Polychlorinated biphenyls (PCBs)

by Larry W. Robertson PhD and Avima Ruder PhD

Citation for most recent IARC review

IARC Supplement 7, 1987

In IARC Monograph Volume 100 F (in press), PCB 126 is *carcinogenic to humans (Group 1)* (Baan et al 2009). In making the evaluations, the Working Group considered the following mechanistic arguments: There is strong evidence to support a receptor-mediated mechanism for PCB 126 carcinogenesis in humans based upon evidence of carcinogenicity in experimental animals and upon extensive evidence showing activity identical to 2,3,7,8-TCDD for every step of the mechanism described for 2,3,7,8-TCDD carcinogenesis in humans including receptor binding, gene expression, protein activity changes, cellular replication, oxidative stress, promotion in initiation-promotion studies and complete carcinogenesis in laboratory animals. (http://monographs.iarc.fr/ENG/Meetings/vol100F-evaluations.pdf)

Current evaluation

Conclusion from the previous review:
There is *limited evidence for the carcinogenicity to humans of polychlorinated biphenyls (Group 2A).* The available studies suggest an association between cancer and exposure to PCBs. The increased risk from hepatobiliary cancer emerged consistently in different studies. Since, however, the numbers were small, dose-response relationships could not be evaluated, and the role of compounds other than PCBs could not be excluded, the evidence was considered to be limited.

Environmental Exposure and Biomonitoring

PCBs were widely used from the 1930's through the 1980's and later, with an estimated total production of about 1.3 million metric tons (Breivik et al., 2002). Exposure continues from leaks from transformers and capacitors, volatilization of PCBs in cites, in buildings, from sewage, landfills and waste sites, and combustion of materials containing PCBs (Dyke et al., 2003).

Occupational Exposure

PCB exposures associated with occupational settings have greatly diminished since the 1970's, due to the ban on new uses for PCBs. Since the production of PCBs ended worldwide in 1993 (Breivik et al., 2002), new occupational exposure has been confined to four groups of workers: personnel replacing or repairing transformers and capacitors still containing PCB dielectric fluid (Altenkirch et al., 1996; Hay and Tarrel 1997; Shalat et al., 1989; Wolff et al., 1992); first responders to incidents where a transformer has exploded (Kelly et al., 2002); construction workers removing old paint, plaster, caulk, or floor finishes containing PCBs (Fromme et al., 1996; Herrick et al., 2004; Herrick et al., 2007; Piloty and Koppl 1993; Rudel and Perovich 2009; Rudel et al., 2008), and workers at hazardous waste disposal sites (Gonzalez et al., 2000; Zhao et al., 2006). The serum levels of workers engaged in sealant removal were 2-10 times higher at the end of these activities than they had been one year before (Kontsas et al., 2004). Workers exposed occupationally while PCBs were still in general use have a body burden of PCBs from their former exposure (Schecter et al., 2002; Wolff 1985). A large number of capacitors and transformers filled with PCBs is still in use

(Environmental Protection Agency and Environment Canada 2005) so potential occupational exposure continues.

Foodborne Exposure

Food chain exposure incidents by accident or malice have also occurred; one is reminded of Yusho (1968), Yu-Cheng (1979), the French cheese contamination episode (1976) and the Belgium "Dioxin" Crisis of 1999 (Covaci et al., 2008). Lower levels of PCBs are broadly prevalent in foodstuffs, and a great deal of attention has been paid to these PCBs, especially higher chlorinated ones, those more resistant to metabolic transformation, and those from certain food sources, especially in fish (Ludewig et al., 2007). People living on or near PCB-contaminated soil or near PCB-contaminated water, those eating contaminated foods, and those living in old homes being renovated continue to be exposed (Patterson et al., 2009; Rudel et al., 2008; Weintraub and Birnbaum 2008; Zheng et al., 2008).

Airborne Exposure

Air as a source of environmental PCB exposure was nearly completely ignored until about a decade ago. Systematic measurements of atmospheric PCBs started only in the 1990's. The first urban monitoring site was installed in Chicago in 1995. The level of PCB contamination in the air is strongly influenced by temperature. In Chicago air concentrations between 100-300 pg/m^3 in winter and up to 5,000-16,000 pg/m^3 on hot summer days were reported (Green et al., 2000). 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 constructed in the 1970's using joint sealants that contained 4-9% PCBs. Indoor air 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 guideline of 1 $\mu g/m^3$ (NIOSH, 2004) for occupational settings. Other possible sources for indoor PCBs are believed to be data screen terminals (Digernes and Astrup 1982), ceiling tiles and fluorescent light capacitors (Harris 1985). It was reported that the concentration of PCBs in indoor air can be at least an order of magnitude higher than in outdoor air (Balfanz et al., 1993; Vorhees et al., 1997; Wallace et al., 1996); however, regional outdoor levels can be very high due to activities like building renovations, dredging, or contamination from cement factory exhaust. Thus under certain circumstances the intake from inhalation exposure exceeds PCB intake from food.

PCBs in foods, like fish or mothers' milk, and in human adipose tissue are usually the higher chlorinated ones, where congeners like PCB153, PCB180, PCB183 and others predominate. Airborne PCBs are very different, since they require volatilization. Major congeners in Chicago air are PCB5/PCB8 (co-elute), PCB18, PCB28, PCB44, PCB52, PCB77/PCB110 (co-elute), PCB95, PCB101, to name a few (Hu et al., 2008; Zhao et al., 2009). Of two populations in Italy the more urban group had significantly higher levels of lower chlorinated PCBs (PCB52 was about 100-fold higher) than the population in a more rural environment (Turci et al., 2006). In Germany, 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 these schools compared to non-contaminated schools, whereas the mean blood levels of higher chlorinated PCBs, i.e. PCB138, PCB153 and PCB180

were almost identical (Schwenk et al., 2002). Children in schools with 690-20,800 ng PCB/m^3 air had median levels of 6, 9, and 5 ng/l PCB28, PCB52, and PCB101, respectively, whereas children in non-contaminated schools had levels below the detection level of 1 ng/L (Liebl et al., 2004). Both groups had no significant differences in PCB138, PCB153, and PCB180 levels, indicating that indoor air exposure contributed to the PCB body burden. In Germany the non-occupational tolerable indoor total PCB concentration was limited to 300 ng/m^3 (PCB Guideline 1995) based on a tolerable daily intake (TDI) of a total of 1 µg/kg body weight. Not only were these levels exceeded in several schools, but this TDI was based on a chronic toxicity study with a commercial PCB mixture, which measured hepatic enzyme induction as endpoint (Chen and Dubois 1973). Airborne PCB profiles are distinctly different from those of commercial PCB mixtures like Aroclor 1254, and enzyme induction in the liver is most likely a completely inappropriate endpoint of toxicity for inhalation exposure.

