Polychlorinated Biphenyls: Sources, Fate, Effects on Birds and Mammals, and Mechanisms of Action

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Chemistry and History of PCBs

PCBs are a family of chlorinated hydrocarbons that were commercially manufactured in the United States starting in the 1920s for use in hydraulic fluids, die-casting equipment, the manufacture of transformers, and as insulating fluid for the assembly of capacitors (Frame et al., 1996a, 1996b; Safe, 1990; reviewed in Jensen, 1972; Rice et al., 2003). The general molecular structure of these compounds consists of two joined phenyl rings with 1–10 chlorine substitutions around both rings. There are 209 possible congeners of PCBs, which are determined by the number and placement of chlorine substitution. Steric hindrance is caused by close placement of chlorine atoms to the connecting carbons, in the ortho-positions on the ring. As a result, the phenyl rings of these ortho-substituted congeners do not lie in the same plane with each other and are therefore referred to as being nonplanar. Conversely, low steric hindrance, as caused by chlorine substitution in the meta- and para-positions, allows the rings to lie in the same plane (Brunström and Halldin, 1998; Rice et al., 2003; Van den Berg et al., 1994). This conformation makes these congeners structurally similar to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, i.e., "dioxin"). Accordingly, they behave similarly to TCDD in biological matrices by activating the aryl hydrocarbon receptor (AhR) and are subsequently referred to as being dioxin-like.

PCBs rarely, if ever, exist as single congeners in the fluids and matrices that contain them (Niimi, 1996; Rice et al., 2003). Most are mixtures of several congeners, and many such mixtures were manufactured and commercially available. They have been marketed under several different commercial names including Aroclor (US), Pheneclor (France), Kaneclor (Japan), and Sovol (Russia; Frame et al., 1996a; Rice et al., 2003). Estimated total worldwide production of PCBs through 1976 was 6.1×10^{11} kg, primarily produced by Monsanto (Durfee et al., 1976); roughly 6.36364×10^{8} kg of these halogenated biphenyls manufactured worldwide have been reported (Safe, 1984). The U.S. Toxic Substances Control Act (TSCA) was enacted in 1976 and authorized the U.S. Environmental Protection Agency (USEPA) to regulate chemicals that were determined to cause unreasonable risk to public health or the environment. In July 1979, TSCA banned the manufacture, processing, and distribution of PCBs in the United States and regulated the phase-out of their existing uses (Bremer, 1983; USEPA, 2010). In March 1985, the Helsinki Convention adopted the directive to stop production and marketing of products and equipment containing PCBs effective from 1987 (for review, see Full and the HELCOM Project Team, 2001).

Mechanisms of Action of PCBs

In general, many dioxin-like compounds bind the aryl hydrocarbon receptor (AhR), including 12 out of 209 PCB congeners that are known to activate the AhR. The ligand-bound AhR causes transcriptional upregulation of any number of cytochrome P450 (CYP450) enzymes, but specifically the CYP450 1A enzymes that have detoxification activity through several biochemical reactions, including deethylation and hydroxylation, among others (Hutzinger et al., 1972; Safe, 1984). In mammals, the *CYP1A1* and *CYP1A2* genes are involved with these detoxification reactions, while the *CYP1A4* and *CYP1A5* genes are their orthologs in birds (Gilday et al., 1996; Kubota et al., 2006; Mahajan and Rifkind, 1999; reviewed in Head and Kennedy, 2007).

Ethoxyresorufin-*O*-deethylase (EROD), a hepatic Phase I biotransformation enzyme involved in detoxification (Melancon, 2003; Melancon et al., 2006; Wright and Welbourn, 2002), is one of several enzymes that is upregulated in response to exposure to dioxin-like compounds (Sanderson et al., 1998) and its induction is correlated with exposure to coplanar PCBs (Rattner et al., 1994, 1996). EROD activity is catalyzed by the enzyme encoded by the *CYP1A4* gene, which is upregulated via the AhR (Head and Kennedy, 2007). Therefore, it is has been considered a reliable and sensitive measure of exposure to dioxin-like compounds (Head and Kennedy, 2007; Rattner et al., 1989); however, more recent advances in transcriptomics and other molecular tools of gene expression have allowed for direct measurement of RNA levels to assess AhR-mediated induction of detoxifying enzymes by PCBs and other exogenous AhR-binding chemicals (Hervé et al., 2010; Bohannon et al., unpublished data).

