environment (Tanabe, 1988). The volatility of PCBs allows their evaporation from source containers and movement through the atmosphere, resulting in widespread environmental dispersal (Headrick et al., 1999). PCDDs and PCDFs are released into the atmosphere primarily by combustion sources and by evaporation from PCDD/PCDF-containing soils and water. Similar to PCBs, the PCDDs and PCDFs can be transported long distances by winds, contributing both to general background concentrations and contamination of remote areas far from the original source. PCBs, PCDDs, and PCDFs are removed from the atmosphere by physical processes such as wet and dry deposition and vapor uptake and are deposited on soils, surface waters, and plant surfaces. Most of the PCBs, PCDDs, and PCDFs that are deposited on surface waters sorb onto suspended sediments. Once bound to soil and sediment, these chemicals generally remain fixed except for bulk transport because of soil erosion, flooding, and dredging (Dickson and Buzik, 1993). Ingestion of these

compounds by animals results in their preferential bioaccumulation and biomagnification in the food chain (Safe, 1994).

Because PBBs were manufactured for a relatively short time and because of their restricted use, PBBs are not considered to be a significant environmental contaminant with the exception of specific locations in Michigan, where PBBs were produced and used. Environmental removal of PBBs from manufacturing sites were estimated to be 0.11% into the atmosphere as particulate matter, negligible losses to storm sewers, and 5% as solid waste to landfills. Similar to PCBs, PCDDs, and PCDFs, PBBs are very stable and persist in the environment. Studies have indicated that PBBs have a high affinity for soil and undergo very little degradation and translocation into vegetation (Fries, 1985). PBBs are also very lipophilic and have the potential to bioaccumulate and biomagnify in the food chain (Damstra et al., 1982).

PCBs, PBBs, PCDDs, and PCDFs in environmental samples are complex mixtures of congeners. Because of various physical and biological processes, the composition of the commercial PCB mixture and an environmental PCB/PCDD/PCDF mixture may vary significantly from one another. Thus, the impacts of these chemicals on the environment and biota are because of the individual components of these mixtures and their additive and/or nonadditive (synergistic/antagonistic) interactions with themselves and other classes of pollutants (Safe, 1994; Loganathan and Masunaga, 2015).

Exposure

There are a number of ways by which animals can be and have been exposed to PCBs, PCDDs/PCDFs, and PBBs. Some of the scenarios described involve ingestion of low concentrations of these chemicals through consumption of environmentally contaminated feed or feed components, whereas other scenarios involve accidental incorporation of the chemical into the feed resulting in exposure to relatively high concentrations of the contaminant. During the 1940s and 1950s, silos constructed with concrete were sealed with a PCB-containing paint, which eventually peeled off from the walls resulting in contaminated silage. Dairy and beef cattle were exposed to the paint in the feed resulting in accumulation of PCBs in adipose tissue. As a result, food products such as milk and meat contained detectable concentrations of PCBs. Examples of other exposure incidents resulting in PCB residues in food animals were summarized by Headrick et al. (1999). These include consumption of tar paper by veal calves, consumption of fish viscera by swine, pullet consumption of feed containing PCB-contaminated fat added during processing, exposure of chickens to ceiling insulation and fiberglass insulation that contained PCBs, and treatment of boars with a topical pesticide containing PCB-contaminated oil.

Several dioxin contamination incidents have occurred in Europe. In 1998, during routine monitoring, dairy products were identified that had dioxin concentrations that were two to four times higher than background concentrations. The source of the contamination was traced to contaminated citrus pulp used as a cattle feed component. The citrus pulp and contaminated feeds were immediately removed from the market. In another incident, PCB/PCDD/PCDF-contaminated oil was added to recycled fat used as an additive in animal feeds. The affected feeds contaminated Belgian poultry, dairy, and meat and were discovered only after toxic effects characteristic of "chick edema disease" were seen in chickens (Van Larebeke et al., 2001; Bernard et al., 2002; Huwe, 2002).

PCBs in Public School Buildings

A significant number of buildings that were constructed between the 1950s and 1970s (when PCBs were being used frequently throughout the world) were found to contain PCB-contaminated building materials (Herrick et al., 2004; Kohler et al., 2005; Priha et al., 2005). The building materials considered to be primary sources of PCBs include caulking, fluorescent light ballasts, transformers, capacitors, coolants, lubricants, plasticizers, inks, flame retardants, paints, and adhesives. However, of these materials, the most culpable seemed to be caulking, which contained PCBs as plasticizing agents (Thomas et al., 2012). As mentioned earlier in this chapter, the volatility of PCBs enables them to travel from a primary source to areas throughout the environment. In this instance, PCBs were found to be capable of spreading throughout the building's interior and exterior by attaching to air, dust, or soil creating multiple opportunities in which PCB exposure was possible.

One of the first chemical substance that fell under the Toxic Substances Control Act criteria was PCBs. In an effort to better regulate PCBs that remained in use, scientists first had to acquire the ambient/background levels of PCBs present in a particular environment. In one study, various locations such as public buildings, homes, and even schools were tested for PCB levels in indoor and outdoor air samples. Not surprisingly, the results revealed a higher amount of PCBs in the indoor air when compared with outdoor air (MacLeod, 1981). Although the amount of PCBs measured was under what is considered safe for the workplace, a standard set by the National Institute for Occupational Safety and Health (1 $\mu g/m^3$ over an 8–10 h workday, 40 h

per week; NIOSH, 1977), the presence of PCBs in publicly accessible buildings still raises concern. It should also be noted that buildings containing PCB-laden materials during or shortly before the testing period of these studies caused an elevation in the amount of PCBs measured, which only strengthens the argument for remediation of primary PCB sources within these buildings (Oatman and Roy, 1986).

Although materials such as fluorescent light ballasts and transformers were found to be primary sources of PCBs, it was the caulking tested in these buildings that sparked a high concern because of the potential for direct contact. In Greater Boston area, MA, USA, 33% of the buildings tested contained PCB-laden caulking at a concentration over 50 parts per million (ppm), which would classify it as a bulk PCB waste product as specified by the US Environmental Protection Agency (Herrick et al., 2004). In Switzerland, a large-scale study involving the collaboration of 17 laboratories throughout the country found that of the 1348 samples collected from joint sealants used in the construction of concrete buildings between the years 1966–1971, 21% contained PCB-laden material at a concentration greater than 10 g/kg by weight, whereas 9.6% of the samples contained PCBs greater than 100 g/kg by weight. In total, 48% of the samples collected during the study contained measurable amounts of PCBs (Kohler et al., 2005).

Not all the PCBs measured inside buildings remained aerosolized. In another study conducted by Coghlan et al. (2002), dust from unit ventilators was taken from a university building that was void of any obvious PCB source historically and presently. Interestingly, 78% of the dust samples assayed contained PCBs that ranged from undetectable to 81 ppm, which is well above the 50-ppm limit mentioned earlier. Also, dust from the ventilation system at a high school in New Bedford, MA, USA, was thought to have had a significant role in the elevated PCBs measured during air sampling (Sullivan et al., 2008). Aroclor 1248 and 1254 and Clophen A50 were among the most frequent mixtures detected, with smaller amounts of high and low chlorinated mixtures also present (Coghlan et al., 2002; Kohler et al., 2005). Because the PCB-laden caulking was used on both the inside and outside of these buildings, some PCB content could leach into the outdoor environment. In soil samples collected from areas surrounding the buildings, high amounts of PCBs were found closer to the building with smaller amounts detected as the distance increased. This trend indicates that the contamination could have occurred because of natural weathering or during building maintenance, which involved removal of old caulking using methods that would leave considerable amounts of PCBs behind (Priha et al., 2005).

PBB Incident in Michigan

FireMaster BP-6 was a mixture of PBB congeners containing two to eight bromines. The major constituents were 2,2′,4,4′,5,5′-hexabromobiphenyl (56%) and 2,2′,3,4,4′,5,5′-heptabromobiphenyl (27%) (Damstra et al., 1982). In 1972, the formulation was changed by grinding BP-6 and adding 2% calcium silicate as an anticaking agent. This new formulation, now called FireMaster FF-1, was a white powder as opposed to brown flakes, which was the appearance of BP-6 (Fries, 1985). In May 1973, 650 pounds of FF-1 were mistakenly included in a shipment of feed-grade magnesium oxide to a feed mill in Climax, MI. This Company, in addition to producing FireMaster FF-1, also produced the magnesium oxide product, which had an appearance identical to FireMaster FF-1 and was sold under the trade name NutriMaster. Normally, the two products were packaged in paper bags with unique color codes. However, during a paper shortage, both FireMaster and NutriMaster were packaged in plain brown bags differentiated only by the product names stenciled on the bags. Both products were stored in the same warehouse (Dunckel, 1975; Fries, 1985). A portion of the magnesium oxide that was shipped to the Climax feed mill was used to mix feeds primarily for dairy cattle. The remaining magnesium oxide was shipped to other mills within the state and used in feeds mixed at those locations. Feeds that were not formulated to contain magnesium oxide also became contaminated because of carryover from the contaminated feed-mixing equipment (Fries, 1985).

Most of the high-level exposures occurred during the fall of 1973 before sale of the initial batch of feed was stopped in December 1973 because of dairy producer complaints of animal health problems. Three initial feed preparations containing different concentrations of PBBs were Feed No. 405, which had 2.4 μg/g (ppm) PBBs, No. 410 having 1790 ppm PBBs, and No. 407 containing 4300 ppm PBBs. The highest feed concentration reported was 13,500 ppm PBBs (DiCarlo et al., 1978; Damstra et al., 1982). Low-level contamination of feed continued beyond the chance identification of PBBs as the contaminant in April 1974 because of their persistence. In 1974, 68% of 1770 feed samples collected in Michigan contained PBB residues. Resampling in 1975 indicated that 6% of 1208 feed samples were contaminated, and in 1976, only 0.3% of 663 samples contained PBBs (DiCarlo et al., 1978). Shortly after PBBs were identified as the feed contaminant, the US Food and Drug Administration (US FDA) set a temporary guideline of 1 ppm PBBs in milk fat, meat and poultry, 0.1 ppm in whole eggs, and 0.3 ppm in animal feeds. Because of the long half-life of PBBs, the decision was made to depopulate affected farms and to dispose off the animals at a

burial site in northern Michigan (Damstra et al., 1982; Fries, 1985; Headrick et al., 1999). In October 1974, the FDA lowered the guidelines for PBBs in milk and meat from 1 ppm to 0.3 ppm, which resulted in disposal of additional cattle, swine, and sheep (Damstra et al., 1982). In response to increasing concerns about the effects of PBBs on human and animal health, the Michigan legislature lowered the PBB tolerance to 0.02 ppm in body fat of all dairy cattle offered for slaughter in 1977. A small number of dairy producers who had repopulated after the initial quarantine in 1974 continued to have violative cattle because of residual contamination on their facilities, although this number was less than 2% of all culled cows.

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PCBs and PBBs

Because commercial PCB and PBB products are mixtures of individual congeners that differ in the number and position of chlorine or bromine atoms and thus differ in terms of their biological activities, it is difficult to accurately assess their absorption, distribution, metabolism, and elimination. A number of experiments have been conducted with a variety of species including cows, pigs, rats, and birds on the absorption, distribution, metabolism, and elimination of the commercial PBB mixture, FireMaster BP-6, which have been summarized in an extensive review by Fries (1985). Because of the similarities between PCBs and PBBs, information pertaining to one can generally be applied to the other.

In general, PBBs are rapidly and extensively absorbed, with absorption being inversely dependent on the number of bromine atoms (Damstra et al., 1982; Fries, 1985). For example, less than 10% of an oral dose of C^{14}-labeled 2,2′,4,4′,5,5′-hexabromobiphenyl was eliminated in rats (Matthews et al., 1977; Fries, 1985), indicating almost complete absorption compared with 62% fecal elimination of C^{14}-labeled octobromobiphenyl 24 h after dosing, suggesting incomplete absorption (Norris et al., 1975; Fries, 1985). PBBs are widely distributed throughout the body of all species studied. Initial concentrations are generally greatest in the liver and adipose tissue with highest equilibrium concentrations on a wet tissue basis being adipose tissue (Damstra et al., 1982; Fries, 1985). Concentrations of PBBs in muscle and other tissues are usually an order of magnitude lower (Fries, 1985) compared with adipose tissue. Generally, differences in concentration between tissues can be attributed, at least in part, to variations in their fat content.

Individual PBB congeners in FireMaster BP-6 undergo hydroxylation by metabolic routes that are similar for the related PCBs with the rate of metabolism being determined primarily by the position of bromine atoms on the ring and secondarily by the bromine content of the molecule (Damstra et al., 1982). In vivo studies suggest that, similar to PCBs, metabolism can occur if there are two adjacent unbrominated positions (Matthews et al., 1978; Fries, 1985).

Elimination of individual PBB congeners occurs at different rates with those congeners that are more slowly removed becoming more concentrated in tissues relative to those congeners that are more rapidly eliminated. PBBs are eliminated primarily by biliary excretion into the feces, but fecal concentrations are low compared with whole-body concentrations (Damstra et al., 1982; Fries, 1985). For example, less than 7% of an intravenous dose of 2,2′,4,4′,5,5′-hexabromobiphenyl was eliminated by rats over a 42-day period (Matthews et al., 1977; Fries, 1985) and rhesus monkeys excreted on a daily basis approximately 0.5% of a single oral dose of the same congener from 10 to 42 days postdosing (Rozman et al., 1982; Fries, 1985).

Placental transfer of PBBs occurs to some extent, but the levels in fetal or offspring tissues are relatively lower compared with levels in maternal tissues. Transfer of PBBs to the offspring during nursing results in much greater whole-body concentrations compared with PBB transfer during gestation. For example, pigs that were fed PBBs during gestation and lactation had a five-fold increase in body burden during the 4-week lactation period with residues accumulated during lactation accounting for 95% of the total body burden (Werner and Sleight, 1981; Fries, 1985).

As suggested above, milk is the major route of elimination of PBBs for lactating mammals, although in the case of females nursing their young, the contaminant is simply transferred from one animal to another. Concentrations of PBBs in milk fat generally exceed dietary levels, with bovine milk fat concentrations exceeding dietary levels by three- to four-fold (Fries and Marrow, 1975; Willett and Irving, 1976; Damstra et al., 1982; Fries, 1985).