The importance of airborne PCBs is now understood. Very little is known, however, about the toxicity of these airborne PCBs and the consequences of exposure by inhalation compared to ingestion. Airborne PCBs are lower chlorinated. Our daily exposure to these airborne PCBs may be low under most circumstances, but children playing near Superfund sites in hot summer days, workers moving dried dredging material, or families living unknowingly in buildings with high indoor PCB concentrations, may be exposed to significant amounts of airborne PCBs for extended times.

Cancer in humans

(*limited***,** Supplement 7, 1987)

The previous IARC review included three occupational epidemiology cohort studies. All three of those studies have been updated (one was expanded) and an additional nine cohort studies have been published. There have also been twelve case-control studies published since the previous IARC review. Many of these used industrial hygienist reviews of participant occupational histories to estimate relative PCB exposure. The strongest studies determined PCB levels in plasma or serum from case and control specimens stored before the cases were diagnosed. No relation was observed between Janus Serum Bank specimen PCB levels and breast cancer diagnoses from the Norwegian Cancer Registry (Ward et al., 2000). Engel and colleagues found a statistically significant trend of higher odds ratios (OR) for non-Hodgkin lymphoma with higher total stored serum PCBs, in three different populations (Engel et al., 2007; Rothman et al., 1997). A significant inverse relation between total PCBs and risk of testicular germ cell tumors was seen in a study linking PCB levels of stored U.S. Department of Defense (DOD) blood specimens with DOD Medical Surveillance (McGlynn et al., 2009).

Recent papers have reviewed much of the epidemiologic literature (Faroon et al., 2003; Faroon et al., 2001; Golden and Kimbrough 2009). The lack of congruity in the cohort results may be due to all occupational PCB exposure having been to mixtures of congeners, with the proportion of each congener varying from batch to batch (Hopf et al., 2009). Because there are so many PCB congeners, some co-planar and some not, some estrogenic and some not (Fiedler 1998), it seems plausible that a variety of tumor types could arise from exposure to various congeners, or their metabolites.

Cancer in experimental animals

(*sufficient*, Supplement 7, 1987)

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 were noted in male rats only for the higher-chlorinated mixture Aroclor 1260, while all four commercial products produced an elevated incidence of hepatocellular carcinomas in female rats. It should be noted that Aroclor 1016 averages only three chlorines per biphenyl. These data indicate that commercial mixtures of chlorinated biphenyls are complete carcinogens, especially in the female rat (Mayes et al., 1998).

Mechanisms of carcinogenicity:

Metabolic Activation of Lower Chlorinated PCBs to Reactive Intermediates

It has long been recognized that biphenyl and halogenated biphenyls, particularly the lower halogenated congeners, are hydroxylated *in vivo* and *in vitro* (see review by (Safe 1989)). These hydroxylation reactions are primarily catalyzed by isoforms of cytochrome P-450. Experiments with PCB3 (4-chlorobiphenyl) and rat liver microsomes showed that five **mono-** and three **di-hydroxy metabolites** were formed (McLean et al., 1996a). The metabolism of PCB3 by cytochrome P-450 probably involves an **arene oxide intermediate** (McLean et al., 1996b; Safe 1989). Other arene oxides could be involved in the oxidation of the mono- to the di-hydroxy forms. Arene oxides are strong electrophiles which may react with critical cellular targets. The dihydroxybiphenyls can be further oxidized by various enzymes like peroxidases, prostaglandin synthase and cytochrome P-450s to the corresponding **quinone** with the formation of a **semiquinone intermediate** (Wangpradit et al., 2009). The formation of *ortho*- and *para*-quinones from diOH-PCB3 *in vitro* has been demonstrated as has their reactivity toward nitrogen and sulfur nucleophiles (Amaro et al., 1996). Other experiments demonstrated that the microsomal metabolism of PCB3 resulted in the formation of adducts with nucleotides *in vitro*, preferentially with purines rather than pyrimidines (McLean et al., 1996b). Most likely at least 1 of the 4 adducts seen is derived from an arene oxide intermediate, the 3 other adducts after further oxidation probably from a (semi)quinone (McLean et al., 1996b). These results suggest that several metabolic pathways and chemical species could be involved in PCB-induced DNA adduction. Very recent publications on oxidative DNA adducts arising from PCB exposure have appeared (Jeong et al., 2008; Spencer et al., 2009).

Oxidative DNA Damage

Lower chlorinated PCBs produce reactive oxygen species (ROS) and intracellular oxidative stress (Oakley et al., 1996; Srinivasan et al., 2001). Free radicals, particularly hydroxyl radicals, may produce 8-oxodeoxyguanosine (8-oxodG), a DNA lesion that is highly mutagenic, producing G $\rightarrow$ T transversions (Marnett and Burcham 1993). Hydroxyl radicals

can also attack fatty acids (linoleic acid, linolinic acid, oleic acid, etc) and form lipid peroxidation-derived enals, such as acrolein, crotonaldehyde, *trans*-4-OH-2-nonenal (4-HNE), and malondialdehyde (MDA) (Nair et al., 1999). These products can then modify DNA bases, resulting in cyclic adducts by interaction of their difunctional groups with NH_2 group in dA, dG or dC residues in DNA (Chaudhary et al., 1994; Chung et al., 1996; Winter et al., 1986). These cyclic adducts are mutagenic, producing base substitutions and deletions, for example G → T mutations from propano-dG and C → A mutations from various etheno adducts (Basu et al., 1993; Marnett and Burcham 1993; Nath et al., 1996). Therefore the question of mutagenicity of PCBs, especially congeners that are prone to metabolic activation like airborne PCBs, should be re-analyzed.