The CYP450 superfamily of enzymes encompass many physiological roles throughout the body beyond Phase I detoxification, including steroidogenesis. For example, the *CYP19* gene codes for aromatase, the enzyme involved in converting testosterone to estradiol (Ojeda et al., 2004). At nonsteroidogenic sites such as the liver, aromatase is involved in steroid metabolism in order to clear steroids, specifically estrogen, from the body when they are no longer needed. Aromatase has been shown to be upregulated in response to compounds such as DDE (You et al., 2001). It is theorized that in the same way that hormones must be metabolized to stop exerting biological effects lest they become toxic, many exogenous chemicals also induce hepatic hormone-metabolic enzymes to detoxify them (Ptak et al., 2006; Liu et al., 2012). Other such enzymes that are induced for hormone metabolism and clearance are deiodinases, which convert triiodotyrosine (T3) into diiodothyronine (T2) and convert thyroxine (T4) into reverse T3. In addition, it has been shown that there is cross-talk between the AhR and the estrogen receptor (ER), in that these two receptors

will dimerize with each other to cause either transcriptional increases in estrogen-responsive genes, or to cause the ubiquitination of the ER for proteosomal breakdown, thereby causing transcription decrease in estrogen-responsive genes (Ohtake et al., 2003). These data demonstrate significant potential for dioxin-like PCBs to modulate the endocrine system through AhR activation.

In contrast, noncoplanar PCBs are less likely to activate the AhR but instead may act through non-AhR-related systems (Fisher et al., 1998). These systems include oxidative damage and the antioxidant enzymes that respond to it. Oxidative stress refers to the overall effects of reactive oxygen species (ROS) build-up in an organism (Mitchelmore et al., 1998; Gutierrez et al., 2000; reviewed in Andreyev et al., 2005). This process is exacerbated by exposure to environmental pollutants, which accelerate ROS accumulation through hydroxylation reactions of the contaminant by the CYP450 enzymes (Wright and Welbourn, 2002). This combined with impairment of the oxidative phosphorylation cascade by organic contaminants and metals will result in synergistically elevated levels of ROS, eventually leading to intracellular oxidation and subsequent reduction of the xenobiotic compound to a free radical. This cycle repeats because the parent compound can be subsequently reformed as is the case with quinone-based anticancer drugs, with a superoxide anion being a by-product of the reaction. This redox cycling of the compound leads to hydrogen peroxide and hydroxyl ion production (Gutierrez et al., 2000). Accumulated ROS react with many biological macromolecules, leading to DNA damage, cancer, pulmonary disease, and neurodegenerative disorders (Ames et al., 1993; Cadenas and Davies, 2000; Ghio et al., 2012; Mitchelmore et al., 1998). Glutathione aids in the recycling of ROS through many roles, including being an intermediate step in reduction reactions via glutathione peroxidase (GPx) and glutathione reductase (GR) reactions. Studies have found an increase in these enzymes in response to PCB exposure (Aly and Domènech, 2009; Brown et al., 2007; Hoffman et al., 1998). It stands to reason that this enzyme system is controlled through transcriptional regulation, since it has been demonstrated that there is already one genomic receptor system—the AhR—that regulates enzymes transcriptionally upon exposure and subsequent activation by PCBs.