PBBs can have a relatively long biological half-life in animals. Data suggested that only 10% of the total dose of 2,2′,4,4′,5,5′-hexabromobiphenyl would be eliminated during the lifetime of a rat (Matthews et al., 1977; DiCarlo et al., 1978). Rats receiving a single dose of C^{14}-octabromobiphenyl had biphasic fecal excretion with the initial half-life being less than 24 h and second phase half-life being greater than 16 days (Norris et al., 1975; DiCarlo et al., 1978). Studies with cows suggested biphasic elimination of PBBs via the milk with an initial half-life of 11 days and a second half-life of 58 days (Gutenmann and Lisk, 1975; DiCarlo et al., 1978). In cases where observation periods were long, a biological half-life of 180 days was estimated for lactating cows

(Fries, 1985). It was estimated that the concentration of FireMaster BP-6 in bovine milk fat would decrease from approximately 300–0.3 ppm in 120 weeks (DiCarlo et al., 1978). A half-life of 17 days was calculated for FireMaster FF-1 in the eggs of chickens fed a diet containing the commercial mixture. Half-lives of 17 days and 31 days were calculated for muscle and liver, respectively, and the concentration of PBBs in adipose tissue was essentially unchanged after 56 days (Ringer and Polin, 1977; Polin and Ringer, 1978b). It was estimated that a chicken exposed to 1 ppm PBB in the feed for at least 10 days (the minimum time required to attain a steady-state concentration in the contents of a whole egg) would require 87 days of feeding uncontaminated feed to reach a concentration of 0.05 ppm in the whole egg (Ringer and Polin, 1977). The half-life of hexabromobiphenyl was 28 days and that of heptabromobiphenyl was 20 days in chicken eggs (Fries et al., 1976; DiCarlo et al., 1978).

PCDDs and PCDFs

The absorption, distribution, metabolism, and elimination of PCDDs and PCDFs have been extensively reviewed by Van den Berg et al. (1994). Absorption from the gastrointestinal tract of mammals is effective and can exceed 75% of the dose for the lower chlorinated congeners. With increasing molecular size, absorption from the intestines is greatly reduced, which is most apparent for the hepta- and octachlorinated congeners. The liver and adipose tissue are the major storage sites of PCDDs and PCDFs for most mammalian and avian species. Whole-body half-lives of the group of 2,3,7,8-substituted congeners in rodents range from a few to more than 100 days.

The absorption of PCDDs and PCDFs from the gastrointestinal tract has been studied for a number of individual congers. The extent of absorption of TCDD or related compounds is variable, depending on the vehicle and the substitution pattern of the congener. There seem to be no differences between species in terms of absorption of these compounds through the gastrointestinal tract. Studies with rats, mice, hamsters, guinea pigs, cows, and chickens, in general, indicate that tetra- and pentachlorinated congeners are well absorbed from the gastrointestinal tract (50–90%), but octa is absorbed only to a limited extent (2–15%) (Pohjanvirta and Tuomisto, 1994; Van den Berg et al., 1994).

The tissue distribution of PCDDs and PCDFs has been extensively studied in laboratory experiments using rodents and nonhuman primates. Upon absorption, 2,3,7,8-substituted PCDDs and PCDFs are bound to chylomicrons, lipoproteins, and other serum proteins and transported throughout the circulatory system. The liver and adipose tissue are the major storage sites of PCDDs and PCDFs for most mammalian and avian species, whereas, depending on species, the skin and adrenals can also act as primary sites for deposition. Few studies suggest that the tissue distribution of TCDD and related compounds is dose dependent in that as the dose increases, so does the liver:adipose distribution ratio. In the liver, TCDD induces both cytochromes CYP1A1 and CYP1A2. Induced CYP1A2, in turn, seems to be a crucial binding species for TCDD and related compounds in rodents. The hepatocellular binding of TCDD to CYP1A1 is so strong that only a very limited amount will be released back into the circulation. Placental transfer of 2,3,7,8-substituted PCDDs and PCDFs was found to be strongly dependent on molecular size with TCDD being retained to the greatest extent in the fetus. In mammalian species, the transfer of PCDDs and PCDFs from the mother to the offspring via lactation is quantitatively more important than transport to the fetus across the placenta. Excretion via lactation generally decreases as chlorine content increases, being most pronounced for the hepta- and octachlorinated congeners (Pohjanvirta and Tuomisto, 1994; Van den Berg et al., 1994).

As in mammals, the liver and adipose tissue of avian species are the major sites for storage and accumulation of 2,3,7,8-substitiuted PCDDs and PCDFs. Hepatic deposition of 2,3,7,8-substituted PCDDs and PCDFs seemed to increase with increasing chlorination, resulting in a limited transfer of the more highly chlorinated congeners to the egg (Van den Berg et al., 1994). Metabolism of TCDD and related compounds is necessary for urinary and biliary elimination, thus playing a major role in regulating the rate of excretion of these compounds. The induction of CYP1A1 and CYP1A2 enzyme activities by 2,3,7,8-substituted PCDDs and PCDFs has been shown to be one of the most sensitive parameters for biological activity of these compounds. The enzymes most studied are the CYP1A1-dependent ethoxyresorufin-*o*-deethylase and aryl hydrocarbon hydroxylase (AHH). In addition, 2,3,7,8-substituted PCDDs and PCDFs also induce Phase II enzymes (Van den Berg et al., 1994).

In mammals, the liver and adipose tissue are the major compartments for the deposition of PCDDs and PCDFs. The elimination of polar metabolites of 2,3,7,8-substituted PCDDs and PCDFs occurs predominantly via the bile and feces, with urinary excretion playing a minor role. However, urinary elimination plays an important role in the hamster. In rats, the whole-body half-lives of PCDDs and PCDFs range from 17 to 31 days, depending on the dose and strain of rat used, whereas in hamsters and mice, the whole-body half-lives range from 11 to 15 days and from 11 to 24 days, respectively. In rats, it was shown that lactation can

reduce the half-life of these compounds, whereas egg-laying can reduce the half-life in avian species. In lactating cows, mean half-lives ranged from 40 to 50 days for tetra- to hepta-CDDs and CDFs. The compound 2,3,7,8-tetrachlorodibenzofuran (TCDF) is eliminated more rapidly than TCDD, having a whole-body half-life of 2 days, and this rapid elimination is thought to be because of its rapid metabolism. In contrast, 2,3,4,7,8-penta-CDF is eliminated very slowly in the rat, with a whole-body half-life of 64 days. The slow elimination rate is probably because of tight binding of this congener by CYP1A2, in addition to limited metabolism. As chlorine content increases, the rate of elimination of PCDDs and PCDFs decreases (Pohjanvirta and Tuomisto, 1994; Van den Berg et al., 1994).

Interspecies differences in toxicity can only be partly explained by differences in toxicokinetics. The hamster is the species most resistant to the acute toxicity of TCDD. Although the elimination rate of TCDD is two- to three-fold greater in the hamster than the rat and mouse, this does not explain entirely the 10- to 100-fold difference in acute toxicity between the hamster and other rodent species. The guinea pig is most sensitive to the acute effects of TCDD, and it is the species with the slowest metabolism and elimination of TCDD, suggesting that toxicokinetics in part explains the unique sensitivity of the guinea pig to the acute toxicity of TCDD and 2,3,7,8-TCDF (Van den Berg et al., 1994).

HEALTH EFFECTS

General Considerations

Exposure to PBBs, PCBs, PCDDs, and PCDFs has been linked with a broad spectrum of effects, both in vivo and in vitro, which vary depending on method/age of exposure, sex of the individual, and dose/duration of exposure (Steinberg et al., 2008). Fetal and early developmental exposures to these chemicals are particularly devastating and can have different outcomes from adult exposure (Crews et al., 2000). As stated by Steinberg et al. (2008), latent effects of early exposures include, but are not limited to, depressed circulating thyroid hormone and abnormal thyroid cytology (Porterfield, 1994; Goldey et al., 1995; Morse et al., 1996a; Chauhan et al., 2000; Bansal et al., 2005); developmental effects of the heart, palate, and kidney (Foster et al., 2010); delayed cognitive development (Chen et al., 1992; Jacobson and Jacobson, 1997); altered sensory and motor abilities (Bowman et al., 1981; Lasky et al., 2002; Roegge et al., 2004); reproductive impairment (Sager and Girard, 1994; Arnold et al., 1995; Meerts et al., 2004; Yang et al., 2005); and compromised neural function (Morse et al., 1996b; Provost et al., 1999; Donahue et al., 2004; Seegal et al., 2005).

Significant differences in toxic effects will result depending upon when exposure occurs during gestation (Miller et al., 2004). For example, in the mouse, gestational development begins with fertilization, cleavage, and blastulation that occur in the oviduct between gestation day (GD) 0 and 5. Between GD 5 and 10, the ectoderm, mesoderm, and endoderm are formed and early organogenesis is initiated. At GD 9, the heart begins to beat, the neuropore closes, and organogenesis continues through GD 15. Primordial germ cells enter the genital ridges at GD 11, and sexual differentiation of the gonads occurs at GD 12.5. Further fetal growth and development and bone formation occur from GD 14 through 19. Exposure to chemicals before GD 12 will have a significant effect on organogenesis and sex-appropriate differentiation of the gonads. Exposures that occur after GD 14 will predominantly affect the overall growth of the fetus because the organ systems have essentially developed before this point.

Many environmental contaminants may mediate their effects by receptor binding, modulation of hormone-regulated mechanisms, or direct toxic effects. TCDD and related compounds such as the non–ortho PCBs are considered to be antiestrogenic, whereas ortho-substituted PCBs have estrogenic properties (Pflieger-Bruss and Schill, 2000; Pflieger-Bruss et al., 2004). A number of studies have demonstrated PBB-induced reproductive effects in a variety of species. Exposure of poultry to deleterious concentrations of PBBs resulted in an initial decrease in feed consumption accompanied by a decrease in egg production. At sufficiently high concentrations, there was a dose-related decrease in egg hatchability with embryo mortality occurring late in incubation (Fries, 1985). Dietary concentrations of FireMaster FF-1 greater than 30 ppm resulted in a significant decrease in egg production of chickens, which returned to control values from 2 to 6 weeks after withdrawal of the contaminated feed. Concentrations above 30 ppm also had an adverse effect on hatchability and subsequent survivability of chicks (Ringer and Polin, 1977; Polin and Ringer, 1978a). Edema of the abdominal and cervical regions of the chicken embryos and hatchlings was the prevalent pathological condition observed and was the only effect that had an increased incidence compared with the incidence of abnormalities observed in controls (Ringer and Polin, 1977; Polin and Ringer, 1978b). A dietary PBB concentration of 20 ppm had no effect on egg production and hatchability in Japanese quail, but 100 ppm resulted in a significant decrease in both parameters (Babish et al., 1975). Rats administered FireMaster BP-6 at doses of 5 or 25 mg/kg body weight every other day for 14 days had a reduced number

of implantation sites compared with controls at both doses and increased resorptions and fetal deaths at the higher dose (Beaudoin, 1977; Damstra et al., 1982). Single doses of 400- and 800-mg/kg body weight caused a decrease in rat fetal weights at term and an increased incidence of cleft palate and diaphragmatic hernia. Pregnant rats fed diets containing 100 and 1000 ppm FireMaster BP-6 experienced increased fetal mortality and reduced fetal weights at term (Corbett et al., 1975, 1978; Damstra et al., 1982). Mice exposed to 200-ppm dietary PBBs on GD 4 through 16 had an increase in fetal deaths and resorptions and reduced fetal weights at term (Preache et al., 1976; Damstra et al., 1982). Exencephaly and cleft palate were observed in offspring of mice fed FireMaster BP-6 at concentrations up to 1000 ppm (Corbett et al., 1975, 1978; DiCarlo et al., 1978). Pigs that were fed diets containing up to 200 ppm PBBs during gestation had normal young but as the offspring nursed, there was 50% mortality in those receiving 200 ppm via lactation and significant growth depression in offspring consuming 100 ppm in the milk (Werner and Sleight, 1981; Fries, 1985). Rhesus monkeys fed diets containing 0.3 ppm FireMaster FF-1 for 7 months before breeding had prolonged menstrual cycles and decreased progesterone concentrations. Offspring had depressed birth weights and growth rates through 16 weeks of age (Allen and Lambrecht, 1978; Damstra et al., 1982). Mink were fed diets containing FireMaster FF-1 at concentrations ranging from 1.0 to 15.6 ppm, a diet containing contaminated chicken that provided a PBB concentration of 1.5 ppm or a diet containing contaminated beef that provided a PBB concentration of 12.0 ppm for up to 10 months. Adults experienced increased mortality at dietary concentrations of 6.25 ppm or greater. There was also a significant decrease in litter size, birth weights, and kit survivability through 4 weeks of age at dietary concentrations from 1.0 to 2.5 ppm and greater. Results indicated that the PBB-contaminated poultry and beef were more toxic than the commercial mixture. A concentration lethal to 50% of the population for FireMaster FF-1 was estimated to be 4 ppm when fed for more than 300 days. This study indicated that PBBs were not as fetotoxic as two commercial PCB mixtures (Aroclors 1242 and 1254) but were lethal to adults at a lower dietary concentration (Aulerich and Ringer, 1979).

Male Reproductive Effects

TCDD and related compounds decrease testis and accessory sex organ weight, cause abnormal testicular morphology, decrease spermatogenesis, and reduce fertility when given to adult animals in doses sufficient to reduce feed intake and/or body weight. Some of these effects have been reported in chickens, rhesus monkeys, rats, guinea pigs, and mice treated with toxic doses of these chemicals. TCDD effects on spermatogenesis are characterized by loss of germ cells, the appearance of degenerating spermatocytes and mature spermatozoa within the lumens of seminiferous tubules, and a reduction in the number of tubules containing mature spermatozoa. Effects of TCDD on the male reproductive system are thought to be in part because of an androgen deficiency, caused by decreased plasma testosterone and dihydrotestosterone levels and unchanged plasma clearance of androgens and luteinizing hormone (LH) (Peterson et al., 1993; Safe, 1994). Pflieger-Bruss et al. (2004) provide a review of the effects of various endocrine-disrupting chemicals, including PCBs/PCDDs/PCDFs, on the male reproductive system.