Hydroxylated PCBs

Stable hydroxylated metabolites of PCBs (OHPCBs) are routinely found in human blood. The percentage of total OHPCBs to total PCBs in human blood ranges from 13 to 44% (Fangstrom et al., 2002; Masuda and Haraguchi 2004; Sandanger et al., 2004; Sandau et al., 2000). 4-OHPCB187, 4-OHPCB146, 4-OHPCB107, 3'-OHPCB138 and 3-OHPCB153 are the five metabolites with the highest concentration in blood. There is increasing evidence that OHPCBs are important in the toxicities associated with PCBs. Along with studies over the last decade or so establishing the presence of OHPCBs in humans and other animals, there is increasing interest in the further metabolism of OHPCBs (Sacco et al., 2008; Wang et al., 2005; Wang et al., 2006). While sulfation has often been considered as a detoxication reaction, the OHPCBs also inhibit sulfotransferases that are important in metabolism of hormones and other endogenous molecules. This has been seen for human estrogen sulfotransferases (Kester et al., 2000), sulfotransferases active with thyroid hormone sulfation (Schuur et al., 1998), and, most recently, for human hydroxysteroid sulfotransferase (Liu et al., 2006). Thus, these molecules may potentially be involved in endocrine disruption and other responses relevant to carcinogenesis. The roles of metabolism of OHPCBs (e.g., further oxidation, sulfation, and other metabolic reactions) in the disposition and toxicity of these compounds represent a significant gap in our knowledge about mechanisms for carcinogenesis and other toxic responses to PCBs.

PCBs as mutagens/genotoxins

(*In vitro* studies)
Comprehensive reviews of the genotoxic actions of PCBs have been published (Ludewig 2001; Ludewig et al., 2008; Silberhorn et al., 1990). Recently a series of PCB3 metabolites were tested in various genotoxicity assays to determine their activity and genotoxicity profile. Both the 3,4- and particularly the 2,5-quinone of PCB3 were efficacious and potent inducers of gene mutations at the HPRT locus. Neither the corresponding dihydroxy metabolites nor the phenols had any activity in this assay. The 2,5-quinone was also by far the most potent and efficacious inducer of chromosome breaks as determined by CREST-negative (immunofluorescent antikinetochore staining using the CREST antibody) micronuclei induction (Miller et al., 1991). This suggests that at least some of the HPRT gene mutations may be due to breaks in the X-chromosome. The *ortho*-quinone, 3,4- and 2,5-dihydroxy and 4-monohydroxy metabolites induced some chromosome breaks at the highest concentration tested, but their by far stronger activity was the induction of chromosome loss (CREST-positive micronuclei). In this respect 4-OH and 2-OH

were the most efficacious metabolites tested, while the dihydroxy and quinone metabolites produced significant chromosome loss at more than 10-fold lower concentrations (Zettner et al., 2007). Of all PCB3 metabolites, only the 3,4-catechol induced sister chromatid exchanges (SCE), and only the 2,5-hydroquinone caused tetraploidization of cells, and this with an efficacy of nearly 90% at 7.5µM concentration. The HQ of PCB2 had the same effect, while PCB1-HQ and a PCB3 catechol, 3,4-Cat, were completely inactive. These results illustrate a strict structure-activity relationship (coplanar ring position, *para*-orientation of the two OH-moieties) for this effect.

These results show that metabolites of PCB3 are indeed genotoxic and that each metabolite induces its own, specific type of DNA damage. What these results did not explain was 1) the mechanism of genotoxicity for the individual endpoints, 2) whether this is of any importance *in vivo* or 3) which metabolic activation pathway(s) lead to these effects. These questions were addressed in the following experiments.

(*In vivo* studies)
A series of experiments was conducted with synthetic PCB3 metabolites in an effort to determine the metabolic activation pathway and the ultimate initiating carcinogen. For these experiments a modified Solt–Farber protocol was used to test whether PCB3 and its metabolic progeny can initiate carcinogenesis in the livers of exposed rats (Farber et al., 1977). For this experiment a fasting/refeeding protocol and 20 mg/kg DEN as positive control was used. Test compounds included the 2-OH-, 3-OH-, 4-OH-, 2,3-diOH-, 3,4-diOH-, 2,5-diOH-, 2,3-quinone, 3,4-quinone, and 2,5-quinone metabolites of PCB3. To summarize the results: the 4-OH- and 3,4-quinone metabolites of PCB3 significantly increased the number of gamma-glutamyl transpeptidase (GGT)-positive foci/cm^3, the number of foci per liver and the focal volume (% of liver). In fact, 100 mol/kg 3,4-quinone of PCB3 was more active than 20 mg/kg DEN with respect to foci number. None of the other PCB3 metabolites had a significant effect on either foci number or foci volume (Espandiari et al., 2004). The conclusion is that the 3,4-ortho-quinone of PCB3 is the ultimate initiating metabolite. It is noteworthy that formation of 3,4-quinone-derived protein adducts in the liver and brain of rats treated with PCB52 were reported (Lin et al., 2000).

Male transgenic BigBlue rats were injected intraperitoneally with PCB3, 4OHPCB3, 3-methylcholanthrene (3-MC), or corn oil and the induction of point mutations analyzed in the lacI indicator gene. PCB3 increased the mutation frequency in the liver (significantly P=0.03) (Lehmann et al., 2007) and lung (non-significantly P=0.244) (Maddox et al., 2008) of BigBlue rats, and changed the mutation spectrum in both organs from predominantly transitions to predominantly GC→TA transversions. 4OHPCB3 had a similar, but smaller effect that was below the level of statistical significance (liver P=0.18; lung P=0.208) (Lehmann et al., 2007; Maddox et al., 2008). These data demonstrate that this PCB congener is mutagenic *in vivo* in the target organ liver and less active in the lung. However, these data do not explain the mechanism of genotoxicity (DNA adduction or ROS).

Very recently Jeong and colleagues (Jeong et al., 2008) identified M_1dG DNA adducts after chronic exposure to PCB126/PCB153. These and several additional biomarkers of oxidative DNA damage including 8-OHdG, N2,3-ethenoguanine, and 1N6-ethenodoxyadenosine

indicate a role for oxidative DNA damage in the carcinogenic action of PCBs in rodent liver. In general these increases are associated with the higher exposures, which are also where the increases in liver tumors occur. More research is needed with this mode of action and with cell proliferation, as the two could drive the induction of mutations and subsequent carcinogenicity. Specific attention needs to focus on dose-response.