PCBs in the Environment

PCBs have spread globally largely via entry into aquatic systems and subsequent biological uptake and aerosolizing. PCB concentrations in the Arctic Ocean ranged from <2 to 6 pg/L in 1992, 42–72 pg/L in the Antarctic Ocean in 1982, and 40–590 pg/L in the Pacific Ocean in 1984 (Hargrave et al., 1992; Tanabe et al., 1983, 1984). A newer effort to monitor PCB levels globally takes advantage of the growing plastics pollution and the hydrophobic nature of many persistent organic pollutants to measure PCB levels and other contaminants in the ocean (Endo et al., 2005). While this effort does not reflect the exact PCB levels in a given matrix since the history of each pellet is unknown (such as how long it has been in an aquatic environment, if it has been in the sediment or in the water column, if it was eaten, etc.), it relies on volunteers from around the world to collect beached plastic and ship it for analysis, which reduces cost dramatically and allows for continual sampling. Ongoing global PCB-pellet sampling data can be viewed at International Pellet Watch (International Pellet Watch, 2005–2017; Mato et al., 2001). This website reports means of 573 ng/g pellet collected on Lake Erie in Cleveland, OH; 314 ng/g pellet in Sandy Hook, NJ; 94 ng/g pellet from Elk Neck State Park, MD; 28 ng/g pellet collected on the Gulf of Mexico near New Orleans, LA; and 48–605 ng/g pellet in several sampling sites on the Pacific Ocean around Los Angeles, CA. Worldwide, the highest reported concentration of 2746 ng/g pellet is reported from Normandy, France.

From the water column, the less chlorinated congeners can disperse into the air due to their relatively high volatility. Atmospheric dispersion causes rapid and widespread movement of PCBs to otherwise pristine ecosystems such as the Arctic, where they then have deleterious effects after moving back into the water and/or soil, and from there moving into biota (reviewed in Wright and Welbourn, 2002). It should be noted that incineration has been a low source of environmental exposure, since only 4.4% of PCBs purchased by US industry until 1976 were incinerated (Durfee et al., 1976). Instead of aerosolizing, most PCBs will settle into the sediment where they are taken up by microbes and other invertebrate biota in the soil. PCBs will then move up the food chain until they reach tertiary consumers including higher trophic level fish, birds such as raptors and seabirds, many terrestrial and aquatic animals such as marine mammals, and also humans. As a result, bioaccumulation and biomagnification cause higher trophic-level organisms to be at greater risk to the damaging effects of PCBs. For example, Safe (1990) reported that in Lake Ontario, concentrations increased from 0.05 ng/g PCB in water to 150 ng/g in sediment, 1800 ng/g in plankton, 11,580 ng/g in catfish, and finally 3,530,000 ng/g in herring gull. Similarly, Chiu et al. (2000) tracked PCB bioaccumulation in the food chain from algae to polar bears in three steps. Biomagnification is exacerbated by higher concentrations of residues stored in fat stores due to their lipophilicity, therefore high-trophic level animals, and especially those that live in extremely cold climates, that is, the Arctic and Antarctic, are of concern because polar species have higher body fat percentages compared with more equatorial species. These animals will include polar bears, seals, whales, and penguins, among others. Bustnes et al. (2006) reported average total PCB loads of 124 ng/g wet weight with a range of 9.8–781.2 in the subarctic great black-backed gull (*Larus marinus*), compared to an average of 448.7 ng/g wet weight with a range of 84.3–1576.1 in the arctic glaucous gull (*Larus hyperboreus*).

Although PCBs were released and persist as mixtures in the environment, the congeners are metabolized as they move through the food chain into lower-substituted congeners, thereby altering the composition of the remaining environmental mixtures (Brown et al., 1987). As a result, the specific congener profile that toxicologists encounter in the field is characteristic of a particular site based on the source mixture and transforming biota. The mixtures found as body burdens in animals differ between species at the same contaminated site (Custer et al., 1999; Echols et al., 2004). Since individual congeners have different effects (for example the activation of the AhR by the more dioxin-like congeners), specific mixtures have different net effects, thereby confounding the problem of studying the effects at a particular site, or of predicting the effects of a particular mixture.