Sexual Development

Aoki (2001) summarized effects in humans exposed to PCBs and PCDFs in contaminated rice oil resulting in Yusho disease (Japan) and Yucheng disease (Taiwan). Sexual development of Yucheng boys was delayed and was thought to be because of altered hormonal status caused by PCBs and related congeners (Guo et al., 1995). Den Hond et al. (2002) reported that fewer adolescent males living near two waste incinerators had achieved adult stages of genital development and pubic hair growth compared with youth living in the control area and that there was a negative association between serum concentrations of PCB 153 and genital development.

Sperm Quality

Epidemiological studies have indicated variable effects of potential PCB/PCDD/PCDF exposure on semen quality and sperm counts. Guo et al. (2000) reported that sperm collected from males exposed prenatally to PCBs and PCDFs as a result of consumption of contaminated rice oil in Yucheng, Taiwan, between 1978 and 1979 was characterized by abnormal morphology, reduced motility, and reduced capacity to penetrate hamster oocytes. Hsu et al. (2003a) reported a higher percentage of oligospermia, abnormal sperm morphology, and reduced sperm-binding capability and penetration in the same cohort. Decreases in sperm concentration and sperm motility (Van Waeleghem et al., 1996) and total sperm count (Comhaire et al., 2007) were reported in males exposed to TCDD in Belgium. Mocarelli et al. (2008) reported that men exposed to TCDD during the 1976 Seveso incident before puberty had reduced sperm count and motility as adults, whereas males exposed during adolescence exhibited increased sperm counts and motility. White and Birnbaum (2009) point out that these effects occurred at a concentration [less than

69 parts per trillion (ppt) in serum lipid in 1976] that is within an order of magnitude of the present mean concentrations (15 ppt on a serum lipid basis).

Foster et al. (2010) state that of the reproductive/developmental effects of TCDD, decreased sperm counts are considered to be the most sensitive outcome. A single exposure of rats to 0.064 μg TCDD/kg body weight on GD 15 resulted in a significant decrease (36%) in epididymal sperm counts (Mably et al., 1992). The World Health Organization (WHO) used this endpoint in establishing a tolerable daily intake for TCDD of approximately 2 pg/kg body weight/day (Foster et al., 2010). However, results of recent studies generally have not indicated an effect of in utero exposure to TCDD on epididymal sperm counts. Foster et al. (2010) propose that the primary effect of TCDD in relation to reduced sperm counts is because of developmental abnormalities of the male reproductive tract and epididymal structure and/or function. Foster et al. (2010) also demonstrated that developmental exposure to TCDD has been consistently linked with decreased cauda epididymal and ejaculatory sperm counts in different rodent species, although doses at which the effects occur vary. They further state that the evidence linking in utero TCDD exposure and spermatogenesis is less convincing and that effects of TCDD on androgen signaling, reproductive organ weights, and sperm transit through the epididymis may better account for the decrease in epididymal sperm counts.

Studies that have examined the functional effects of exposure to commercial PCB mixtures in male rodents indicate that the effects on testis weight and fertility depend on the test congener or mixture, the dosage, the developmental stage during exposure, and the age of the animal at the time of examination and species and strain (Fielden et al., 2001). For example, Sager (1983), Sager et al. (1987), and Sager and Girard (1994) reported that male Holtzman rats exposed to Aroclor 1254 from birth to 9 days of age had decreased fertility at 18 weeks of age and increased testis weight at 23 weeks of age. Epididymal sperm count and sperm morphology and motility were not adversely affected, but there was a decline in the ability of sperm to fertilize eggs. Cooke et al. (1996) demonstrated that neonatal exposure (from birth to day 25 of age) of Sprague–Dawley rats to Aroclor 1254 and Aroclor 1242 increased testis weight and daily sperm production at 19 weeks of age that were attributed to PCB-induced hypothyroidism. In contrast to the reduced fertility of Aroclor 1254 exposed pups reported by Sager (1983) and Sager et al. (1987, 1991), all Aroclor 1242–treated male pups bred successfully. Fielden et al. (2001) conducted a study to determine if gestational and lactational exposure of B6D2F1 mice to Aroclor 1242 could induce alterations in organ development and sperm quality and fertility in young adult (16 weeks of age) male offspring and to determine if the effects persisted into middle age (45 weeks of age). They reported no increase in testes size and epididymal sperm count at either age. However, in vitro sperm fertilizing ability was significantly decreased at both 16 and 45 weeks of age, suggesting that fertility in the adult mouse is susceptible to developmental exposure to Aroclor 1242 and that it is independent of testis weight or epididymal sperm count.

Hsu et al. (2003a,b) reported that a single dose (9.6 or 96 mg/kg body weight) of 2,2′,3,3′,4,6′-hexachlorobiphenyl (PCB 132) or 2,2′,3,4′,5′,6-hexachlorobiphenyl (PCB 149) at 21 days of age resulted in decreased sperm motility, velocity, and the ability of sperm to penetrate oocytes. Hsu et al. (2007) reported that prenatal exposure to PCB 132 (1 or 10 mg/kg body weight) resulted in a decrease in cauda epididymal weight, epididymal sperm count, and motile epididymal sperm count in adult offspring. Kuriyama and Chahoud (2004) reported that a single dose of 375 μg 2,3′,4,4′,5-pentachlorobiphenyl (PCB 118)/kg body weight given to pregnant Sprague–Dawley rats on GD 6 exhibited smaller testes, epididymides, and seminal vesicles (absolute and relative weights) when assessed at postnatal day (PND) 170. Decreases in sperm and spermatid numbers and impairment of daily sperm production were also observed, although there was no effect on fertility.

Female Reproductive Effects

TCDD and approximate stereoisomers have been shown to affect female reproductive endpoints in a variety of animal studies. Among the effects reported are a decrease in the number of females mated in rats, mink, and monkeys; fewer completed pregnancies in rats, mink, and monkeys; lower maternal weight gain during pregnancy in rats, rabbits, and monkeys; decreased litter size in rats, rabbits, mink, and swine; effects on female gonads in guinea pigs and mice; and altered estrous and menstrual cycles in mice, rats, and monkeys. Decreased egg production and hatchability occur in a number of avian species. Numerous studies have indicated that TCDD and related chemicals are antiestrogenic presumably because of increased metabolism of estrogen and a decreased number of estrogen receptors (ERs). One manifestation of the antiestrogenic effect is the TCDD-induced decrease in uterine weight in rats. Occasionally, some signs of ovarian dysfunction such as anovulation and suppression of the estrous cycle have been reported (Golub et al., 1991; Peterson et al., 1993; Safe, 1994). Specific topics are addressed below.

Sexual Development

Den Hond et al. (2002) reported that fewer adolescent females living near two waste incinerators in Flanders, Belgium, had achieved the adult stage of breast development compared with youth living in the control area. There was a negative association between serum concentrations of TCDD-like chemicals and breast development. In the second cycle of the Flemish human biomonitoring survey on adolescents (14–15 years old), the level of dioxin-like compounds showed positive (boys) and negative (girls) associations with sexual development markers (genital, breast, pubic hair development, age at menarche, etc.). The study further suggested that even relatively low concentrations (picogram) found in the adolescents can have significant effects on sexual developments (Croes et al., 2014).

Fecundity

Buck et al. (2000) reported that consumption of PCB-contaminated fish from Lake Ontario by women of reproductive age was associated with a reduction in fecundity. Specifically, women who consumed fish for 3–6 years or who reported recent consumption of at least one monthly fish meal were approximately 25% less likely to become pregnant per cycle than women who did not consume fish. Mendola et al. (1997) reported a significant reduction in menstrual cycle length associated with consumption of PCB-contaminated sport fish from Lake Ontario. Abnormal menstrual bleeding was reported to be higher among women exposed to PCBs and PCDFs as a result of consumption of contaminated rice oil in Yucheng, Taiwan, between 1978 and 1979 (Yu et al., 2000). Chao et al. (2007) indicated that placental toxic equivalency (TEQ) PCDD/PCDF concentrations in Taiwanese women were greater with an irregular menstrual cycle. Yang et al. (2008) reported prolonged time to pregnancy and reduced fertility in women exposed in utero to PCBs and PCDFs during the Yucheng incident. Eskenazi et al. (2010) also reported that the serum dioxin levels in women from Seveso, Italy, were associated with a dose-related increase in time to pregnancy and infertility.

Embryotoxicity

Oral administration of Aroclor 1260 to female rabbits (4 mg/kg body weight for 14 weeks) resulted in a significant accumulation of PCBs in 6-day-old blastocysts and increased preimplantation embryo mortality (Seiler et al., 1994). Lindenau and Fischer (1996) reported that 1-day-old cleavage stages and 3-day-old rabbit morulae exposed to 5.0 or 50 μg Aroclor 1260/mL culture medium for 24 h displayed dose-related developmental arrest or degeneration of embryos.

Endometriosis

Endometriosis is a common gynecological disorder affecting at least 5–10% of the reproductive-age women in the United States and is characterized by the presence of endometrial glands and stroma outside the uterus (Bruner-Tran and Osteen, 2010). Rier et al. (1993) were the first to report an association between chronic exposure to TCDD-like chemicals and endometrosis in rhesus monkeys. This finding prompted epidemiological studies that attempted to correlate the body burden of TCDD and PCBs and the incidence of endometriosis in humans. Bruner-Tran and Osteen (2010) stated that these studies do not come to consistent conclusions. An association between endometriosis and TCDD was observed in a case–control study conducted in Israel (Mayani et al., 1997), whereas no significant association was demonstrated in a Belgian case–control study (Pauwels et al., 2001). In a study carried out on a large group of women exposed to TCDD in Seveso, Italy, a two-fold nonsignificant risk for endometriosis was observed among women with TCDD concentrations equal to or greater than 100 ng/L (ppt), but there was no evidence of a dose–response relationship (Eskenazi et al., 2002). De Felip et al. (2004) reported no significant differences in body burdens of TCDD-like chemicals in Belgian and Italian women with and without endometriosis, despite the observation that the incidence and severity of endometriosis and exposure to TCDD-like chemicals in Belgium is greater compared with other industrialized countries. Recently, Martínez-Zamora et al. (2015) reported that patients with deep-infiltrating endometriosis have higher levels of dioxins and PCBs in adipose tissue from the omentum, a layer of peritoneum that surrounds abdominal organs.

Ovary

The ovary is responsible for oocyte and follicle development and synthesis of steroid hormones. Thus, chemicals affecting the ovary may affect fertility, menstrual/estrous cyclicity, timing of puberty, and menopause. Because the ovary is composed of multiple cell types and follicles are in different stages of development, it can be difficult to identify those cell types that may be targeted by a specific toxicant (Miller et al., 2004). Synthesis of the ovarian steroid hormones (estrogen and progesterone) is regulated by follicle-stimulating hormone (FSH) and LH, which in turn are synthesized and released from the anterior pituitary in response to gonadotropin-releasing hormone (GnRH), which is released from the hypothalamus. As a result, hormonal regulation of follicular growth and ovulation is regulated by the neuroendocrine system acting on the ovary. Ovarian toxicity can

thus result from direct action of the chemical on the ovary or indirectly through modulation of the neuroendocrine system.

TCDD-induced ovarian toxicity seems to be strain dependent. Long–Evans rats exposed to 1 μg TCDD/kg body weight on GD 8 had reduced ovarian weight, a decline in fertility, and persistent vaginal estrous, which can lead to infertility. Exposure to 0.8 μg TCDD/kg body weight on GD 15 resulted in fewer functional effects compared with exposure on GD 8 (Gray and Otsby, 1995; Gray et al., 1997). In Sprague–Dawley rats, exposure to TCDD on GD 15 resulted in a decrease in the number of days spent in estrous (1 μg/kg body weight) and a decrease in or elimination of ovulation (2.5 μg/kg body weight) (Salisbury and Marcinkiewicz, 2002). Recently, Baldridge et al. (2015) reported that TCDD at femtomolar level significantly decrease E2 (estradiol-17β) production by human luteinizing granulose cells obtained from women stimulated for in vitro fertilization. The non–ortho PCB congener 3,3′,4,4′-tetrachlorobiphenyl (PCB 77) resulted in a reduction in germ cells and follicles of all stages in the ovaries of C57B1/6 mice on PND 28 when injected on GD 13 (Ronnback, 1991). Salisbury and Marcinkiewicz (2002) reported that a single dose of 1 or 10 μg 2,3,4,7,8-pentachlorodibenzofuran/kg body weight injected on GD 15 caused periods of diestrus and corresponding irregular cyclicity. Additionally, ovulation rates were reduced (1 μg/kg body weight) or ovulation was eliminated entirely (10 μg/kg body weight). Miller et al. (2004), in evaluating the previous studies, summarized a number of possible mechanisms of action for the TCDD-like chemicals that included alteration of steroidogenic and transcriptional pathways because of binding of the ligand to the ovarian AhR, reduction of the responsiveness of the developing follicle to gonadotropins, and blocking of the LH surge needed to induce ovulation.

Placenta

The placenta, a highly vascularized tissue that develops during early gestation, is involved in the circulation of blood, oxygen, glucose, and nutrients between the mother and fetus. The placenta synthesizes estrogen and progesterone and other hormones associated with pregnancy. Exposure to toxicants during pregnancy can compromise placental development and function and alter hormonal signaling that is critical for in utero development of the fetus (Miller et al., 2004). It has been suggested that in utero exposure to TCDD results in placental hypoxia. The hypoxic response to TCDD is thought to be the ultimate cause of death in Holtzman rats exposed to 1.6 μg/kg body weight on GD 15 (Ishimura et al., 2002a). Additionally, placental exposure to TCDD may interfere with glucose kinetics in that tissue. Because glucose transport from the mother to the fetus is facilitated by the placenta and because the placenta itself uses glucose, disruption of glucose kinetics could lead to an increase in fetal deaths in late pregnancy as suggested by Ishimura et al. (2002b) who administered a single 1.6-μg/kg body weight dose to Holtzman rats on GD 15. Placental angiogenesis is dramatically increased during pregnancy in association with the elevated placental blood flows to support the rapidly growing fetus. Li et al. (2015) reported that TCDD suppresses proliferation and migration of human umbilical cord vein and artery endothelial cells and inhibits fetoplacental angiogenesis, leading to negative pregnancy outcomes.