PCBs as Promoters of Hepatocarcinogenesis

PCBs promote liver tumors in rodents (reviewed in Glauert 2001; Glauert et al., 2008b; Silberhorn et al., 1990). Studies have shown that PCBs increase oxidative stress in the liver, including lipid peroxidation, oxidative DNA damage, and NF-κB activation. Recent studies were conducted to determine if the promoting activities of PCBs could be inhibited by dietary antioxidants (vitamin E, selenium, or phytochemicals) or by knocking out the p50 subunit of NF-κB. In the antioxidant studies, female rats were first injected with DEN (150 mg/kg) and then administered four biweekly intraperitoneal injections (300 μmol/kg/injection) of PCB77, PCB153, or vehicle; the number and volume of placental glutathione S-transferase (PGST)-positive foci were then quantified. Vitamin E did not influence the promoting activities of PCBs (Glauert et al., 2005). Increasing dietary selenium above the recommended intake increased the number of foci induced but decreased their volume (Stemm et al., 2008). Most of the phytochemicals examined (N-acetyl cysteine, ß-carotene, resveratrol, EGCG) had no significant effect on the promoting activity of PCB77. Ellagic acid increased and lycopene decreased the number of foci; ellagic acid, CoQ10, and curcumin decreased the volume of foci (Tharappel et al., 2008). In the NF-κB knockout study, male mice were first injected with DEN (90 mg/kg); controls not receiving DEN were also studied. Both p50 −/− and wild-type mice were then injected biweekly 20 times with PCB153 (300 μmol/kg). In DEN-treated and DEN+ PCB-treated mice, the incidence of tumors was lower in the p50 −/− mice than in wild-type mice. In mice receiving PCB153, the tumor incidence and tumor volume were higher. The volume of tumors that were positive for glutamine synthetase was increased in mice administered PCB153. These studies show that the promotion of hepatocarcinogenesis in rodents by PCBs is largely unaffected by dietary antioxidants but is diminished when NF-κB activation is impaired by the absence of the p50 subunit (Glauert et al., 2008a).

Biomarkers of exposure:

The analytical capabilities for congener-specific quantitation of PCBs and metabolites have improved greatly in past decades (ATSDR 2000). Higher halogenated biphenyls are themselves relatively resistant to metabolic breakdown and appear to be stable indicators of past exposure, with half-lives of 2 – 6 years (discussed in (Martin-Jimenez and Hansen 2008)). For individuals who received historic, high-level exposures, PCBs levels have diminished over time. On the other hand, for most individuals PCB exposure is low-level and continuing. For this group, body burdens of these industrial chemicals generally increase with age.

Higher halogenated metabolites, OHPCBs with five or more chlorines, also persist in human blood but little is known about their kinetics.

On the other hand, PCBs like those found in the air of buildings, in cities on a hot day, and near waste sites, are generally much more labile, are susceptible to metabolic attack, and may

disappear from blood with half lives of less than 30 days. These short lived congeners have been termed "episodic" (Martin-Jimenez and Hansen 2008). The fate of these residues, whether excreted without causing harm, or converted to metabolites that are toxic, or if bound covalently to tissues, is unknown.

Biomarkers of effect

Individual PCB congeners are ligands for a number of cellular receptors. The binding of "co-planar" PCBs to the aryl hydrocarbon (Ah) receptor was demonstrated almost three decades ago (Bandiera et al., 1982). The receptor binding/activation leads to increased transcription of a number of proteins, including a range of carcinogen – metabolizing enzymes. One notable effect is the increased expression of a cytochrome P-450-dependent monooxygenase CYP1A1, and its associated enzyme activity ethoxyresorufin-O-deethylase (EROD), routinely reported as a bioindicator for PCB-exposure, especially for coplanar PCBs and related chemicals. Other PCBs may alter the expression of other CYPs through actions on other receptors (Bandiera 2001).

Dioxin-like and Non-Dioxin-like PCBs:

Both dioxin-like (Ah receptor agonists) and non-dioxin-like PCBs (NDL-PCBs) may greatly alter gene expression controlled by estrogen receptors. A series of PCB congeners were evaluated for their estrogenic or anti-estrogenic potencies using in vitro reporter gene assay. The results suggest that some lower-chlorinated congeners exhibit weakly estrogenic effects, while higher-chlorinated ones are primarily anti-estrogenic (Pliskova et al., 2005).

Activation of AhR by dioxin-like PCBs led to a release of epithelial cells from contact inhibition of cell proliferation. The disruption of cell cycle control in liver progenitor cells might be linked to tumor promotion (Vondracek et al., 2005).

Non-dioxinlike PCBs have been shown to exert a host of rapid non-genomic effects, possibly linked to cell membrane changes. These newly discovered processes may be closely related to tumor promotion and progression. PCB153, a model NDL congener disrupted gap junction intercellular communication in liver epithelial "progenitor-like" cellular model WB-F344, which was associated with connexin43 degradation (Simeckova et al., 2009a). PCB153 and PCB47 congeners were found to induce a significant release of arachidonic acid from membranes of WB-F344 cells (Umannova et al., 2008). Moreover, PCB153 decreased protein levels of several adherens junction proteins, including E-cadherin, beta-catenin and gamma-catenin; the enhanced beta-catenin degradation led to disruption of Wnt/beta-catenin-dependent signaling (Simeckova et al., 2009b).

Research needs and recommendations:

1. Gaps in understanding related to PCB sources and exposures:

a. Mechanisms for human exposure to Aroclor and non-Aroclor PCBs. Recently non-Aroclor PCBs have been identified in air. PCB11, for example, appears to be associated with paint (Hu et al., 2008). There is a need to identify sources, including volatilization from paint. Mechanisms that deserve study include inhalation, child consumption of paint chips and other

building materials, accumulation in food, accumulation in fish, occupational exposures, for example, during building demolition.

b. Distribution of airborne PCB sources in cities.

c. Storage of Aroclors, particularly in cities. There is a great need for reasonably accurate inventories of stored PCBs. Some countries do a much better job of tracking their old transformers than others.

d. Human exposure to PCB degradation products and metabolites (these compounds are not well studied but are found in human tissues and in the environment).

