PRINT: ISSN 0972-3757 ONLINE: 2456-6360
Int J Hum Genet, 4(4): 281-290 (2004)
DOI: 10.31901/24566330.2004/04.04.10

Toxicity of Polychlorobiphenyls and its Bioremediation

Supriyo De[1], Saroj K. Pramanik[2], Arthur L. Williams[2] and Sisir K. Dutta[1]

1. Department of Biology, Howard University, 415 College Street, NW, Washington DC – 20059, USA
2. Department of Biology, Morgan State University, 1700E Cold Spring Lane, Baltimore, MD – 21251, USA
Supriyo De E-mail address: supriyo_de@yahoo.com
Saroj K. Pramanik E-mail address: pramanis@jewel.morgan.edu
Arthur L. Williams E-mail address: awillia5@jewel.morgan.edu
Sisir K. Dutta E-mail address: sdutta@howard.edu
Phone: (202) 806-4170; Fax: (202) 806-4176

KEYWORDS PCB; neurotoxicity; hepatotoxicity; immunomodulation; apoptosis; reductive dechlorination; biodegradation

ABSTRACT PCBs are chlorinated aromatic compounds known to have toxic effects on humans and animals. They are well known mutagens and carcinogens and are known to affect almost all systems of the body. They have neurotoxic effects especially on infants and children and may have persisting effects in adults as well. The mechanism of action of neurotoxicity is probably through disruption of Ca^{2+} homeostasis. PCBs are known to cause hepatocarcinoma through genotoxicity and through receptor mediated deregulation of apoptosis. They have deleterious effects on the endocrine system because of their structural similarity with the steroid hormones especially estrogen. They also act as goitrogens and increase the thyroid stimulating hormone level. Polychlorobiphenyls also down-regulate the immune system probably through apoptotic pathways. As they cross placenta neurotoxic, immunomodulatory and other developmental effects are seen on the new born. Infants are also affected by PCB secreted through breast milk. PCBs are also found to cause atherosclerosis through oxidative stress and endothelial cell dysfunction. Available methods of PCB removal through incineration may lead to formation of more toxic dioxin like compounds. Partial metabolites of PCB are also equally harmful. Complete biodegradation may be the only way to clean up the PCB contamination which is persistent in the environment even after being banned from USA since 1977. Many bacteria and fungus which can degrade PCB have been identified. Since the prevalent hypothesis of degradation, first by dechlorination by anaerobic organisms followed by ring cleavage by aerobic organism has inherent difficulties, a novel finding of reductive dechlorination by the white rot fungus may help to accelerate PCB biodegradation.

INTRODUCTION

Polychlorobiphenyls (PCBs) are a class of hazardous compounds which were widely used in the manufacture for capacitors, transformers, cooling liquids, hydraulic fluid, lubricants, pesticides and copy paper because of their excellent dielectric properties and chemical stability. The physical and chemical natures of the PCBs including lipophilicity, heat resistance, and inertness are the main reasons for all health problems. Though PCBs were banned from USA since 1977, our biosphere contains approximately 750,000 tons of released PCBs (http://bio.nagaokaut.ac.jp/~mitsui-l/BphC/background.html), known to cause reproductive (Loch-Caruso 2002), neurological (Schantz et al. 2003), endocrinal (Portigal et al. 2002) and other defects. These also adversely effect fetal and infant developments (Winneke et al. 2002). It is also highly mutagenic and carcinogenic (Faroon et al. 2001a; Faroon et al. 2001b; Faroon et al. 2001c; Laden et al. 2002). Currently, there are numerous PCB contaminated sites on the EPA, USA Superfund Priority List. The 209 congeners of PCB differ in the position of the chlorine atoms. The generalized structure of PCB is shown in Figure 1.

As PCBs are mostly insoluble in water, its incomplete breakdown by several micro-organisms like *Pseudomonas* and *Clostridium* in the human body makes it water soluble and there by helping it getting absorbed which in turn increases its toxic effects. PCBs can be degraded by high heat (1200ºC); however, one of the degraded products (dioxin) is more notorious than the original PCBs (Amend and Lederman 1992). While aggressive engineering methods, e.g., excavation, are suitable for "hot spot" removal, less expensive containment and treatment

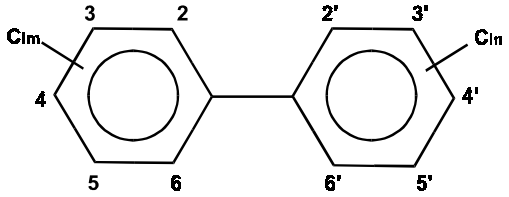

Fig. 1. The numbers represent the different positions of the chlorine (m+n=10 or less). From the health hazard point of view, one of the most important PCB congener is the 2,2',4,4',5,5' hexachloride biphenyl which is primarily used for research in our lab (De 2003).

technologies are required for remediation of surrounding areas or lower level contaminated sites. Bioremediation may provide a safe and cost effective alternative to current methods for PCB cleanup. Bacteria have been shown to degrade PCB compounds directly and co-metabolically under anaerobic and aerobic conditions in both laboratory and field conditions. The first step of all PCB degradation is the removal of the chlorine atoms by anaerobic organisms and then is breaking open of the phenyl ring by the aerobic organisms. (Commandeur and Parsons 1990; Copley 1997; Morris et al. 1992). The final degradation product is CO_2 (Freudenberg and Neish 1968).