Uterus, Vagina, and Cervix

The ability of a female to maintain pregnancy can be compromised by structural abnormalities in the uterus, vagina, and cervix. Miller et al. (2004) summarized a number of studies that have examined the effects of TCDD and related compounds on external genitalia of rats. Gray and Otsby (1995) reported a decline in fertility in female offspring of Long–Evans rats administered a single dose of 1 μg TCDD/kg body weight on GD 8 because of an increase in endometrial hyperplasia. When an identical dose of TCDD was administered on GD 15, female progeny of Long–Evans and Holtzman rats had a delay in puberty because of phallic clefting and the presence of a vaginal thread. Gray et al. (1997) reported that there was a dose-related increase in the incidence of these effects in female offspring of Long–Evans rats injected with single doses of TCDD ranging from 0.2 to 1.0 μg/kg body weight. Wolf et al. (1999) reported delayed vaginal opening, reduced fertility, and increased incidence of cleft phallus in hamsters, although at a dose that was greater than the effective dose in rats. Vaginal threads were not apparent in hamsters.

Mammary Gland

As a source of nourishment for perinatal mammalian offspring, the mammary gland is an important part of the female reproductive system. Although the majority of development of the gland occurs in adult females during pregnancy, the organ is formed during embryonic development (Miller et al., 2004). Fenton et al. (2002) and Fenton (2006) describe the development of the rodent mammary gland. The mammary epithelial bud is formed by GD 12–14 followed by a short period of inactivity. At GD 16–17, the epithelial bud begins to migrate and fill the stromal portion of the gland. At parturition, the ductal tree is formed, consisting of several ducts and lateral branches from each primary duct. The time period between parturition and puberty is characterized by slow growth of the

gland as the fat pad and epithelium from the nipple extend backward. During puberty, growth of the gland is exponential with rapid development of terminal-end buds at the ends of the lateral ductal branches. This phase is then followed by a period whereby terminal-end buds are replaced by terminal ducts and small lobules. Exposure to toxicants during gestation can directly affect the development of the mammary gland and influence the ability of the female to adequately nurse her young (Miller et al., 2004).

Fenton et al. (2002) reported that in utero and lactational exposure of Long–Evans rats to TCDD (1 μg/kg body weight) on GD 15 delayed maturation of the mammary gland and could increase the incidence of breast cancer because of increased susceptibility of the gland to carcinogens. Exposure to TCDD on GD 20 had no effect on the mammary gland. When TCDD-exposed female rat offspring were bred and subsequently raised their offspring, the mammary glands of the second-generation females were smaller compared with control females. Brown et al. (1998) treated Sprague–Dawley rats prenatally with 1 μg TCDD/kg body weight, which resulted in more terminal-end buds and less lobules II at PND 50 compared with controls. Because terminal-end buds are less mature than lobules, they are more susceptible to chemical carcinogens. Brown et al. (1998) demonstrated that rats exposed to TCDD prenatally had twice as many mammary adenocarcinomas as a result of subsequent exposure to dimethylbenzanthracene compared with rats treated with dimethylbenzanthracene only. Prenatal exposure to non–ortho PCB congeners also results in similar effects. Sprague–Dawley rats exposed prenatally to PCB 126 at a dose of 0.25 μg/kg body weight resulted in an increase in the number of terminal-end buds and a decrease in alveolar buds and lobules, which in turn was associated with an increase in incidence of postnatal dimethylbenzanthracene-induced mammary carcinomas (Muto et al., 2002). Exposure to TCDD-like chemicals during the time of puberty can also influence mammary gland development. Brown and Lamartiniere (1995) reported that when TCDD (2.5 μg/kg body weight) was administered to rats on PND 25, 27, 29, and 31, there was an inhibition of mammary epithelial outgrowth and fewer terminal-end buds on PND 32 compared with controls.

Another critical time of mammary gland development that can be affected by exposure to TCDD-like chemicals is during pregnancy as the gland prepares for lactation (Fenton, 2006). Vorderstrasse et al. (2004) reported that pregnant C57B1/6 mice exposed to 5 μg TCDD/kg body weight on GDs 0, 7, and 14 and assessed for mammary gland effects on GDs 9, 12, and 17 and on the day of parturition had stunted gland growth, decreased branching, and poor formation of lobular alveolar structures. In addition, expression of a specific milk protein in the gland was suppressed, and all pups born to TCDD-treated dams died within 24 h of birth. TCDD has been implicated in impaired mammary differentiation and lactation. Basham et al. (2015) demonstrated that TCDD directly block lactogenesis in isolated mammary epithelial cells of mouse, and the aryl hydrocarbon receptor repressor (AHRR) mediates this response. The study suggested a model in which AHRR induction promotes formation of AHRR/ARNT heterodimers, which transcriptionally inhibits β-casein production.

Developmental and Teratogenic Effects

Exposure to TCDD during pregnancy causes prenatal mortality in the mouse, rat, guinea pig, hamster, rabbit, mink, and monkey. The time period during which exposure of the embryo/fetus to TCDD occurs is just as important as the dose of TCDD administered in terms of prenatal mortality. In most laboratory mammals, gestational exposure to TCDD produces a characteristic pattern of fetotoxic responses that consists of thymic hypoplasia, subcutaneous edema, decreased fetal growth, and prenatal mortality. In addition to these common fetotoxic effects, there are other effects of TCDD that are highly species specific. Such effects include cleft palate formation in the mouse and intestinal hemorrhage in the rat. In the mouse, hydronephrosis is the sensitive sign of prenatal toxicity, followed by cleft palate formation and atrophy of the thymus at higher doses, and by subcutaneous edema and mortality at maternally toxic doses. In the rat, TCDD prenatal toxicity is characterized by intestinal hemorrhage, subcutaneous edema, decreased fetal growth, and mortality. Structural abnormalities occur in the rat only at relatively large doses. In the hamster fetus, hydronephrosis and renal congestion are the most sensitive effects, followed by subcutaneous edema and mortality. In the rabbit, an increased incidence of extra ribs and prenatal mortality is found, whereas in the guinea pig and rhesus monkey, prenatal mortality is seen (Dickson and Buzik, 1993; Peterson et al., 1993).

Avian embryos are more sensitive to TCDD toxicity compared with mammals based on LD_{50} values (a dose that kills 50% of a group of test animals). Among bird species, most of the developmental toxicity research has been done on the chicken, which is considered to be the most sensitive avian species to TCDD-like chemicals. Clinical signs in turkey embryos include microophthalmia, beak deformities, and embryo mortality, but not liver lesions, edema, or thymic hypoplasia, whereas ring-necked pheasant embryos experienced only mortality. Thus, the clinical signs of toxicity of TCDD and its approximate stereoisomers are species dependent with embryo mortality being the only common effect (Peterson et al., 1993).

Cardiovascular Effects

The developing cardiovascular system is a sensitive target of many environmental pollutants, including TCDD and TCDD-like chemicals. Kopf and Walker (2009) reviewed studies of the effects of TCDD and TCDD-like PCBs on the developing heart. These studies have shown that fish, avian, and mammalian embryos exhibit cardiovascular structural changes and functional deficits, although the specific characteristics vary with species. Fish models typically exhibit reduced blood flow, altered heart looping, and reduced heart size and contraction rate. The chick embryo exhibits extensive cardiac dilation, thinner ventricle walls, and reduced responsiveness to chronotropic stimuli, whereas the mouse embryo exhibits reduced heart size. In all the models, the TCDD-induced cardioteratogenicity is associated with increases in cardiovascular apoptosis and decreases in cardiocyte proliferation. Although the cardioteratogenicity in fish and avian species is associated with overt morbidity and mortality, this is not the case for the mouse embryo. However, murine offspring exposed during development to TCDD exhibit cardiac hypertrophy and an increased sensitivity to a second cardiovascular insult in adulthood. Thus, although the mammalian embryo is less sensitive to cardiovascular defects induced by TCDD and TCDD-like compounds, developmental exposure increases the risk of cardiovascular disease later in life. The impact of developmental exposure to TCDD-like chemicals on human cardiovascular disease susceptibility is not known. However, recent animal studies confirmed human epidemiology studies that exposure to TCDD in adulthood is associated with hypertension and cardiovascular disease (Kopf and Walker, 2009).

Studies characterizing the developmental cardiovascular toxicity of TCDD in mammalian species have focused on mice. Because fetal exposure to TCDD in mice does not cause overt toxicity and mortality when exposure occurs after the fusion of the palate, studies assessing the developmental effects of TCDD on the heart have conducted exposures on GD 14.5, a developmental window of cardiomyocyte proliferation (Kopf and Walker, 2009). Fetal heart:body weight ratio is decreased on GD 17.5, with a reduction in myocyte proliferation. This decreased heart:body weight ratio persisted with a trend in PND 7 pups. However, PND 21 pups from TCDD-treated litters exhibited a significant increase in heart:body weight ratios, which was associated with an increase in atrial natriuretic factor (ANF) mRNA expression (Thackaberry et al., 2005). ANF is an indicator of cardiac stress and hypertrophy (Kopf and Walker, 2009). In addition, basal heart rate is decreased in TCDD-exposed mice on PND 21 (Thackaberry et al., 2005). Expression of extracellular matrix remodeling genes and cardiac hypertrophy genes were dysregulated in both fetal (GD 17.5) and adult (3 months) hearts, suggestive of cardiac remodeling that persists into adulthood. Additionally, adult mice exposed to TCDD in utero had increased left ventricle weight, mild hydronephrosis in the kidney, and decreased plasma volume (Aragon et al., 2008).

Carreira et al. (2015) studied the molecular, structural, ultrastructural, functional, and pathological cardiac phenotypes of adult naïve *Ahr* knockout mice ($Ahr^{-/-}$) and wild-type ($Ahr^{+/+}$) adult mice exposed in utero to TCDD or vehicle. The results indicated that abnormalities induced by AhR disruption in utero persist long after removal of the inducing agent and have a significant effect on predisposing the adult to cardiac disease. The study further suggested that the congenital heart defects induced by AhR disruption in the mouse embryo may be a cause of cardiac insufficiency in the adult, in analogy to the human congenital heart disease.

Hydronephrosis and Cleft Palate

The teratogenic responses induced by TCDD-like chemicals are species and strain specific. The induction of terata is one of the most sensitive indicators of TCDD toxicity in mice, as hydronephrosis and cleft palate are induced at doses below those resulting in either maternal or embryo/fetal toxicity (Couture et al., 1990). Indices of maternal and embryo/fetal toxicity classically reported for TCDD-exposed mice include increased maternal mortality, overt clinical signs of maternal toxicity, decreased maternal weight gain, increased maternal liver:body weight ratios, increased fetal mortality, and decreased fetal weight (Neubert and Dillman, 1972; Courtney, 1976). In susceptible strains of mice, such as the C57BL strain, the teratogenic response is tissue specific in that only the kidney, secondary palate, and thymus show alterations. Hydronephrosis is induced in the absence of palatal clefting; thus, the urinary tract is more sensitive to TCDD than is the secondary palate (Birnbaum et al., 1989; Couture et al., 1990). In addition, although palatal sensitivity to TCDD increases with gestational age at days 6–12 in the C57BL/6N mouse, the urinary tract seems to be equally sensitive throughout the major period of organogenesis (Couture et al., 1990).

Cleft palate in mice has been studied extensively (Abbott and Birnbaum, 1991; Abbott et al., 1992; Moriguchi et al., 2003). To form a barrier between the oral and nasal cavities, two opposing palatal shelves normally meet and fuse. The opposing medial edges consist of an outer layer of continuously shed periderm that overlays a layer of basal cells resting on basal lamina. Before fusion, the lamina disappears, and basal cells of the

opposing medial seam lose epithelial characteristics, extend filopodia into the adjacent connective tissue, and gain fibroblast-like features (epithelial to mesenchyme transformation). In this way, a single fused tissue is formed. TCDD-exposed murine palatal shelves grow and make contact, but the subsequent process of epithelial-to-mesenchyme transformation does not occur. Therefore, a cleft is formed as the palatal shelves continue to grow without fusing (Bock and Kohle, 2006). Human embryonic palatal shelves are similarly affected, but at a much higher TCDD concentration (Abbott and Birnbaum, 1991). The observation that humans are less sensitive than mice is supported by studies with AhR-transgenic "humanized" mice (Moriguchi et al., 2003).

Neuroendocrine Effects

Hypothalamo–Pituitary–Gonadal Axis

PCBs are the most studied class of endocrine-disrupting chemicals; yet, the neuroendocrine effects of PCBs have not enjoyed the same attention. As mentioned earlier, the reproductive and thyroid axes are organized with hierarchical control at hypothalamic, pituitary, and gonadal/thyroid levels. Neuroendocrine systems within the hypothalamus depend on a relatively more porous blood–brain barrier at the hypothalamic–pituitary interface and are subject to regulatory feedback from peripheral hormones. Therefore, it is likely that PCBs produce part of their actions via neuroendocrine disruption. PCB effects are often sexually dimorphic, affecting male and female parameters differently and with unequal intensity. Early-life PCB exposure also results in long-term changes in reproductive capacity. There is mounting evidence that PCBs impact reproductive function via neurotoxic/chemical actions on multiple aspects of hypothalamic neuroendocrine circuitry. In combination, the evidence suggests that neurotransmitters, neurotrophic factors, steroid hormone receptors, and metabolic factors involved in the control of GnRH activity are altered by PCB exposure (Bell, 2014).

Reproductive physiology and behavior are regulated by the preoptic–hypothalamic forebrain known as the hypothalamo–pituitary–gonadal (HPG) axis. Sexually dimorphic differentiation of a hypothalamic neuronal network of GnRH cells within the preoptic area is critical for genderization of brain (Gore, 2008). This occurs first during fetal and postnatal development and is followed by further refinement in puberty. In mammals, sexual differentiation of the hypothalamus is due, in large part, to sex differences in testosterone (from fetal testis in males). Aromatization of testosterone to estradiol is also critical for perinatal brain masculinization and depends on the gender-specific expression pattern of aromatase and 5-alpha-reductases during brain development (Colciago et al., 2009). Demasculinization of the brain in females depends on alpha-fetoprotein in addition to estrogens and androgens (Gore, 2008).