2. Research needs related to mechanisms of action/toxicity:

a. There is an overwhelming need to investigate the metabolic fate of lower chlorinated PCBs found in buildings, in cities, near waste sites, and in schools. What are the reaction products? Are they mutagenic? Are any of these accessible/stable such that they may serve as biomarkers of exposure/effect? Can we prevent or abrogate the negative impacts of exposure? The fate of these residues, whether excreted without causing harm, or converted to metabolites that are toxic, or if bound covalently to tissues, is unknown.

b. The roles of metabolism of OHPCBs (e.g., further oxidation, sulfation, and other metabolic reactions) in the disposition and toxicity represent a significant gap in our knowledge about mechanisms for carcinogenesis and other toxic responses to PCBs.

c. Many mechanisms of genotoxicity/carcinogenicity for PCBs appear to involve issue of reactive oxygen species/oxidative stress, including the appearance of oxidative DNA damage. The recent identification of M_1dG DNA adducts after chronic exposure to PCBs 126/153 (Jeong et al., 2008), and the recent report by Spencer and colleagues (Spencer et al., 2009) demonstrate a role for oxidative DNA damage in the carcinogenic action of PCBs in rodent liver. In general these increases are associated with the higher exposures, which are also where the increases in liver tumors occur. More research is needed with this mode of action and with cell proliferation, as the two could drive the induction of mutations and subsequent carcinogenicity. Specific attention needs to focus on dose-response. More PCB congeners need to be studied!!

3. Airborne PCB profiles are distinctly different from those of commercial PCB mixtures like Aroclor 1254, and enzyme induction in the liver is most likely a completely inappropriate endpoint of toxicity for inhalation exposure. There is a great need to identify appropriate **biomarkers for exposure /effect/susceptibility** for airborne PCB exposure. Issues of dietary/nutritional deficiencies, *in utero* exposures, and developmental impacts are all unknown.

4. The existing epidemiologic literature, most of it produced since the last review of PCBs in 1987, may suffice for a re-evaluation of the carcinogenicity by an IARC working group. Possible studies include cancer incidence of the large (>26,000 workers) NIOSH cohort, which is under way. Nested case-control studies within this cohort and/or those in Sweden and Italy, evaluating current PCB blood levels in cases and controls, might be informative. A large population of individuals living in Aniston, Alabama, around the former PCB manufacturing facility received high levels of exposure through various routes. This group may be a useful study population.

Selected relevant publications since IARC review:

Altenkirch H, Stoltenburg G, Haller D, Hopmann D, Walter G. Clinical data on three cases of occupationally induced PCB-intoxication. *Neurotoxicology* 1996; 17: 639-643.

Amaro AR, Oakley GG, Bauer U, Spielmann HP, Robertson LW. Metabolic activation of PCBs to quinones: reactivity toward nitrogen and sulfur nucleophiles and influence of superoxide dismutase. *Chem Res Toxicol* 1996; 9: 623-629.

ATSDR. 2000. Toxicological Profile for Polychlorinated Biphenyls (PCBs).

Baan R, Grosse Y, Straif K, et al. A review of human carcinogens--Part F: chemical agents and related occupations. *Lancet Oncol.* 2009; 10: 1143-1144.

Balfanz E, Fuchs J, Kieper H. Sampling and analysis of polychlorinated biphenyls (PCB) in indoor air due to permanently elastic sealants. *Chemosphere* 1993; 26: 871-880.

Bandiera S. 2001. Cytochrome P450 enzymes as biomarkers of PCB exposure and modulators of toxicity. In: Robertson LW, Hansen LG, editors. PCBs: Recent Advances in the Environmental Toxicology and Health Effects. Lexington, Kentucky: University Press of Kentucky. p 185–192.

Bandiera S, Safe S, Okey AB. Binding of polychlorinated biphenyls classified as either phenobarbitone-, 3-methylcholanthrene- or mixed-type inducers to cytosolic Ah receptor. *Chem Biol Interact* 1982; 39: 259-77.

Basu AK, Wood ML, Niedernhofer LJ, Ramos LA, Essigmann JM. Mutagenic and genotoxic effects of three vinyl chloride-induced DNA lesions: 1,N6-ethenoadenine, 3,N4-ethenocytosine, and 4-amino-5-(imidazol-2-yl)imidazole. *Biochemistry* 1993; 32: 12793-12801.

Breivik K, Sweetman A, Pacyna JM, Jones KC. Towards a global historical emission inventory for selected PCB congeners--a mass balance approach. 1. Global production and consumption. *Sci Total Environ* 2002; 290: 181-198.

Chaudhary AK, Nokubo M, Marnett LJ, Blair IA. Analysis of the malondialdehyde-2'-deoxyguanosine adduct in rat liver DNA by gas chromatography/electron capture negative chemical ionization mass spectrometry. *Biol Mass Spectrom* 1994; 23: 457-464.

Chen T, Dubois K. Studies on the enzyme inducing effects of PCBs. *Toxicol Appl Pharmacol* 1973; 26: 504-512.

Chung FL, Chen HJ, Nath RG. Lipid peroxidation as a potential endogenous source for the formation of exocyclic DNA adducts. *Carcinogenesis* 1996; 17: 2105-2111.

Digernes V, Astrup EG. Are datascreen terminals a source of increased PCB-concentrations in the working atmosphere? *Int Arch Occup Environ Health* 1982; 49: 193-197.

Dyke PH, Foan C, Fiedler H. PCB and PAH releases from power stations and waste incineration processes in the UK. *Chemosphere* 2003; 50: 469-480.

Engel LS, Laden F, Andersen A, et al. Polychlorinated biphenyl levels in peripheral blood and non-Hodgkin's lymphoma: a report from three cohorts. *Cancer Res* 2007; 67: 5545-52.

Environmental Protection Agency, Environment Canada. 2005. Great Lakes Binational Toxics Strategy 2004 Progress Report.

Espandiari P, Glauert HP, Lehmler HJ, Lee EY, Srinivasan C, Robertson LW. Initiating activity of 4-chlorobiphenyl metabolites in the resistant hepatocyte model. *Toxicol Sci* 2004; 79: 41-46.