Biological Effects of PCBs in Birds and Mammals

Avian populations started declining in the 1960s as a response to chemicals such as dichlorodiphenyltrichloroethane (DDT), and since then the effects of man-made compounds on birds has been of interest to toxicologists (Scanes and McNabb, 2003). The effects of avian exposure to PCBs have been studied both in the field and in the laboratory. PCB-related effects in birds have been reported to include decreased reproduction and developmental abnormalities (Fernie and Bortolotti, 2003; Fernie et al., 2003b), altered behavior (Fernie and Bortolotti, 2003) reduced hatchability in both maternal feeding and egg injection studies (Brunström, 1989), embryo mortality (Halldin et al., 2005), endocrine disruption affecting thyroid function (McNabb, 2005; Webb and McNabb, 2008), gonadal effects including reduced testis weight and imbalanced sex hormones (Biessmann, 1982), and hypothalamic hormone level changes (reviewed in Ottinger et al., 2009a,b). Other studies have reported hepatic cytochrome P450 (CYP450) enzyme induction (via AhR; Brunström, 1989; Brunström and Halldin, 1998; Elliott et al., 1990; Elliott et al., 1997; Melancon, 2003) and differential expression of related genes (Head and Kennedy, 2007); immune effects including follicle and cell count reduction in the bursa and thymus (Nikolaidis et al., 1989) immunosuppression (Lavoie and Grasman 2007); and changes in song production (Hoogesteijn et al., 2008). Many of these effects can emerge in the second generation after parental exposure (Fernie et al., 2003a).

PCBs have been implicated in adverse biological effects in several terrestrial and marine mammal species including seals, polar bears, mink, otters, and cetaceans (Basu et al., 2007; Beland et al., 1988; Chiu et al., 2000; Harding et al., 1999; Mos et al., 2007). This demonstrates further that these compounds are ubiquitous across the earth and through all biota.

Knowledge about the general effects of PCBs have been gained from extensive laboratory studies using mice and rats, yielding similar results to those found in birds and other mammals, including decreased and altered detoxification, as well as impacts on immune, reproductive, neurological, and endocrine system function. Mariussen and Fonnum (2006) review a number of neurochemical effects in laboratory animals, including changes to neurotransmitters, behavior, the neuroendocrine system, long-term potentiation, signal transduction pathways, and calcium homeostasis. Brown et al. (2007) reported increased hepatic tumors, superoxide production, and CYP450 induction, which changed in a correlated manner with PCB dose. Aly and Domènech (2009) found that rat hepatocytes exposed to Aroclor 1254 produced increased ROS and lipid peroxidation; in addition, GR, glutathione peroxidase (GPx), and glutathione (GSH) production decreased. There was a correlated increase in ethoxyresorufin-*O*-deethylase (EROD) and pentoxyresorufin-*O*-deethylase (PROD) activities, which provide biomarkers of exposure.

Transcriptional changes were found in rats in which doses of TCDD and Aroclor 1254 with similar TEQs were given to rats in order to elucidate gender-specific differences in the carcinogenicity of Aroclor 1254, specifically where mechanisms of action for carcinogenicity are concerned (Silkworth et al., 2008). Aroclor 1254 exposure is related to a higher rate of liver tumors in females than in males. Ultimately, this study revealed several interesting profiles of gene expression along gender lines, as well as showing that the TEQs, as defined by the World Health Organization (reviewed in Silkworth et al., 2008), did not explain those gender differences.

The Upper Hudson River: A Case Study

Historic manufacturing and disposal facilities have been identified as the chief point-sources of PCBs to the environment. The upper Hudson River in New York State is one of the largest sites of PCB contamination. Between 1940 and 1977, two General Electric (GE) capacitor manufacturing plants located in the towns of Fort Edward and Hudson Falls, NY, released 95,000–604,500 kg PCBs into the river (NYSDEC; Carlson et al., 2009; Feng et al., 1998; Sanders, 1989). As a result, the entire 200 mile downstream stretch of the river, between Hudson Falls and the Battery in New York City, was placed on the USEPA's National Priorities List in 1984 and is now a Superfund site (Feng et al., 1998; Hettling et al., 1978; Sanders, 1989).