After puberty, the neuroendocrine control of female reproduction depends on a reproductive cycle with a preovulatory surge of GnRH in response to positive steroid feedback. GnRH triggers the anterior pituitary to release the gonadotropins (FSH and LH) on proestrus, which stimulate follicular development and regulate the timing of ovulation and estrogen output from the ovary. Peripheral gonadal steroid hormones such as estrogen contribute to imprinting effects on the organization of the sexually differentiated GnRH neuronal network, in part, by interaction with ERs. These early organizational processes are critically important for the attainment and maintenance of adult reproductive functions. Abnormal timing or balance in the exposure to the sex steroid hormones testosterone or estradiol can masculinize the HPG and alter estrous cycling in female and possibly lead to female sexual precocity (Gorski, 1968; Matagne et al., 2004).

Neuroendocrine disruption of HPG components by PCBs has been studied using acutely prepared dispersed anterior pituitary cells and with cultured hypothalamic GT1-7 cells, an in vitro model of GnRH neuroendocrine cells, which synthesize and secrete the key hormone, GnRH. The effects of the PCB mixture Aroclor 1242 (0.1–50 ppm) on the former preparation mimics those of estradiol, i.e., enhanced gonadotropin responses to GnRH as compared with control (Jansen et al., 1993). Similar results have been found when using GT1-7 cells and Aroclor 1221 (A1221), a reconstituted mixture containing the three most prevalent PCBs in humans (PCBs 138, 153, and 180). In this case, stimulatory effects were reported on GnRH mRNA and peptide levels. In contrast, Aroclor 1254 reduces GnRH mRNA levels. The effects of both PCB mixtures are sensitive to co-treatment with an ER antagonist suggesting that PCB effects may be mediated via ERs (Gore et al., 2002).

PCBs can increase the responsiveness of the pituitary gland to GnRH in female sheep. In utero and lactational exposure to PCB 153 in female sheep can significantly enhance LH secretion induced by GnRH in PND 60 offspring (Kraugerud et al., 2012). These effects may result from interactions with developmental HPG processes, with adult HPG processes, or a combination of both. Estrogenic PCBs can also alter LH levels on proestrus, the day of the preovulatory GnRH/gonadotropin surge in female Sprague–Dawley rats. Specifically, prenatal exposure to Aroclor 1221 on gestational days 16 and 18 substantially suppresses the proestrus LH surge in F2 female offspring and commensurately reduces uterine and ovarian weights on estrus. Notably, an opposite and less robust effect on LH levels (apparent increase) was noted in F1 female offspring. In both

generations, litter sex ratio was preferentially biased toward females. These profound transgenerational effects of PCBs on the reproductive axis of female rats may represent epigenetic reprogramming actions of their estrogenic activity (Steinberg et al., 2008).

Further evidence that prenatal exposure to estrogenic PCBs can alter programming of the hypothalamus includes the ability of A1221 exposure during the third trimester of pregnancy to masculinize the female preoptic hypothalamic area (POA) in adulthood. This is manifested as a significant reduction in markers of the GnRH neuronal network, i.e., the number of ERα-positive neurons, kisspeptin fiber density, and GnRH-Fos co-expression in comparison to vehicle-injected rats (Dickerson et al., 2011). Commensurate with this reduced GnRH activity, A1221 has a general developmental apoptotic effect in the female anteroventral periventricular nucleus, a sexually dimorphic preoptic hypothalamic region involved in the regulation of reproductive neuroendocrine function (Dickerson et al., 2011). POA transcript levels of the androgen receptor, hypothalamic growth factors, and synaptic receptors were also significantly downregulated after exposure to A1221.

Neuroendocrine disruption may contribute to disrupted reproductive physiology. For example, gestational exposure to A1221 (GDs 16 and 18) has been associated with delayed timing of puberty and decreased serum LH in male rats at PD15 (Walker et al., 2014). In utero and lactational exposure to a reconstituted mixture (10 mg/kg) of four indicator congeners (PCB 126, 138, 153, and 180) in Sprague–Dawley rats delayed testicular descent in males and advanced the onset of puberty in females (Colciago et al., 2009). Aroclor 1254 (10 mg/kg) when given from GD 10–18 can increase the percentage of irregular estrous cycles in female Long–Evans rats (Faass et al., 2013). In female goat kids, PCB 153 exposure during gestation and lactation lowers prepubertal LH concentration and delays puberty (Lyche et al., 2004). Several studies have also shown masculinization in the anogenital distance (larger) in exposed female offspring at birth. These effects are consistent with abnormal reproductive parameters reported for other EDCs and have important implications for reproductive health and fertility of wildlife and humans exposed to PCBs (Parent et al., 2011).

Disruption of sex-specific organization of neuroendocrine circuits by PCBs may result in improper reproductive behavior in adulthood. Low ecologically relevant doses of A1221 (1 mg/kg) increased the number of female-paced trials required to mate successfully and increased audible vocalizations, a potential index of stress during mating (Steinberg et al., 2007). These results are consistent with decreased fecundity in humans with high body burdens of PCBs. The timing of copulatory behavior is significantly delayed in male offspring exposed to a reconstituted mixture (10 mg/kg) of four indicator congeners (PCB 126, 138, 153, and 180) daily from GD 15 to 19 and then twice a week until weanling (Colciago et al., 2009). No treatment effects were observed for female lordosis behavior (sexual receptivity). In contrast, neonatal exposure to Aroclor 1254 (2.5 mg/kg/day) or PCB 77 (0.25 mg/kg) from GD 7 to 15 decreased lordosis in ovariectomized Long–Evans females (receiving estrogen and progesterone replacement therapy) (Chung et al., 2001; Wang et al., 2002).

PCB interference of developmental sexual differentiation of the brain has also been demonstrated in birds and fish (Ottinger et al., 2009). For example, a reduction of the LH response to synthetic GnRH after a 30-day exposure to the PCB mixture Aroclor 1254 has been reported in the male Atlantic croaker (Khan et al., 2001). Other effects of Aroclor 1254 include reduced GnRH content in the pre-optic anterior hypothalamus (but not pituitary) and number of pituitary GnRH receptors, further suggesting an impairment of normal maturation of the GnRH-LH system in these fish (Khan et al., 2001). PCBs may produce this effect by decreasing protein content of hypothalamic tryptophan hydroxylase, the rate-limiting enzyme in serotonin (5-hydroxytryptamine, 5-HT) synthesis and reducing the availability of neuronal 5-HT, which modulates the gonadotropin release in response to LH-releasing hormone in Atlantic croaker (Khan and Thomas, 1997, 2004).

Hypothalamo–Pituitary–Thyroid Axis

Hypothyroid and hyperthyroid actions of PCBs have been reported, suggesting complex interaction between PCBs and the thyroid axis. For example, low-chlorinated PCB exposure during late gestation and lactation may significantly decrease total T3 levels in 3-week-old children (Darnerud et al., 2010). In contrast, exaggerated circulating T3 has been shown in adolescent children belonging to baby–mother pairs associated with high body burdens of dioxin-like PCBs in the Netherlands from 1987 to 1991 (Leijs et al., 2012). The latter finding suggests that developmental effects of PCBs on thyroid function may continue to impact physiology in adolescence. Studies on experimental animals suggest that inhibitory and stimulatory actions of PCBs arise because of a reduction in circulating thyroid hormone (TH) levels and reducing pituitary sensitivity to thyrotropin-releasing hormone (TRH) and/or thyroid hormone receptor agonism, respectively (Bansal and Zoeller, 2008).

Thyroid function is controlled by the hypothalamo–pituitary–thyroid (HPT) axis. Neuroendocrine cells within the paraventricular nucleus of the hypothalamus secrete TRH from their terminals in the median eminence into the hypophysial portal circulation. TRH then triggers the secretion of thyroid-stimulating

hormone (TSH) from thyrotrophs in the anterior pituitary via the TRH receptor, TRHR1. Circulating TSH, in turn, stimulates the synthesis and secretion of thyroxine (T4) from the thyroid. Conversion of T4 to triiodothyronine (T3), its biologically active form, occurs by the action of deiodinases in peripheral tissues and the hypothalamus. In the hypothalamus, T4 is taken up from the circulation by TH transporters on tanycytes that convert it to T3 by deiodinase D2. T3 is then released in the surrounding neuropil and taken up by neurons (Tu et al., 1997). Thyroid hormones are transported to target tissues in the blood by T4-bound globulin, transthyretin (TTR), or albumin.

Activity of the HPT is regulated by the end products (T3 and T4) at multiple steps of the HPT axis (Joseph-Bravo et al., 2015). Negative feedback of circulating TH on TRH neurons of the PVN and on TSH via actions on TSH-secreting thyrotrophs in the pituitary are both observed. TH inhibits TSH secretion faster than inhibition on TRH or TSH transcription. With regard to feedback inhibition of TRH, this process is aided by T4-induced upregulation of TRH-degrading enzymes in tanycytes of the median eminence.

PCBs share a striking structural similarity with THs making it likely that PCBs interfere with biosynthesis and metabolism, transport of thyroid hormones and/or action at TH receptors. Chlorinated hydrocarbons such as PCBs result in T3 reduction and a compensatory increase in TSH secretion and thyroid hypertrophy and increased incidence of tumors (Capen, 1994). Similarly, acute oral exposure of adult male Sprague–Dawley rats to PCB 126 (75 and 275 μg/kg body weight) increased serum TSH and reduced serum T4 and T3 (Fisher et al., 2006). Administration of 2,3,6-2′,5′-pentachlorinated biphenyl (PCB 95; 32 mg/kg/day, i.p.) to early-weaned male rats on PND 15 and 16 reduced serum concentrations of T4 and T3 and increased the serum concentration of TSH at PND 17 and 18 compared with controls. This hypothyroid state was correlated with higher serum concentrations of leptin and adiponectin, and lower serum concentrations of insulin compared with the control group, pointing to a possible link between PCB-induced hypothyroidism and metabolic disease (Ahmed, 2013).

Fetal and neonatal neurons express TH receptors before the fetal thyroid is functional, suggesting a role for maternal thyroid hormones. An important question is whether developmental exposure to PCBs interferes with TH signaling indirectly by producing maternal hypothyroidism or directly by disrupting the HPT of the offspring. Nevertheless, maternal exposure to PCBs is associated with lower TSH during pregnancy, warning of adverse consequences for maternal health and fetal development (Lv et al., 2015). In the case of hydroxylated PCB isomers, neonatal TSH has been positively associated with maternal exposure during the first trimester of pregnancy (Hisada et al., 2014). Several other studies showed no changes in TSH even though free and total T4 and T3 concentrations were suppressed (Morse et al., 1996a; Khan et al., 2002). The varied outcomes of these studies is probably because of differences in the PCB mixtures and doses used, length and time of exposure, and/or gender differences. It is unclear if altered TSH levels resulting after PCB exposure is, in part, because of a reduction of neuroendocrine stimulation by TRH or from an increase in the peripheral metabolism of thyroid hormones through induction of hepatic microsomal enzymes or by other mechanisms.

There is some evidence that PCBs reduce functionality of HPT via central neuroendocrine actions. For example, acute exposure to ortho-PCB congeners 95 and 101 interferes with the performance of the HPT axis in female weanling rats. Specifically, evoked secretion of TSH and T4 by exogenous TRH is submaximal after treatment with ortho-substituted PCB congeners: PCB 95 (2,3,6-2′,5′) reduces TSH and T4 and PCB 101 (2,4,5-2′,5′) reduces T4 (Khan and Hansen, 2003). These data suggest that PCBs can impact the thyroid axis by reducing the TRH sensitivity of pituitary thyrotrophs.

PCBs seem to have a direct agonist action on TH receptors. Gestational exposure to Aroclor 1254 (1 and 4 mg/kg/day) produced stimulatory actions on thyroid hormone–responsive genes (RC3/neurogranin, Oct-1) in the fetal cortex of GD 16 rat embryos (Gauger et al., 2004). These effects are contradictory to those seen in exposed pregnant dams (reduced T3 and T4), suggesting that PCBs can have direct stimulatory actions on the fetus aside from inhibitory actions produced via maternal hypothyroidism. Further evidence for these dual effects of PCBs is that PCBs do not mimic the effect of low TH concentrations. For example, treatment with Aroclor 1254 counteracts the effects of hypothyroidism, namely, it reverses the reduced Purkinje cell protein-2 expression and thickness of the cerebellar external granule layer. Moreover, both hypothyroidism and PCB treatment reduce serum free and total T4 on PND 15 but only hypothyroidism increased pituitary TSH-beta expression (Bansal and Zoeller, 2008).

Thyroid Hormone Effects

Thyroid hormone homeostasis plays an important role in vertebrate metabolism, growth, and development. Thyroid hormones are also necessary for normal brain development in the human fetus and newborn infant because deficits in pregnant women result in neurological disorders accompanied by severe cognitive and/or mental deficits in their offspring. The thyroid system operates in basically the same way in all vertebrates including humans. The thyroid gland produces predominantly thyroxine (T4), which is

transported to target tissues by the serum transport proteins, TTR, thyroxine-binding globulin, and albumins (Kashiwagi et al., 2009). PCBs are structurally similar to the thyroid hormones and have been documented to disrupt normal thyroid function in laboratory animals (Brouwer et al., 1998). Exposure to PCBs and related compounds causes a reduction in thyroid hormones in developing and adult animals (Kodavanti et al., 1998; Kodavanti and Curras-Collazo, 2010).

The following mechanisms have been summarized (Kashiwagi et al., 2009; Kodavanti and Loganathan, 2014) to explain how PCBs and related chemicals alter thyroid function. Because these chemicals are structurally similar to thyroid hormones, PCBs especially non–dioxin-like ones bind to TTR and displace T4. This free T4 in serum is subjected to hepatic metabolism and elimination. PCBs that are bound to TTR will be transported to the target sites where it can bind to thyroid hormone receptors to elicit a physiological response. Dioxin-like PCBs act through the AhR. These PCBs can bind to the AhR and induce hepatic uridine diphosphate glucuronyl transferases, leading to biliary excretion and elimination of T4. Consistent with these multiple modes of action of PCBs on the thyroid axis and the importance of thyroid status during neural development, functional studies have also demonstrated delayed hippocampal and cerebellar development and altered dendritogenesis after neonatal exposure to Aroclor 1254 (Lein et al., 2007; Royland et al., 2008; Yang et al., 2008). Disruption of dendritic growth by PCBs could explain, in part, the impairment in learning and memory after perinatal exposure (Yang et al., 2008; Parent et al., 2011).