Fangstrom B, Athanasiadou M, Grandjean P, Weihe P, Bergman A. Hydroxylated PCB metabolites and PCBs in serum from pregnant Faroese women. *Environ Health Perspect* 2002; 110: 895-899.

Farber E, Solt D, Cameron R, Laishes B, Ogawa K, Medline A. Newer insights into the pathogenesis of liver cancer. *Am J Pathol* 1977; 89: 477-482.

Faroon OM, Keith LS, Smith-Simon C, de Rosa CT. 2003. Polychlorinated biphenyls: human health aspects. Geneva, Switzerland: World Health Organization, International Programme on Chemical Safety.

Faroon OM, Keith S, Jones D, De Rosa C. Carcinogenic effects of polychlorinated biphenyls. *Toxicol Ind Health* 2001; 17: 41-62.

Fiedler H. Polychlorinated Biphenyls (PCBs): Uses and Environmental Releases; 1998; St. Petersburg, Russian Federation, 1–4 July 1997. p 81-129.

Fromme H, Baldauf AM, Klautke O, Piloty M, Bohrer L. (Polychlorinated biphenyls (PCB) in caulking compounds of buildings--assessment of current status in Berlin and new indoor air sources). *Gesundheitswesen* 1996; 58: 666-672.

Glauert HP. 2001. PCBs and Tumor Promotion. In: Robertson LW, Hansen LG, editors. PCBs: Recent Advances in Environmental Toxicology and Health Effects. Lexington: University Press of Kentucky. p 355-371.

Glauert HP, Lu Z, Kumar A, et al. Dietary vitamin E does not inhibit the promotion of liver carcinogenesis by polychlorinated biphenyls in rats. *J Nutr* 2005; 135: 283-286.

Glauert HP, Tharappel JC, Banerjee S, et al. Inhibition of the promotion of hepatocarcinogenesis by 2,2',4,4',5,5'-hexachlorobiphenyl (PCB-153) by the deletion of the p50 subunit of NF-kappa B in mice. *Toxicol Appl Pharmacol* 2008a; 232: 302-308.

Glauert HP, Tharappel JC, Lu Z, et al. Role of Oxidative Stress in the Promoting Activities of PCBs. *Environ Toxicol Pharmacol* 2008b; 25: 247-250.

Golden R, Kimbrough R. Weight of evidence evaluation of potential human cancer risks from exposure to polychlorinated biphenyls: an update based on studies published since 2003. *Crit Rev Toxicol* 2009; 39: 299-331.

Gonzalez CA, Kogevinas M, Gadea E, et al. Biomonitoring study of people living near or working at a municipal solid-waste incinerator before and after two years of operation. *Arch Environ Health* 2000; 55: 259-267.

Green ML, Depinto JV, Sweet C, Hornbuckle KC. Regional Spatial and Temporal Interpolation of Atmospheric PCBs: Interpretation of Lake Michigan Mass Balance Data. *Environ Sci Technol* 2000; 34: 1833-1841.

Harris MG. PCB exposure from fluorescent lights. *Am J Public Health* 1985; 75: 892.

Hay A, Tarrel J. Mortality of power workers exposed to phenoxy herbicides and polychlorinated biphenyls in waste transformer oil. *Ann N Y Acad Sci* 1997; 837: 138-156.

Herrick RF, McClean MD, Meeker JD, Baxter LK, Weymouth GA. An unrecognized source of PCB contamination in schools and other buildings. *Environ Health Perspect* 2004; 112: 1051-1053.

Herrick RF, Meeker JD, Hauser R, Altshul L, Weymouth GA. Serum PCB levels and congener profiles among US construction workers. *Environ Health* 2007; 6:25.

Hopf NB, Waters MA, Ruder AM. Cumulative exposure estimates for polychlorinated biphenyls using a job-exposure matrix. *Chemosphere* 2009; 76: 185-193.

Hu D, Martinez A, Hornbuckle KC. Discovery of non-aroclor PCB (3,3'-dichlorobiphenyl) in Chicago air. *Environ Sci Technol* 2008; 42: 7873-7877.

Jeong YC, Walker NJ, Burgin DE, et al. Accumulation of M1dG DNA adducts after chronic exposure to PCBs, but not from acute exposure to polychlorinated aromatic hydrocarbons. *Free Radic Biol Med* 2008; 45: 585-591.

Kelly KJ, Connelly E, Reinhold GA, Byrne M, Prezant DJ. Assessment of health effects in New York City firefighters after exposure to polychlorinated biphenyls (PCBs) and polychlorinated dibenzofurans (PCDFs): the Staten Island Transformer Fire Health Surveillance Project. *Arch Environ Health* 2002; 57: 282-293.

Kester MH, Bulduk S, Tibboel D, et al. Potent inhibition of estrogen sulfotransferase by hydroxylated PCB metabolites: a novel pathway explaining the estrogenic activity of PCBs. *Endocrinology* 2000; 141: 1897-1900.

Kohler M, Zennegg M, Waeber R. Coplanar polychlorinated biphenyls (PCB) in indoor air. *Environ Sci Technol* 2002; 36: 4735-4740.

Kontsas H, Pekari K, Riala R, Back B, Rantio T, Priha E. Worker exposure to polychlorinated biphenyls in elastic polysulphide sealant renovation. *Ann Occup Hyg* 2004; 48: 51-55.

Lehmann L, Esch HL, Kirby PA, Robertson LW, Ludewig G. 4-monochlorobiphenyl (PCB3) induces mutations in the livers of transgenic Fisher 344 rats. *Carcinogenesis* 2007; 28: 471-478.

Liebl B, Schettgen T, Kerscher G, et al. Evidence for increased internal exposure to lower chlorinated polychlorinated biphenyls (PCB) in pupils attending a contaminated school. *Int J Hyg Environ Health* 2004; 207: 315-324.

Lin PH, Sangaiah R, Ranasinghe A, et al. Formation of quinonoid-derived protein adducts in the liver and brain of Sprague-Dawley rats treated with 2,2',5, 5'-tetrachlorobiphenyl. *Chem Res Toxicol* 2000; 13: 710-718.

Liu Y, Apak TI, Lehmler HJ, Robertson LW, Duffel MW. Hydroxylated polychlorinated biphenyls are substrates and inhibitors of human hydroxysteroid sulfotransferase SULT2A1. *Chem Res Toxicol* 2006; 19: 1420-1425.

