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Metabolic activation of PCBs to carcinogens *in vivo*—A review

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Abstract

Many higher-chlorinated biphenyls, persistent and predominant in foods, are active as promoters in hepatocarcinogenesis. Lower-chlorinated biphenyls, predominating in indoor and outdoor air, are more readily metabolized and therefore shorter lived, 'episodic' contaminants. Thus inhalation of such lower-chlorinated biphenyls may expose humans to reactive, possibly genotoxic/carcinogenic intermediates. Lower-chlorinated biphenyls may be metabolized via arene-oxides to mono- and di-hydroxylated intermediates and further to (semi)quinones, highly reactive intermediates. Covalently bound lower-chlorinated biphenyls have been detected in rodent tissues *in vivo*. We recently showed using the modified Solt–Farber foci assay that several mono- to tetrachlorinated biphenyls have initiating activity in the livers of rats. In a follow-up study of PCB3 (4-chlorobiphenyl) metabolites, only one monohydroxy- and one quinoid-metabolite showed initiating activity, indicating that the metabolic activation of PCB3 proceeds via hydroxylation and oxidation to the 3,4-quinone, the ultimate carcinogen. Since cancer initiation is based on genotoxic event(s), we hypothesized that PCB3 and/or its metabolite(s) are mutagenic in rat liver *in vivo*. To investigate this, BigBlue® rats, transgenic for the *lacI* reporter gene, were exposed to PCB3 and 4-hydroxy-PCB3 (4-HO-PCB3). In male rats the mutant frequency (MF) of *lacI* in the liver was significantly elevated and the mutation spectrum differed significantly from the control. 4-HO-PCB3 caused a non-significant ($p = 0.115$) doubling of the MF compared to the control. These studies prove that lower halogenated biphenyls may be metabolically activated *in vivo* to genotoxic and initiating intermediates.

Keywords: PCB; Carcinogenesis; *In vivo*; Mutation; Transgenic rat; 4-monochlorobiphenyl; Metabolic activation

1. Background and routes of exposure

PCBs were commercially manufactured for about 50 years and used as dielectrics in transformers and capacitors as cooling fluids, in hydraulic systems, in the formulation of lubricating and cutting oils, in pesticides and flame retardants, and as plasticizers in paints, copy paper, adhesives, sealants and plastics (WHO, 1976). Although the U.S. sales and distribution of PCBs ended in the late 1970s, a significant portion of PCBs purchased by industry are still in use, mostly within capacitors and transformers. The stability of these compounds, once the major reason for their widespread commercial use, has led to their worldwide distribution in the environment, as first reported by Jensen (1966). Although the production of PCBs peaked in 1970s and has steadily declined thereafter as most countries throughout the world have banned their production, these compounds remain in our environment and are routinely found in human serum, breast milk and adipose samples.

Regular monitoring of environmental PCBs in water, fish, and sediment of the Great Lakes and other regions in the USA started in the 1980s (Schneider et al., 2001). PCBs have also been found in at least 500 of the 1598 National Priorities List sites identified by the Environmental Protection Agency (EPA) (http://www.atsdr.cdc.gov/tfacts17.html). High PCB levels in fish have resulted in fish advisories for rivers and lakes in nearly all states of the USA. The PCBs in these fish samples are mostly higher-chlorinated congeners. It is therefore assumed that diet, especially fish, is the major source of the higher-chlorinated biphenyls that are found in human tissue samples.

Non-atmospheric sources of PCBs are carefully monitored and regulated. Air as a source of lower-chlorinated biphenyls, however, was nearly completely ignored until a decade ago. Systematic measurements of atmospheric PCBs started only in the 1990s. The first urban monitoring site was installed in Chicago in

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1995. The level of PCB contamination in the air is strongly influenced by temperature. In Chicago air concentrations between 100 and 300 pg/m^3 in winter and up to 5000–16,000 pg/m^3 on hot summer days were reported (1996–2002 IADN Data; http://epa.gov/glnpo/monitoring/air/iadn/reports/IADN_1996.pdf).