Dentition Effects

Dental deformities and periodontal diseases have been documented in humans exposed to PCBs and related chemicals. A case report of a 12-year-old Japanese girl with Yusho poisoning determined that her periodontal disease and alveolar bone resorption were caused by the consumption of contaminated rice bran oil at 6 years of age (Shimizu et al., 1992). Wang et al. (2003) demonstrated a dose–response relationship between perinatal PCBs and PCDF exposure and dental defects in Yucheng children. The developmental defects were directly impacted by the maternal serum concentrations of contaminants and were apparent when total PCB concentrations were less than 10 μg/L (parts per billion or ppb). In a 14-year follow-up study of Yucheng victims, focusing on people exposed as children rather than in utero or via breast milk, gum pigmentation, gum swelling, and broken teeth were prevalent (Guo et al., 1999). Similarly, a Finnish study of children (Alaluusua et al., 1999) determined that hypomineralized enamel defects of molar teeth could be the best available biomarker for TCDD exposure because the defects were present after low exposure through breast milk. Continued research in Finland supplied further evidence of the relationship between PCDDs and PCDFs and dental defects in children. Women living along the heavily polluted Kymijoki River, which eventually empties into the Gulf of Finland (part of the Baltic Sea), had breast milk international TEQ between 10.9 and 13.4 pg/g fat. The duration of breastfeeding was positively correlated with prevalence of dental defects in children (Holtta et al., 2001). The effects of long-term exposure to PCBs on developmental dental defects were also examined in children from Slovakia demonstrating a dose–response relationship between PCB exposure and developmental enamel defects of permanent teeth in children (Jan et al., 2007).

Neurobehavioral and Neurochemical Effects

In humans and wildlife, laboratory along with epidemiological studies have shown that exposure to TCDD-like compounds can impair cognitive functions, motor development, and gender-related behavior (Schantz et al., 2003). Furthermore, these studies have indicated that the behavioral effects associated with PCB exposure seem to be species independent and that the most susceptible period of exposure is during development and nursing. In children of mothers who consumed contaminated rice oil in Japan (Yusho) and Taiwan (Yucheng), developmental and cognitive dysfunctions were observed that were associated with exposure to complex mixtures of TCDD-like chemicals including PCBs, PCDFs, and polychlorinated quarterphenyls in the rice (Hsu et al., 1985; Chen et al., 1994). In some patients, exposure to these compounds resulted in several peripheral nervous system symptoms that included decreased nerve conduction velocity, numbness and weakness in limbs, and central nervous system signs that included tiredness and respiratory disturbances (Fonnum and Mariussen, 2009). Children exposed prenatally and/or through breastfeeding to PCBs displayed clinical signs including delayed motor development, defects in short-term memory, and lower scores on intelligence quotient tests (Rogan et al., 1988; Tilson et al., 1990). In several studies conducted in Michigan and North Carolina, there were correlations between the PCB exposure levels in mothers who consumed fish contaminated with PCBs and impairment of their children in terms of behavioral test performance and display of fine motor skills (Fein et al., 1984; Jacobson and Jacobson, 1996; Stewart et al., 2000). However, unlike their children, the mothers exhibited no effects of the exposure. Similar signs have been observed in children exposed to PCBs in the Faroe Islands, Germany,

and the Netherlands where in nearly all cases there was a negative correlation between PCB exposure and cognition in children (Schantz et al., 2003). In a study on 9-year-old boys, it was estimated that for each 1 ng PCB/g increase in placental tissue, full-scale IQ dropped by three points and verbal IQ dropped by four points (Stewart et al., 2008; Fonnum and Mariussen, 2009). Moreover, even when the authors controlled for potential confounders such as prenatal exposure to methyl mercury, dichlorodiphenyltrichloroethane, and lead, this association was still statistically significant.

In humans, most studies of PCB exposure on behavior and cognitive function have focused on young children, but the effects can also be observed in adolescents and adults with adverse effects being memory impairment and decreased motor activity (Schantz and Widholm, 2001; Newman et al., 2006). Animal studies have also demonstrated this phenomenon. In female rats, exposure to ortho-substituted PCB congeners (PCBs 28, 118, or 153) during gestation and lactation resulted in spatial learning deficits but not mnemonic deficits in adulthood, indicating that the effects could be delayed or persist from an earlier exposure (Chen and Hsu, 1994; Schantz et al., 1995). In a study relevant to humans, monkeys exposed to a PCB mixture (7.5 μg/kg body weight/day) from birth to 20 weeks of age had deficits on a spatial delay alteration task and displayed perseverative behavior and the inability to inhibit inappropriate responding when tested between 2.5 and 5 years of age (Rice, 1999).

Several epidemiological studies have indicated that exposure to PCBs can contribute to hyperactivity and may contribute to the prevalence of attention deficit hyperactivity disorder (ADHD) in humans (Bowman et al., 1981; Rice, 2000; Hardell et al., 2002). Exposure to PCBs during brain development has been shown to increase activity levels in rats and mice indicating that PCB exposure could potentially lead to ADHD-like symptoms (Tilson and Cabe, 1979; Eriksson et al., 1991; Eriksson and Fredriksson, 1996, 1998; Berger et al., 2001; Branchi et al., 2005).

Laboratory studies with animals and epidemiological studies with humans have provided extensive evidence for an association between exposure to TCDD-like chemicals and adverse effects on behavior and cognition; however, linkages of these changes to alterations in specific nervous tissue are still a significant challenge for toxicologists. In part, this difficulty is because of these compounds acting on a range of neurochemical and neuroendocrine targets that can vary in their significance depending on the exact nature of the chemical, species, gender, and age. Targets that are most likely to be affected by these chemicals are neurotransmitter processes and systems including neurotransmitter transport and receptors, calcium homeostasis, and oxidative stress (Fonnum and Mariussen, 2009). Additional effects further downstream of the initial interaction with neurotransmitter systems include the activation or inhibition of a variety of signal transduction enzymes including nitric oxide synthase (NOS) and protein kinase C (PKC) and alterations in Ca^{2+} homeostasis and synaptic plasticity (Smith et al., 2002; Kodavanti and Loganathan, 2014).

Numerous studies have shown that ortho-substituted PCBs can alter dopamine (DA) concentrations and turnover in the brain (Seegal, 1996; Giesy and Kannan, 1998; Mariussen and Fonnum, 2006). This PCB-related decrease in brain DA concentrations also seems to be dependent on whether an animal is exposed during development or as an adult. In adult pig-tailed macaques (*Macaca nemestrina*) exposed to Aroclor 1016 or Aroclor 1260 at doses of 0.8, 1.6, or 3.2 mg/kg body weight/day for 20 weeks, significant reductions in DA concentrations were observed in certain regions of the brain where DA synthesis occurs (Seegal, 1996; Seegal et al., 2002). In contrast, offspring of rats exposed up to 25 mg/kg body weight/day of Aroclor 1016 exhibited an increase in brain DA concentrations (Seegal, 1994). In rats exposed to non–ortho PCBs at doses ranging from 1 μg/kg body weight/day to 1 mg/kg body weight/day from GD 6 through weaning, DA concentrations were elevated in the prefrontal cortex, whereas rats exposed to 20 mg/kg body weight/day of ortho-substituted PCBs during the same developmental period exhibited a decrease in brain DA concentrations (Seegal et al., 2002; 2005). This finding is not unique to DA in that brain concentrations of other biogenic amines such as norepinephrine and serotonin also exhibit a similar pattern in rats exposed to different PCB congeners (Chishti et al., 1996; Messeri et al., 1997; Lee and Opanashuk, 2004). The congener-specific effect has also been observed in vitro. In pheochromocytoma (PC12) cells, exposure to Aroclor 1254 reduced DA activity (Greene and Rein, 1977; Seegal et al., 1989). In studies designed to characterize the relationship between the structure of individual PCB congeners and their ability to alter PC12 cellular DA content, di-ortho-substituted through tetra-ortho-substituted congeners were the most potent, whereas non–ortho PCB congeners were ineffective (Shain et al., 1991). Moreover, chlorination in a metaposition decreased the potency of ortho-substituted congeners, but metasubstitution had little effect on congeners with both ortho- and para-substitutions. These results support the hypothesis that PCB congeners predicted to have little TCDD-like activity decreased DA concentrations in the nervous system and that neurotoxicity might be caused by a mechanism independent of AhR activation.

Studies also have shown that PCBs can adversely affect neurotransporter systems including the inhibition

of the vesicular monoamine transporter (VMAT) in synaptic vesicles and the plasma membrane DA transporter (DAT) in synaptosomes (Mariussen and Fonnum, 2001a,b; Mariussen et al., 2001). The VMAT is a common transporter for biogenic amines and is an analog of the so-called multidrug transporter that can transport potential cytotoxic components out of cells (Peter et al., 1995; Yelin and Shuldiner, 1995). It is suggested that the neurodegenerative effects of other VMAT inhibitors such as amphetamines are because of the redistribution of DA to the cytoplasm resulting in an outflow of vesicular DA, generation of oxidative stress, and impairment of intraneuronal metabolism followed by a depletion of intracellular DA concentrations (Gainetdinov and Caron, 2003). Ortho-substituted PCBs can also inhibit plasma membrane DA transport, and this reduction in DAT is correlated with reductions in DA concentrations (Gainetdinov and Caron, 2003). Another factor related to the alteration of DA concentrations is the imbalance between VMAT and DAT inhibition. Strong inhibition of brain VMAT compared with DAT can increase the susceptibility to DA-induced neurotoxicity (Miller et al., 1999). Results from PCB structure–activity studies have indicated that inhibition of the plasma membrane uptake of DA is mainly because of lower chlorinated ortho-substituted PCBs, whereas the inhibition of vesicular uptake is theoretically possible by all ortho-substituted PCBs, independent of chlorination (Mariussen and Fonnum, 2006, 2001a,b). This hypothesis is supported by the results of Seegal et al. (2002), where adult male rats were exposed to 25 mg Aroclor 1254/kg body weight/day and brain DA concentrations were evaluated for 8 weeks. Rat brain DA concentrations increased after 3 days of exposure followed by a sustained decrease in extracellular DA that may have been caused by an acute extracellular overflow of DA because of DAT inhibition followed by a reduction in DA because of inhibition of VMAT and reduced DA synthesis. In rat synaptosomes exposed to different PCB congeners and mixtures, reductions in synaptosomal DA concentrations were thought to be because of VMAT inhibition (Bemis and Seegal, 2004). Richardson and Miller (2004) fed rats a single dose of Aroclor 1260 or Aroclor 1016 (500 mg/kg body weight) and reported that the expression of DAT was primarily reduced by Aroclor 1016, whereas VMAT was primarily reduced by the Aroclor 1260 mixture. PCBs and similar compounds can also alter DA concentrations in the brain by inhibiting its synthesis. It has been proposed that PCBs (greater than 50 μM) can inhibit TH, the rate-limiting enzyme involved in the catalysis of the DA precursor, L-3,4-dihydroxyphenylalanine from tyrosine (Seegal et al., 1991; Schwartz, 1991).

Other neurotransmitters that may be involved in PCB-induced alterations in behavior such as changes in cognitive function and hyperactivity include the cholinergic neurotransmitter system (Eriksson and Norberg, 1986; Eriksson, 1997). Findings from these studies indicated that PCB exposure resulted in increased concentrations of brain muscarinic receptors and a reduction in nicotine receptors in the hippocampus but not the cerebral cortex (Eriksson et al., 1991; Eriksson and Fredriksson, 1998). Other neurotransmitter systems altered by PCB exposure include a reduction in N-methyl-D-aspartic acid receptor–binding sites in the visual cortex of developmentally exposed rats (Altmann et al., 2001). Results from other studies support the concept that PCBs can also influence the glutamatergic, GABAergic, and dopaminergic systems (Myhrer, 2003). PCBs have been shown to be weak inhibitors of vesicular glutamate and GABA uptake (Mariussen et al., 1999) where relatively low concentrations (less than 4 μM) of ortho-substituted PCBs (less than five chlorines) inhibited both glutamine and GABA uptake into synaptosomes whereas ortho-substituted PCBs containing more than five chlorines at low concentrations (5 μM) inhibit glutamate and GABA uptake by about 40% (Mariussen and Fonnum, 2001b). Thus, PCB-mediated reduction in the uptake of glutamate and GABA could result in increased extracellular concentrations and lead to excitotoxicity and neurotoxicity.