Ludewig G. 2001. Cancer Initiation by PCBs. In: Robertson LW, Hansen LG, editors. PCBs: Recent Advances in Environmental Toxicology and Health Effects. Lexington: University Press of Kentucky. p 337-354.

Ludewig G, Esch H, Robertson LW. 2007. Polyhalogenierte Bi- und Terphenyle. In: Dunkelberg H, Gebel T, Hartwig A, editors. Handbuch der Lebensmitteltoxikologie. Weinheim: Wiley-VCH Verlag GmbH & Co. p 1031-1094.

Ludewig G, Lehmann L, Esch H, Robertson LW. Metabolic Activation of PCBs to Carcinogens in Vivo - A Review. *Environ Toxicol Pharmacol* 2008; 25: 241-246.

Maddox C, Wang B, Kirby PA, Wang K, Ludewig G. Mutagenicity of 3-Methylcholanthrene, PCB3, and 4-OH-PCB3 in the Lung of Transgenic Bigblue Rats. *Environ Toxicol Pharmacol* 2008; 25: 260-266.

Marnett LJ, Burcham PC. Endogenous DNA adducts: potential and paradox. *Chem Res Toxicol* 1993; 6: 771-785.

Martin-Jimenez T, Hansen LG. 2008. Toxicokinetic Extrapolation of PCB Exposure in Anniston, Alabama. In: Hansen LG, Robertson LW, editors. PCBs: Human and Environmental Disposition and Toxicology. Urbana and Chicago: University of Illinois Press. p 70-80.

Masuda Y, Haraguchi K. Hydroxy and methylsulfone metabolites of polychlorinated biphenyls in the human blood and tissues. *Organohalogen Compounds* 2004; 66: 3576-3584.

McGlynn KA, Quraishi SM, Graubard BI, Weber JP, Rubertone MV, Erickson RL. Polychlorinated biphenyls and risk of testicular germ cell tumors. *Cancer Res* 2009; 69: 1901-1909.

McLean MR, Bauer U, Amaro AR, Robertson LW. Identification of catechol and hydroquinone metabolites of 4-monochlorobiphenyl. *Chem Res Toxicol* 1996a; 9: 158-164.

McLean MR, Robertson LW, Gupta RC. Detection of PCB adducts by the 32P-postlabeling technique. *Chem Res Toxicol* 1996b; 9: 165-171.

Miller BM, Zitzelsberger HF, Weier HU, Adler ID. Classification of micronuclei in murine erythrocytes: immunofluorescent staining using CREST antibodies compared to in situ hybridization with biotinylated gamma satellite DNA. *Mutagenesis* 1991; 6: 297-302.

Nair J, Barbin A, Velic I, Bartsch H. Etheno DNA-base adducts from endogenous reactive species. *Mutat Res* 1999; 424: 59-69.

Nath RG, Randerath K, Li D, Chung FL. Endogenous production of DNA adducts. *Regul Toxicol Pharmacol* 1996; 23: 22-28.

Oakley GG, Devanaboyina U, Robertson LW, Gupta RC. Oxidative DNA damage induced by activation of polychlorinated biphenyls (PCBs): implications for PCB-induced oxidative stress in breast cancer. *Chem Res Toxicol* 1996; 9: 1285-1292.

Patterson DG, Jr., Wong LY, Turner WE, et al. Levels in the U.S. population of those persistent organic pollutants (2003-2004) included in the Stockholm Convention or in other long range transboundary air pollution agreements. *Environ Sci Technol* 2009; 43: 1211-1218.

Piloty M, Koppl B. (PCB caulking sealants--experiences and results of measures in Berlin and decontamination of a school (Part I)). *Gesundheitswesen* 1993; 55: 577-581.

Pliskova M, Vondracek J, Canton RF, et al. Impact of polychlorinated biphenyls contamination on estrogenic activity in human male serum. *Environ Health Perspect* 2005; 113: 1277-1284.

Rothman N, Cantor KP, Blair A, et al. A nested case-control study of non-Hodgkin lymphoma and serum organochlorine residues. *Lancet* 1997; 350: 240-244.

Rudel RA, Perovich LJ. Endocrine disrupting chemicals in indoor and outdoor air. *Atmos Environ* 2009; 43: 170-181.

Rudel RA, Seryak LM, Brody JG. PCB-containing wood floor finish is a likely source of elevated PCBs in residents' blood, household air and dust: a case study of exposure. *Environ*

Health 2008; 7:2.

Sacco JC, Lehmler HJ, Robertson LW, Li W, James MO. Glucuronidation of polychlorinated biphenylols and UDP-glucuronic acid concentrations in channel catfish liver and intestine. *Drug Metab Dispos* 2008; 36: 623-630.

Safe S. 1989. Metabolism, uptake, storage and bioaccumulation. In: Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins and Related Products. Eds. Kimbrough and Jensen, Elsevier, New York.

Sandanger TM, Dumas P, Berger U, Burkow IC. Analysis of HO-PCBs and PCP in blood plasma from individuals with high PCB exposure living on the Chukotka Peninsula in the Russian Arctic. *J Environ Monit* 2004; 6: 758-765.

Sandau CD, Ayotte P, Dewailly E, Duffe J, Norstrom RJ. Analysis of hydroxylated metabolites of PCBs (OH-PCBs) and other chlorinated phenolic compounds in whole blood from Canadian inuit. *Environ Health Perspect* 2000; 108: 611-616.

Schecter A, Pavuk M, Amirova DA, et al. Characterization of dioxin exposure in firefighters, residents, and chemical workers in the Irkutsk Region of Russian Siberia. *Chemosphere* 2002; 47: 147-156.

Schuur AG, van Leeuwen-Bol I, Jong WM, et al. In vitro inhibition of thyroid hormone sulfation by polychlorobiphenylols: isozyme specificity and inhibition kinetics. *Toxicol Sci* 1998; 45: 188-194.

Schwenk M, Gabrio T, Papke O, Wallenhorst T. Human biomonitoring of polychlorinated biphenyls and polychlorinated dibenzodioxins and dibenzofuranes in teachers working in a PCB-contaminated school. *Chemosphere* 2002; 47: 229-233.