The sources of these atmospheric PCBs are almost completely undefined. Occupational exposure to atmospheric PCBs may be significantly higher. Indeed, inhalation exposure is considered to be a major route of occupational exposure to PCBs, and it was estimated that in capacitor workers, for example, a maximum of 80% of adipose PCBs may have been absorbed by inhalation exposure (Wolff, 1985). Recently even higher levels of PCBs were measured in indoor air in buildings, especially schools, constructed in 1970s where joint sealants containing 4–9% PCBs were used. Concentrations up to 13,000 ng/m^3 were measured in some classrooms of a contaminated school (Kohler et al., 2002), which is more than an order of magnitude above the NIOSH guidelines of 1 μg/m^3 for occupational settings. Other possible sources for indoor PCBs are believed to be data screen terminals (Digernes and Astrup, 1982), ceiling tiles (Anonymus, 1988) and fluorescent lights (Harris, 1985). It was reported that the concentration of PCBs in indoor air can be at least an order of magnitude higher than outdoor air (Balfanz et al., 1993; Vorhees et al., 1997; Wallace et al., 1996), although the sources are not completely clear. A recent publication from Great Britain found a daily average total PCB intake of 0.49 μg/person/day for adults of which 30.6% (4.2–63%) was derived from inhalation exposure (Harrad et al., 2006). The average intake of toddlers was 0.222 μg/toddler/day, with ∼12.6% from inhalation. It is very well possible that under certain circumstances the intake from inhalation exposure may currently be the major source of PCB intake for humans.

Major PCB congeners in Chicago air are PCB 5/8 and 15/17 (co-elute), 18, 28, 31, 33, 44, 52, and 95, to name a few (see Fig. 1). Hexa- or higher-chlorinated PCB congeners are only a minor fraction in air samples. PCB28 and PCB52 were the prevailing congeners in indoor air of contaminated schools (Kohler et al., 2002; Schwenk et al., 2002). Elevated levels of PCB28 and PCB52 were measured in the blood of teachers from contaminated schools compared to non-contaminated schools (Kohler et al., 2002; Schwenk et al., 2002), whereas the mean blood levels of higher-chlorinated biphenyls, i.e. PCB138, 153 and 180 were almost identical between groups. Inhalation exposure may also be the reason for higher levels of PCB28 and PCB52 in mothers' milk from women who smoked and/or were living in industrialized areas compared to non-smokers and women living in non-industrialized areas (Angulo et al., 1999). Environmental levels of monochlorinated PCB congeners are usually not determined because of technical reasons, but a recent report identified PCB1 and PCB3 as the major congeners in indoor air in a child care facility (Davis et al., 2002). PCB3 was by far the dominant congener emitted in the emission gases of cement plants (Ishikawa et al., 2007). Unlike higher-chlorinated biphenyls, lower, airborne PCBs do not bioaccumulate to the same extent since they are more readily metabolized and excreted. The true level of human exposure and contamination with lower-chlorinated biphenyls may therefore be grossly underestimated.

2. Implications in carcinogenesis

Studies indicate that PCB mixtures with a higher chlorine content are more potent in inducing nodular hyperplasia and hepatocarcinomas than mixtures with lower chlorination (Silberhorn et al., 1990), especially in male rodents. In a comprehensive chronic toxicity and carcinogenicity study, the effects of four Aroclor products (1016, 1242, 1254, and 1260) were investigated at multiple dietary concentrations, ranging from 25 to 200 ppm, for 24 months in male and female Sprague–Dawley rats. Statistically significant increases in hepatocellular carcinomas was noted in male rats only for the higher-chlorinated

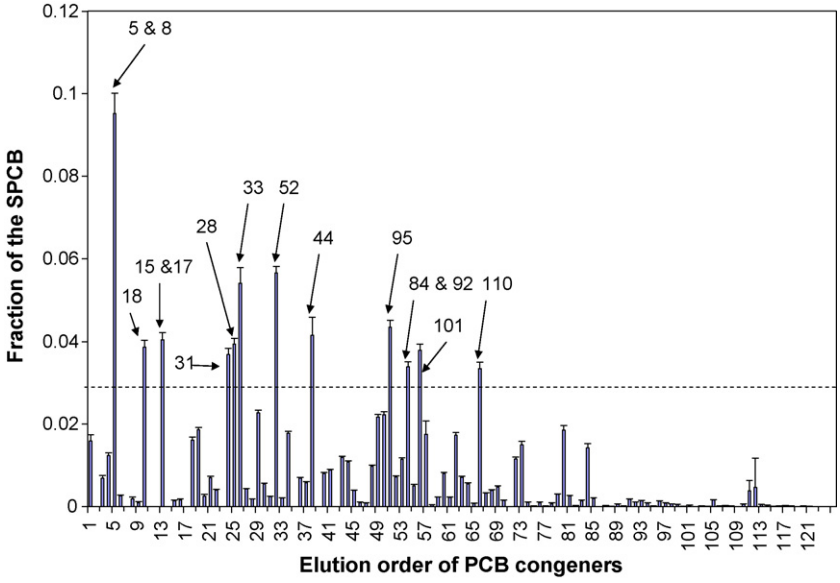

Fig. 1. Mean Chicago ∑ gas-phase PCB signal (1996–2002 IADN Data, IIT. Figure adapted from a report by Hornbuckle et al., 2006. Congeners with a fraction >0.03 of the ∑ PCB are considered to be major components (dotted line).