Investigations of the effects of various PCB congeners on Ca^{2+}-homeostasis and PKC translocation in cerebellar granule cells indicate a similar structure–activity relationship in that the ortho-substituted PCBs have potential to alter Ca^{2+}-homeostasis in the brain, whereas the AhR-active congeners were reported to be inactive (Kodavanti et al., 1998, Fig. 39.3). Disruption of Ca^{2+} homeostatic processes by PCBs can result in various adverse effects including the production of reactive oxygen species; altered neurotransmitter release; activated phosphokinase, phosphatase, phospholipase, and protease activity; enhanced apoptotic processes; alteration of other Ca^{2+}-dependent enzyme activities including NOS; and long-term potentiation (LTP) and synaptic plasticity (Mariussen and Fonnum, 2006). In general, exposure to PCBs results in an influx of extracellular Ca^{2+} from a variety of sources and routes. Routes of extracellular Ca^{2+} into cells include entry via L-type voltage-sensitive Ca^{2+} channels, release of Ca^{2+} from inositol triphosphate (IP3)-sensitive Ca^{2+} stores in the endoplasmic reticulum, influx of Ca^{2+} from store-operated Ca channels and glutamate receptors channels (Inglefield et al., 2001). Ryanodine receptors, which regulate Ca^{2+} release from the ER, may also be involved in PCB-altered Ca^{2+}-homeostasis. Wong et al. (1997) reported that exposure to ortho-substituted PCBs enhanced ryanodine binding in membrane preparations from rat brain hippocampus, cerebellum, and cortex and induced a ryanodine-sensitive Ca^{2+} mobilization in cortex preparations. Schantz et al. (1997) reported decreased ryanodine-specific binding in the hippocampus and an

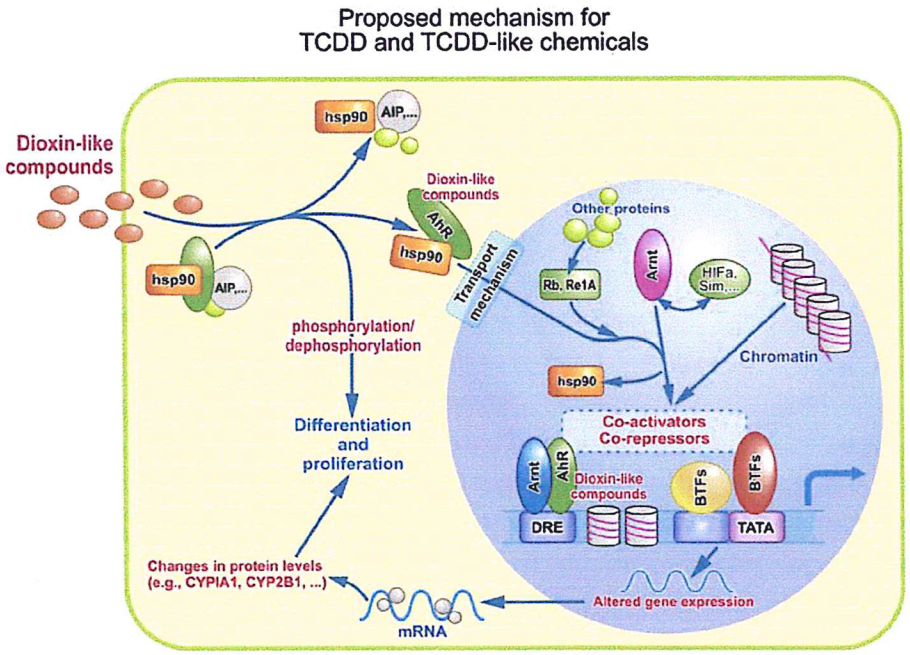

FIGURE 39.2 The proposed molecular mechanism of action of 2,3,7,8-tetrachorinated dibenzo-*p*-dioxin (TCDD) and TCDD-like chemicals. Schematic representation of functioning of the AhR (aryl hydrocarbon receptor) pathway. After entering the cell, the TCDD-like compounds bind to a protein complex in the cytoplasm consisting of AhR, Hsp90, AIP. Upon ligand binding, AIP is released, exposing nuclear localization signal on AhR and leading to translocation of AhR from the cytoplasm to the nucleus. Within in the nucleus, Hsp90 are released and AhR heterodimerizes with the Aryl Receptor Nuclear Translocator (ARNT). The AhR–ARNT complex then binds to multiple enhancer elements in the promotor region of the responsive genes in the AhR battery such as CYP1A. *Adapted from Denison, M.S., Nagy, S.R., 2003. Activation of the aryl hydrocarbon receptor by structurally diverse exogenous and endogenous chemicals. Annu. Rev. Pharmacol. Toxicol. 43, 309–334.*

increase in binding in the cerebral cortex in offspring of rats exposed to PCB 95. Howard et al. (2003) indicated that an ortho-substituted PCB-mediated effect on ryanodine receptors was associated with an increase in apoptosis in rat hippocampal neurons.

PKC has been shown to have an important role in PCB-induced toxicity (Kodavanti et al., 1998, Fig. 39.3). In mammals, there are at least 12 different isoforms of PKC and they influence a range of activities including modulation of neurotransmitter release, synaptic plasticity and LTP, apoptotic processes, and neurological diseases (Sweatt, 1999; Way et al., 2000; Battaini, 2001). Studies have shown that PCBs increase PKC translocation and affect the inositol phosphate accumulation in cerebellar granule cells in vitro (Kodavanti et al., 1994; Shafer et al., 1996) and that the phenomenon is enantiomer selective (Lehmler et al., 2005). Although the exact mechanism for these effects is not yet known, extracellular calcium seems to be necessary. PCBs have been suggested to inhibit brain NOS activity (Kang et al., 2002; Yun et al., 2005). Glutamate receptors and calcium/calmodulin have been shown to regulate NOS activity (Moncada et al., 1991). NOS is involved in LTP and is implicated in oxidative injury (Sweatt, 1999). Thus, inhibition of NOS could influence the generation of LTP and therefore learning and memory deficits. In rats exposed to Aroclor 1254, extracellular DA concentrations in the striatum were decreased, whereas there was an increase in the expression of phosphorylated NOS (Yun et al., 2005).

MECHANISM OF ACTION

Dioxin-Like Chemicals

The AhR is a ligand-activated transcription factor that is involved in the regulation of a number of genes, including those for enzymes that play a role in the metabolism of xenobiotics and genes involved in cell growth regulation and differentiation (Safe, 1994; Hahn, 1998, 2002; Denison et al., 2002; Denison and Nagy, 2003; Mandal, 2005; Kodavanti and Loganathan, 2014). The AhR plays an important role in the altered gene expression and species- and tissue-specific toxicity resulting from exposure to specific PCB congeners and PCDD and PCDF isomers. The toxicity of individual isomers and congeners is closely related to the affinity with which these compounds bind to the AhR with the most toxic compounds being those that bind with the greatest affinity (Okey et al., 1994). There are large species and strain differences in sensitivity to TCDD and related chemicals. Mouse and rat strain differences in sensitivity to TCDD can be partially explained by differences in the ligand-binding affinity of their polymorphic AhR variants.

The AhR is a basic helix-loop-helix (bHLH) and Per-Arnt-Sim (PAS)—containing transcription factor (Denison and Nagy, 2003). In the absence of a ligand, AhR occurs as a soluble multiprotein complex in the cytosol of the cell. The chaperone proteins are two molecules of hsp90 (a heat shock protein of 90 kDa), the X-associated protein 2 (XAP2) and p23 (a co-chaperone protein of 23 kDa). When TCDD or another ligand diffuses across the plasma membrane and binds to the AhR, the ligand AhR complex undergoes a conformational change that exposes a nuclear localization sequence (Fig. 39.2). The complex translocates into the nucleus of the cell and the chaperone proteins dissociate from the complex. The AhR—ligand then binds to the bHLH-PAS nuclear protein, AhR nuclear translocator or Arnt. The formation of this heterodimer initiates conversion of the complex into a form that binds to DNA with high affinity on a specific recognition site called the dioxin-responsive element (DRE). Binding of the ligand—AhR—Arnt complex to the DRE stimulates transcription of genes encoding cytochrome P450 enzymes in the CYP1A1 subfamily and other AhR-responsive genes that are located upstream of the DRE (Denison and Nagy, 2003). Continuous and inappropriate modulation of gene expression is thought to be responsible for a series of biochemical and cellular changes that result in toxicity characteristic of TCDD and related chemicals (Mandal, 2005).

Non—Dioxin-Like Chemicals

The disruption of Ca^{2+}-homeostasis may have a significant effect on other signal transduction pathways [e.g., inositol phosphate (IP) and arachidonic acid (AA) second messengers] regulated or modulated by Ca^{2+}. The noncoplanar PCB, but not coplanar PCB affected basal and carbachol (CB)-stimulated IP accumulation in cerebellar granule cells (Kodavanti et al., 1994). AA is released intracellularly after activation of membrane phospholipases, and AA is an important second messenger in releasing Ca^{2+} from endoplasmic reticulum (Striggow and Ehrlich, 1997). Aroclor 1254 and noncoplanar PCB congener increased [^{3}H]-AA release in cerebellar granule cells, whereas coplanar PCB did not (Kodavanti and Derr-Yellin, 1999); this is in agreement with previous structure—activity relationship studies on Ca^{2+} buffering and PKC translocation (Kodavanti and Tilson, 1997). A similar increase in [^{3}H]-AA was observed with structurally similar chemicals such as polybrominated diphenyl ether mixtures (Kodavanti and Derr-Yellin, 2002).

One of the downstream effects of perturbed Ca^{2+}-homeostasis is translocation of PKC from the cytosol to the membrane where it is activated (Trilivas and Brown, 1989). [^{3}H]-Phorbol ester ([^{3}H]-PDBu) binding has been used as an indicator of PKC translocation. The noncoplanar PCB congener increased [^{3}H]-PDBu binding in a concentration-dependent manner in cerebellar granule cells, whereas coplanar PCB had no effect even at 100 μM (Kodavanti and Tilson, 2000). Experiments with several pharmacological agents revealed that the effects of PCBs are additive with glutamate, and none of the channel (glutamate, calcium, and sodium) antagonists blocked the response of 2,2′-DCB (Kodavanti et al., 1994). Immunoblots of PKC-alpha and epsilon indicated that noncoplanar ortho-PCB decreased the cytosolic form and increased the membrane form significantly at 25 μM (Yang and Kodavanti, 2001). Subsequent structure—activity relationship studies indicated that congeners that are noncoplanar increased PKC translocation, whereas coplanar congeners did not (for review, see Kodavanti and Tilson, 1997, 2000). This was further strengthened by observations with structurally similar chemicals such as polychlorinated diphenyl ethers (Kodavanti et al., 1996). Nitric oxide (NO), which is produced by NOS, is a gaseous neurotransmitter. NO has an important role as a retrograde messenger in LTP, learning and memory processes, and endocrine function (Schuman and Madison, 1994). The congener 2,2′-DCB, but not 4,4′-DCB, inhibited both cytosolic (neuronal nitric oxide synthase; nNOS) and membrane (endothelial nitric oxide synthase; eNOS) forms of NOS (Sharma and Kodavanti, 2002).

These in vitro studies clearly demonstrated that second messenger systems, involved in the development of the nervous system, LTP, and learning and memory, are sensitive targets for the ortho-substituted PCBs and related chemicals. Fig. 39.3 illustrates the intracellular signaling events affected by these chemicals (ortho-PCBs and commercial PCB mixtures) at low micromolar concentrations and shorter exposure periods, where cytotoxicity is not evident. These signaling pathways include calcium homeostasis and PKC translocation. The increase of intracellular free Ca^{2+} is slow, but steady after exposure. This increase in free Ca^{2+} could be because of increased calcium influx, inhibited Ca^{2+}-buffering mechanisms, and/or calcium release from intracellular stores by the products of membrane phospholipases. This increase in free Ca^{2+} could cause translocation of PKC. The coplanar non—ortho PCBs have marginal effects on calcium homeostasis and no effects on PKC translocation. Literature reports indicate that at slightly higher concentrations, commercial PCB mixtures (Aroclors 1221 and 1254) alter neurite outgrowth in PC12 cells (Angus and Contreras, 1995) and in hypothalamic cells (Gore et al., 2002). The possible mode of action for this structural change could be because of changes in intracellular signaling by these chemicals.

In vivo effects of PCBs have been studied with a commercial PCB mixture, Aroclor 1254, given orally from GD

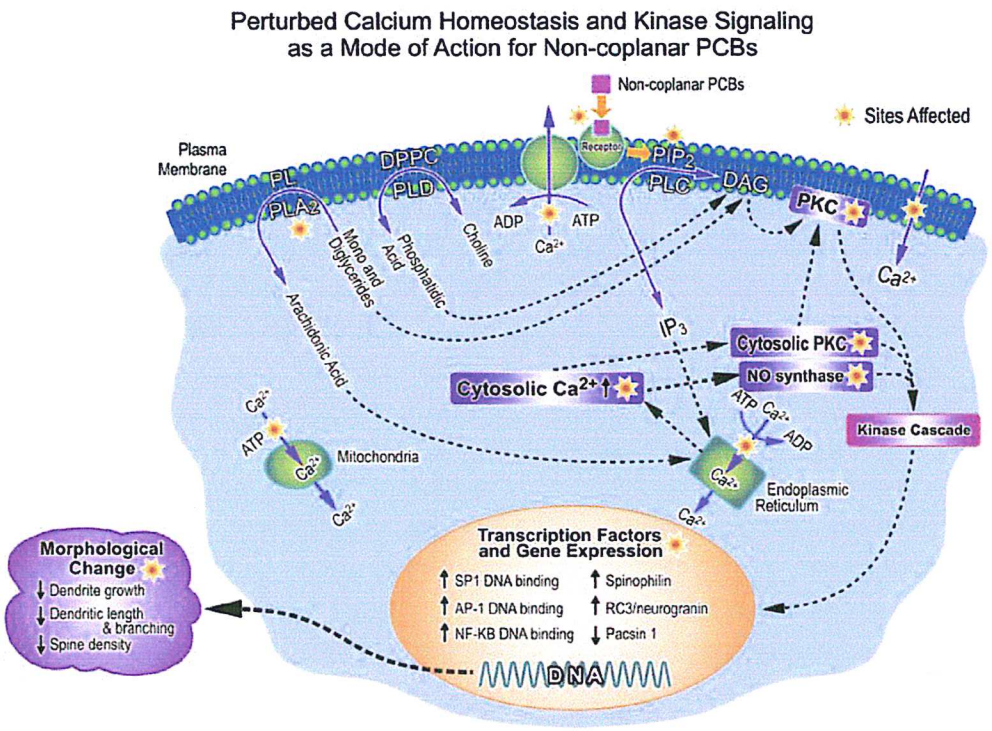

FIGURE 39.3 Schematic showing calcium and kinase signaling as a mode of action for noncoplanar PCBs. The processes by which these compounds disrupt calcium homeostasis and kinase signaling are as follows. First, chemicals bind to the cell surface receptors and activate membrane phospholipases such as phospholipase C (PLC), phospholipase A2 (PLA2), and phospholipase D (PLD). This will result in several second messengers such as arachidonic acid and inositol trisphosphate (IP3), which will release calcium from intracellular stores such as endoplasmic reticulum. After blockage of calcium sequestration mechanisms in mitochondria and endoplasmic reticulum, cytosolic free calcium levels increase. Increased cytosolic calcium levels translocate protein kinases from cytosol to the membrane, where they are activated. This will result in the activation of kinase cascade triggering transcription of genes, which will result in a morphological change. *Adapted from Kodavanti, P.R.S., 2004. Intracellular signaling and developmental neurotoxicity. In: Zawia, N.H. (Ed.), Molecular Neurotoxicology: Environmental Agents and Transcription-Transduction Coupling. CRC Press, Boca Raton, FL, pp. 151–182.*

6 through PND 21. Both calcium homeostasis and PKC activities were significantly affected after developmental exposure to Aroclor 1254 (Kodavanti et al., 2000). Developmental exposure to PCBs also caused significant hypothyroxinemia and age-dependent alterations in the translocation of PKC isozymes; the effects were significant at PND 14 (Yang et al., 2003). The changes in PKC and other second messengers were associated with changes in transcription factors such as specificity protein 1 and nuclear factor kappa-B indicating changes in gene expression after developmental exposure to PCBs (Riyaz Basha et al., 2006). Considering the significant role of PKC signaling in motor behavior, learning, and memory, altered subcellular distribution of PKC isoforms at critical periods of brain development may be associated with activation of transcription factors and subsequent gene expression and may be a possible mechanism of PCB-induced neurotoxic effects. Proteomic studies indicated that chemicals such as Aroclor 1254 may alter protein networks related to energy metabolism and intracellular signaling (Kodavanti et al., 2011).