Although PCB levels in the Hudson River have dropped from 540 ng/L in 1977 to 130 ng/L by 1981 (Sloan et al., 1983), contamination persists to this day. Echols et al. (2004) reported PCB concentrations in insects, tree swallow (*Tachycineta bicolor*, TRES) eggs, and hatchlings at two contaminated sites (Remnant Site 4 and Special Area 13) on the upper Hudson River. Mean concentrations in two families of insects (Odonata and Diptera) at one of the sites were 1.2 and 18 μg/g, respectively. Mean concentrations at another site for those insects were 0.56 and 6.7 μg/g, respectively. Mean tree swallow egg concentrations at those two sites were 24 and 13 μg/g respectively (Echols et al., 2004). Mean day-5 nestling concentrations were 14 and 19 μg/g. Mean day-10 nestling concentrations were 48 and 32 μg/g. Mean day-15 nestling concentrations were 96 and 32 μg/g. Mean adult concentrations at one of the sites were 152 μg/g. This is compared to an upstream reference site where day-10 nestlings and adults had mean concentrations of 1.5 and 53 μg/g, respectively. In 1999, PCB concentrations in TRES eggs were 9.3–29.5 μg/g wet weight (ww), while adults and hatchlings had concentrations at 114 and 3.7–62.2 μg/g ww respectively (Secord et al., 1999). Spotted sandpiper (*Actitis macularius*, SPSA) eggs had a concentration average of 15.2 μg/g ww, while 56.2 μg/g ww was the highest concentration measured in an individual egg (Hudson River Natural Resources Trustees, 2013). Impaired reproduction has been observed in bald eagles, white-tailed eagles, and peregrine falcons with respective egg burdens of 20, 25, and 40 μg/g ww (Custer et al., 2010).

Work on Japanese quail has shown that mortality increased with *in ovo* exposure to two PCB profile mixtures found in spotted sandpiper eggs and tree swallow eggs at the Hudson river Superfund site, respectively (Dean et al., unpublished data).

Transcriptional changes in Japanese quail exposed to the same spotted sandpiper PCB mixture have been found (Bohannon et al., unpublished data). Specifically, gene pathways related to energy balance, metabolism including cellular respiration, endocrine function, immune function, and xenobiotic metabolism were affected.

The Great Lakes: A Case Study

The Great Lakes have been a site of legacy contamination of PCBs and other industrial chemicals such as DDT, leading to the characterization of the Great Lakes embryo mortality, edema, and deformities syndrome (GLEMEDS) caused by these persistent organic pollutants (POPs; Colborn, 1991; Giesy et al., 1994; Gilbertson et al., 1991). PCB concentrations in the Great Lakes have been measured at 1 ng/L in Lake Michigan in 1987, 1–4 ng/L in Lake Superior in the late 1970s, and 1 ng/L in Lake Ontario (Swackhamer and Armstrong, 1987; Capel and Eisenreich, 1985; Oliver and Niimi, 1988). Wan et al. (2010) reported fish having body burdens ranging from 21 to 190 ng/g wet weight in the Saginaw and Tittabawassee Rivers in Michigan. Norstrom and Hebert (2006) report a decline in total PCBs in gull eggs from 1971 to 1982 (from 196 down to 39 μg/kg at Scotch Bonnet Island in Lake Ontario; from 133 down to 34 μg/kg at Big Sister Island in Lake Michigan), possibly reflecting the ban on PCBs in the late 1970s and as residues became buried in sediment. The Bald Eagle Biosentinel Program, ongoing since 1961, has measured steadily declining PCB levels in bald eagle hatchling nesting plasma, which correlates with the recovery of the eagle population bottleneck event caused by the use of PCBs and other chemicals such as DDT in the Great Lakes, however PCB levels were often measured at 213–553 μg/kg in individual birds across the state (Wierda et al., 2016). In 2008, these high numbers remained well above the productivity NOAEC of 36.4 μg/kg as reported by Bowerman et al. (2003). A similar biomonitoring program maintained in Voyageur's National Park found unexplained PCB contamination, however it remains below the productivity NOAEC of 36.4 μg/kg (Pittman et al., 2015).

In summary, PCBs were a technological advance for manufacturing and were rapidly put into use as an insulator and lubricant into a vast array of industrial, equipment and appliance applications. However, as growing concentrations of PCBs entered into the waterways and terrestrial environments, it became a formidable contaminant with both toxic and endocrine disrupting effects. Despite the ban of PCBs, there is tremendous persistence of many PCBs in the environment, thereby posing the continued exposure of wildlife and humans alike. In order to fully assess the potential for adverse outcomes and risk at the population level, with in depth understanding of both toxic and endocrine disruption integrated into the assessment of risk.

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