Shalat SL, True LD, Fleming LE, Pace PE. Kidney cancer in utility workers exposed to polychlorinated biphenyls (PCBs). *Br J Ind Med* 1989; 46: 823-824.

Silberhorn EM, Glauert HP, Robertson LW. Carcinogenicity of polyhalogenated biphenyls: PCBs and PBBs. *Crit Rev Toxicol* 1990; 20: 440-496.

Simeckova P, Vondracek J, Andrysik Z, et al. The 2,2',4,4',5,5'-hexachlorobiphenyl-enhanced degradation of connexin 43 involves both proteasomal and lysosomal activities. *Toxicol Sci* 2009a; 107: 9-18.

Simeckova P, Vondracek J, Prochazkova J, Kozubik A, Krcmar P, Machala M. 2,2',4,4',5,5'-Hexachlorobiphenyl (PCB 153) induces degradation of adherens junction proteins and inhibits beta-catenin-dependent transcription in liver epithelial cells. *Toxicology* 2009b; 260: 104-111.

Spencer WA, Lehmler HJ, Robertson LW, Gupta RC. Oxidative DNA adducts after Cu(2+)-mediated activation of dihydroxy PCBs: role of reactive oxygen species. *Free Radic Biol Med* 2009; 46: 1346-1352.

Srinivasan A, Lehmler HJ, Robertson LW, Ludewig G. Production of DNA strand breaks in vitro and reactive oxygen species in vitro and in HL-60 cells by PCB metabolites. *Toxicol Sci* 2001; 60: 92-102.

Stemm DN, Tharappel JC, Lehmler HJ, et al. Effect of dietary selenium on the promotion of hepatocarcinogenesis by 3,3', 4,4'-tetrachlorobiphenyl and 2,2', 4,4', 5,5'-hexachlorobiphenyl. *Exp Biol Med (Maywood)* 2008; 233: 366-376.

Tharappel JC, Lehmler HJ, Srinivasan C, Robertson LW, Spear BT, Glauert HP. Effect of antioxidant phytochemicals on the hepatic tumor promoting activity of 3,3',4,4'-tetrachlorobiphenyl (PCB-77). *Food Chem Toxicol* 2008; 46: 3467-3474.

Turci R, Finozzi E, Catenacci G, Marinaccio A, Balducci C, Minoia C. Reference values of coplanar and non-coplanar PCBs in serum samples from two Italian population groups. *Toxicol Lett* 2006; 162: 250-255.

Umannova L, Neca J, Andrysik Z, et al. Non-dioxin-like polychlorinated biphenyls induce a release of arachidonic acid in liver epithelial cells: a partial role of cytosolic phospholipase A(2) and extracellular signal-regulated kinases 1/2 signalling. *Toxicology* 2008; 247: 55-60.

Vondracek J, Machala M, Bryja V, et al. Aryl hydrocarbon receptor-activating polychlorinated biphenyls and their hydroxylated metabolites induce cell proliferation in contact-inhibited rat liver epithelial cells. *Toxicol Sci* 2005; 83: 53-63.

Vorhees DJ, Cullen AC, Altshul LM. Exposure to polychlorinated biphenyls in residential indoor air and outdoor air near a Superfund site. *Environ Sci Technol* 1997; 31: 3612-3618.

Wallace JC, Basu I, Hites RA. Sampling and analysis artifacts caused by elevated indoor air polychlorinated biphenyl concentrations. *Environ Sci Technol* 1996; 30: 2730-2734.

Wang LQ, Lehmler HJ, Robertson LW, Falany CN, James MO. In vitro inhibition of human hepatic and cDNA-expressed sulfotransferase activity with 3-hydroxybenzo(a)pyrene by polychlorobiphenylols. *Environ Health Perspect* 2005; 113: 680-687.

Wang LQ, Lehmler HJ, Robertson LW, James MO. Polychlorobiphenylols are selective inhibitors of human phenol sulfotransferase 1A1 with 4-nitrophenol as a substrate. *Chem Biol Interact* 2006; 159: 235-246.

Wangpradit O, Mariappan SV, Teesch LM, et al. Oxidation of 4-chlorobiphenyl metabolites to electrophilic species by prostaglandin H synthase. *Chem Res Toxicol* 2009; 22: 64-71.

Ward EM, Schulte P, Grajewski B, et al. Serum organochlorine levels and breast cancer: a nested case-control study of Norwegian women. *Cancer Epidemiol Biomarkers Prev* 2000; 9(12):1357-1367.

Weintraub M, Birnbaum LS. Catfish consumption as a contributor to elevated PCB levels in a non-Hispanic black subpopulation. *Environ Res* 2008; 107: 412-417.

Winter CK, Segall HJ, Haddon WF. Formation of cyclic adducts of deoxyguanosine with the aldehydes trans-4-hydroxy-2-hexenal and trans-4-hydroxy-2-nonenal in vitro. *Cancer Res* 1986; 46: 5682-5686.

Wolff MS. Occupational exposure to polychlorinated biphenyls (PCBs). *Environ Health Perspect* 1985; 60: 133-138.

Wolff MS, Fischbein A, Selikoff IJ. Changes in PCB serum concentrations among capacitor manufacturing workers. *Environ Res* 1992; 59: 202-216.

Zettner MA, Flor S, Ludewig G, Wagner J, Robertson LW, Lehmann L. Quinoid metabolites of 4-monochlorobiphenyl induce gene mutations in cultured Chinese hamster v79 cells. *Toxicol Sci* 2007; 100: 88-98.

Zhao G, Xu Y, Han G, Ling B. Biotransfer of persistent organic pollutants from a large site in China used for the disassembly of electronic and electrical waste. *Environ Geochem Health* 2006; 28: 341-351.

Zhao HX, Adamcakova-Dodd A, Hu D, Hornbuckle KC, Just CL, Robertson LW, Thorne PS, Lehmler HJ. Development of a synthetic PCB mixture resembling the average polychlorinated biphenyl profile in Chicago air. *Environ Int* 2009; in press.

Zheng GJ, Leung AO, Jiao LP, Wong MH. Polychlorinated dibenzo-*p*-dioxins and dibenzofurans pollution in China: sources, environmental levels and potential human health impacts. *Environ Int* 2008; 34: 1050-1061.