Further studies focused on the structural outcome for changes in the intracellular signaling pathway after developmental PCB exposure. Detailed brain morphometric evaluation was performed by measuring neuronal branching and spine density. Developmental exposure to PCBs affected normal dendritic development of Purkinje cells and CA1 pyramids (Lein et al., 2007). The branching area was significantly smaller in the PCB-exposed rats. When the rats became adults, there is continued neurostructural disruption of the CA1 dendritic arbor after PCB exposure; however, the branching area of the Purkinje cells returned to normal level. Developmental exposure to PCBs also resulted in a significantly smaller spine density in hippocampus, but not in cerebellum. This dysmorphic cytoarchitecture could be the structural basis for long-lasting neurocognitive deficits in PCB-exposed rats (Lein et al., 2007; Yang et al., 2009). Previously, Pruitt et al. (1999) reported a reduced growth of intra- and infrapyramidal mossy fibers after developmental exposure to PCBs. These studies indicate that developmental exposure to a PCB

TABLE 39.1 Summary of World Health Organization (WHO) 2005 Toxic Equivalency Factor (TEF) Values for Mammals

Compound	WHO 2005 TEF
CHLORINATED DIBENZO-P-DIOXINS	
2,3,7,8-TCDD	1
1,2,3,7,8-PeCDD	1
1,2,3,4,7,8-HxCDD	0.1
1,2,3,6,7,8-HxCDD	0.1
1,2,3,7,8,9-HxCDD	0.1
1,2,3,4,6,7,8-HpCDD	0.01
OCDD	0.0003
CHLORINATED DIBENZOFURANS	
2,3,7,8-TCDF	0.1
1,2,3,7,8-PeCDF	0.03
2,3,4,7,8-PeCDF	0.3
1,2,3,4,7,8-HxCDF	0.1
1,2,3,6,7,8-HxCDF	0.1
1,2,3,7,8,9-HxCDF	0.1
2,3,4,6,7,8-HxCDF	0.1
1,2,3,4,6,7,8-HpCDF	0.01
1,2,3,6,7,8,9-HpCDF	0.01
OCDF	0.0003
NON–ORTHO-SUBSTITUTED PCBs	
3,3′,4,4′-tetra-CB (PCB 77)	0.0001
3,4,4′,5-tetra-CB (PCB 81)	0.0003
3,3′,4,4′,5-penta-CB (PCB 126)	0.1
3,3′,4,4′,5,5′-hexa-CB (PCB 169)	0.03
MONO-ORTHO-SUBSTITUTED PCBs	
2,3,3′,4,4′-penta-CB (PCB 105)	0.00003
2,3,4,4′,5-penta-CB (PCB 114)	0.00003
2,3′,4,4′,5-penta-CB (PCB 118)	0.00003
2′,3,4,4′,5-penta-CB (PCB 123)	0.00003
2,3,3′,4,4′,5-hexa-CB (PCB 156)	0.00003
2,3,3′,4,4′,5′-hexa-CB (PCB 157)	0.00003
2,3′,4,4′,5,5′-hexa-CB (PCB 167)	0.00003
2,3,3′,4,4′,5,5′-hepta-CB (PCB 189)	0.00003

TCDF, 2,3,7,8-tetrachlorodibenzofuran.
Adapted from Van den Berg, M., Birnbaum, L.S., Denison, M., et al., 2006. The 2005 World Health Organization re-evaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicol. Sci. 93, 223–241.

mixture resulted in altered cellular distribution of PKC isoforms, which can subsequently disrupt the normal maintenance of signal transduction in developing neurons. The perturbations in intracellular signaling events could lead to structural changes in the brain. These findings suggest that altered subcellular distribution of PKC isoforms may be a possible mode of action for PCB-induced neurotoxicity. Fig. 39.3 illustrates the intracellular signaling events, transcription factors, and brain morphometry affected by these chemicals.

Differential Toxicity of PCBs, PBBs, PCDDs, and PCDFs

Comparison of the relative toxicity of PCDDs, PCDFs, and PCBs as three separate classes suggests that the dioxins are more toxic than the furans, which in turn are more toxic than the PCBs. It was concluded that PBBs are slightly more toxic than their chlorinated counterparts (McConnell, 1985). Studies also suggest that the toxicity of commercial PCB mixtures increases with increasing chlorine content (Aroclor 1221 < 1232 < 1242 < 1248 < 1254), but highly chlorinated Aroclors 1260, 1262, and 1268 are less toxic than Aroclor 1254 (Tanabe, 1988).

TCDD binds with the greatest affinity to the AhR and is the most potent isomer in terms of toxicity. PCDDs and PCDFs substituted with chlorines in at least three of the four lateral positions (2, 3, 7, and 8; Fig. 39.1) bind most strongly to the AhR. If chlorines are removed from these lateral positions or if chlorines are added to the nonlateral positions (1,4,6,9; Fig. 39.1), binding affinities decrease markedly, as do toxicities. There are seven 2,3,7,8-substituted PCDDs (TCDD, 1,2,3,7,8-penta-CDD, 1,2,3,4,7,8-hexa-CDD, 1,2,3,6,7,8-hexa-CDD, 1,2,3,7,8,9-hexa-CDD, 1,2,3,4,6,7,8-hepta-CDD, and octa-CDD) and ten 2,3,7,8-substituted PCDFs (2,3,7,8-TCDF, 1,2,3,7,8-penta-CDF, 2,3,4,7,8-penta-CDF, 1,2,3,4,7,8-hexa-CDF, 1,2,3,6,7,8-hexa-CDF, 1,2,3,7,8,9-hexa-CDF, 2,3,4,6,7,8-hexa-CDF, 1,2,3,4,6,7,8-hepta-CDF, 1,2,3,4,6,7,8-hepta-CDF, and octa-CDF) that induce TCDD-like toxicity. There are 209 theoretically possible PCB congeners having different toxic and biologic responses. The most toxic PCB congeners have four or more chlorine atoms at both the para (4,4′) and meta positions (3,3′,5,5′; Fig. 39.1) in the biphenyl rings, but no chlorine atoms in the ortho positions (2,2′,6,6′; Fig. 39.1). Of the 209 PCB congeners, four PCB congeners [3,3′,4,4′-tetra-CB (PCB 77), 3,4,4′,5-tetra-CB (PCB 81), 3,3′,4,4′,5-penta-CB (PCB 126)and 3,3′,4,4′,5,5′-hexa-CB (PCB 169)] are approximate stereoisomers of the highly toxic TCDD and thus bind to the AhR and elicit toxic and biologic responses typical of TCDD, although this required higher doses. These four congeners

TABLE 39.2 Concentrations of Polychlorinated Dibenzo-*p*-dioxin (PCDD), Polychlorinated Dibenzofuran (PCDF), and Polychlorinated Biphenyl (PCB) Congeners in Terms of Toxicity Equivalence Factors (TEF) Values and Toxic Equivalents (TEQs) in Fish Collected Downstream From a Contaminated Industrial Site

Compound	TEF	Congener and (TEQ) Concentration (pg/g)
PCDDs		
TCDD	1.00000	0.5 (0.5)
1,2,3,7,8-PeCDD	1.00000	0.3 (0.3)
TEQ from PCDDs		0.8
% total TEQ		1.8
PCDFs		
2,3,7,8-TCDF	0.10000	3.0 (0.3)
2,3,4,7,8-PeCDF	0.30000	4.6 (1.4)
TEQ from PCDFs		1.7
% total TEQ		3.8
NON–ORTHO PCBs		
PCB 126	0.10000	410 (41.0)
TEQ from non–ortho PCBs		41.0
% total TEQ		90.5
MONO-ORTHO PCBs		
PCB 123	0.00003	38,000 (1.1)
PCB 156	0.00003	23,000 (0.7)
TEQ from mono-ortho PCBs		1.8
% total TEQ		4.0
Grand total TEQ (pg/g wet weight)		45.3

TCDF, 2,3,7,8-tetrachlorodibenzofuran.

are considered to be coplanar because both rings of the biphenyl molecule lie in the same plane, which enables binding to the AhR (Tanabe, 1988). There are eight PCB congeners with chlorine substitution in one of the ortho positions (2,2′,6,6′). These congeners may have partial coplanarity and thus exhibit lower competitive binding affinities for the AhR and lower toxic potency. The mono-ortho PCB congeners are 2,3,3′,4,4′-penta-CB (PCB 105), 2,3,4,4′,5-penta-CB (PCB 114), 2,3′,4,4′,5-penta-CB (PCB 118), 2′,3,4,4′,5-penta-CB (PCB 123), 2,3,3′,4,4′,5-hexa-CB (PCB 156), 2,3,3′,4,4′,5-hexa-CB (PCB 157), 2,3′,4,4′,5,5′-hexa-CB (PCB 167), and 2,3,3′,4,4′,5,5′-hepta-CB (PCB 189) (Tanabe, 1988; Safe, 1990, 1998; Giesy and Kannan, 1998; Huwe, 2002; Whyte et al., 2004).

Toxic Equivalency Factors

The relationship between the structure of individual PCDD, PCDF, and PCB congeners and their toxicity is the basis of toxicity equivalency factors (TEFs) and the TEQ approach. The TEQ approach is used to determine the toxic potency of complex mixtures of PCDDs, PCDFs, and PCBs found in the environment. Assuming a similar mechanism of action (binding to the AhR), the potency of each chemical in a mixture to cause a particular toxic or biological effect can be expressed as a fraction of the potency of TCDD to cause the same effect. Thus, the TEF is a ratio of half maximal effective concentration (EC_{50}) (TCDD-like chemical)/EC_{50} (TCDD). TCDD has been assigned a TEF value of 1.0. Based on a variety of biological endpoints, relative potency factors (RPFs) are assigned to the different TCDD-like congeners. All RPFs for an individual congener are evaluated to derive a consensus value (the TEF) that describes an order-of-magnitude potency for that congener. The toxic potency of a mixture of PCDDs, PCDFs, and/or PCBs is estimated by multiplying the concentrations of individual congeners by their respective TEFs and summing the products to yield a total TEQ. The total TEQ expresses the toxicity as if the mixture were pure TCDD (Dickson and Buzik, 1993; Safe, 1998; Fries, 1995; Van den Berg et al., 2006; Whyte et al., 2004; Schecter et al., 2006). Several assumptions are made when using the TEF approach: (1) the effects of individual PCDDs, PCDFs, and/or PCBs in a mixture are additive; (2) only tissue and environmentally persistent organochlorine compounds have been assigned TEFs; (3) all these compounds bind to the Ah receptor and elicit receptor-mediated biochemical and toxic responses (Safe, 1998). The TEQ approach and current values (Table 39.1) have been adopted internationally as the most appropriate way to estimate the potential health risk of mixtures of TCDD-like chemicals (Schecter et al., 2006).

Table 39.2 presents the concentrations of various PCDD, PCDF, and PCB congeners found in fish collected downstream from the abandoned site of a manufacturing facility that used PCBs. By multiplying the congener concentration detected in the fish by the appropriate TEF value, the quantity of the TCDD-like toxicity contributed by each congener can be determined. In the example given, the PCDDs contribute 0.8 pg TEQ/g, the PCDFs contribute 3.8 pg TEQs/g, and the non–ortho PCBs contribute 41 pg TEQs/g or over 90% of the TCDD-like activity in the fish sample. Although the relative toxicities of the mono-ortho PCB congeners are relatively low, as indicated by their TEF values, their high concentrations in the fish allow them to contribute a measurable quantity of TCDD-like activity.

CONCLUDING REMARKS AND FUTURE DIRECTIONS

PCBs, PBBs, PCDDs, and PCDFs belong to a group of compounds that are structurally related and are environmentally and biologically persistent. These chemicals have a tendency to bioaccumulate and biomagnify in the food chain. Residues of these chemicals have been detected even in remote areas of the world and in a variety of animal species, including humans. Exposure to these chemicals has been linked to a broad spectrum of effects. Fetal and early developmental exposures are particularly sensitive and can have different outcomes when compared to exposure in adults. Latent effects of early exposures include, but are not limited to, depressed circulating thyroid hormone levels and abnormal thyroid cytology; developmental effects of the heart, palate, and kidney; delayed cognitive development; altered sensory and motor abilities; and reproductive impairment and compromised neural function. Although AhR activation has been attributed to several dioxin-like coplanar compounds, some PCBs that are noncoplanar in nature seem to exert their toxic effects through different mechanisms including calcium signaling, oxidative stress, thyroid hormone perturbations, and neurotransmitter imbalance. Although certain congeners and isomers can pose a very serious threat to the health of animals and humans, environmental exposure situations are generally such that risks of health effects are generally low. The most significant problem by these compounds involved accidental poisoning via food supply or consumption of contaminated food from contaminated areas. Additionally, there are areas of the environment that are heavily contaminated by these chemicals because of past industrial activities. Animals and humans residing in or near contaminated locations certainly are at risk of serious health effects. Efforts must continue to reduce exposure to protect wildlife and humans. The best way to accomplish is to modernize technological processes to prevent the release of these chemicals in to the environment.

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