Toxicity of PCB Neurotoxicity

The neurological effects of polychlorinated biphenyls (PCBs) have been extensively investigated in humans and in animals. The main focus of most of these studies is on the effects in neonates and young children, although studies of adults have also been conducted. One of the most important concern is the low level of PCBs transferred to the fetus across the placenta may induce long-lasting neurological damage. Because PCBs are lipophilic substances, there is also concern that significant amounts might be transferred to nursing infants via breast milk. These studies have provided evidence that PCBs are important contributors to neurobehavioral alterations observed in newborn children such as motor immaturity and hyporeflexia. Some of these alterations even persist during childhood. There is preliminary evidence that highly chlorinated PCB congeners, which accumulate in certain fish, are associated with neurobehavioral alterations seen in some newborn children. Children born to women who accidentally consumed rice oil contaminated with relatively high amounts of PCBs and chlorinated dibenzofurans (CDFs) during pregnancy also had neuro-developmental changes (Faroon et al. 2001a). Children exposed to PCBs during fetal life show IQ deficits, hyperactivity, and attention deficits, known as autism. (Bowman et al. 1981; Levin et al. 1988; Rice 1999; Rice and Hayward 1997). Studies in animals support the human data as neurobehavioral alterations have been also observed in rats and monkeys following prenatal and/or postnatal exposure to commercial Arochlor mixtures (defined experimental congener mixtures), single PCB congeners, and Great Lakes (USA) contaminated fish. In addition, postnatal exposure of monkeys to PCB mixtures similar to that found in human breast milk showed learning deficits long after the exposure had ceased. It appeared that ortho-substituted PCB congeners are more active than coplanar PCBs in modifying cognitive processes. Changes in levels of neurotransmitters in various brain structures such as decrease in dopamine content in basal ganglia and prefrontal cortex areas have also been observed in monkeys, rats, and mice. (Faroon et al. 2001a).

In primates, ortho-substituted PCB congeners having a non-coplanar structure decreases catecholamine levels in certain brain regions and in rat pheochromocytoma cells (PC12 cells) (Seegal et al. 1990). Subsequent work implicated a mechanism involving general disruption of Ca^{2+} homeostasis and altered activity of protein kinase C (Kodavanti and Tilson 1997; Tilson and Kodavanti 1998). Whether non-coplanar PCB's target multiple regulatory proteins and cellular membranes in a non-selective manner (Kodavanti and Tilson 1997) or through a more selective receptor mediated mechanism (Inglefield et al. 2002; Schantz et al. 1997) is not known clearly. Probably, PCBs alter the Ca^{2+} signaling through ryanotidine receptors or through inositol 1,4,5-triphosphate (IP_3) receptors. (Berridge 1998; Berridge et al. 2000)

Hepatotoxicity

PCBs were found to cause liver cancer in the mice (Rumsby et al. 1992). It was found that the environmental pollutants hexachlorobenzene (HCB) and PCBs produce mutations in the Ha-ras proto-oncogene at codon 61. Only two

mutations (causing a focus of altered cells and a trabecular cell carcinoma) from 23 preneoplastic and neoplastic lesions induced by HCB were detected. With Aroclor 1254 no mutations were detected in 28 areas at various stages of carcinogenesis analysed. Sequencing of the two mutations generated by HCB showed a C→T transversion at the first base of codon 61 (carcinoma) and an A→T transversion at the second base (proliferative focus) (Rumsby et al. 1992). The non-mutagenic chemicals are believed to mediate their effects by promoting the clonal outgrowth of initiated cells. Some of these chemicals, such as dibenzo-p-dioxins and certain PCBs have been demonstrated to interact with specific cellular receptors which appear crucial for their tumorogenic activity. Enzyme-altered foci in rat liver may serve as a sensitive means to estimate the promoting activity of these agents in rodents. These data can perhaps be extrapolated to the humans (Schwarz et al. 1995).

The exact mechanism of hepatocarcinogenesis remains controversial. It was found out that in female rats, and to a much lesser extent in male rats, there was pronounced iron accumulation in hepatocytes at the 26th-week when treated with mid- and high-dose of Aroclor-1254 and -1260. At 52 weeks, large accumulations of iron were also present in Kupffer cells of female rats, and dose-related increases in proliferating cell nuclear antigen (PCNA) hepatocyte labeling indices were found in both male and female rats. These changes preceded the formation of liver tumors, which were not generally found until 78 weeks. This study suggested that PCB-induced iron accumulation in hepatocytes was an early event that may be related to tumor formation, especially in female rats (Whysner and Wang 2001). Promotion of altered hepatic foci was evaluated utilizing glutathione-S-transferase bioassay for promoters of hepatocarcinogenesis. GST-P+ foci were quantified by histomorphometry and were reported as areas and numbers of GST-P+ foci within the area of liver examined (Dean et al. 2002). Glutathione S-transferase placental (GSTP) positive foci were present at 52 weeks in high-dose Aroclor-1254 and -1260 female groups, and small foci were found in some Aroclor 1254-exposed female rats at 26 weeks, along with centrilobular hepatocytes expressing GSTP. Consequently, iron accumulations producing oxidative damage, and enhanced cell proliferation resulting in tumor promotion may be components in the mode of action for PCB-induced hepatocarcinogenesis in rodents (Whysner and Wang 2001).

Recent studies made progress towards elucidating the mechanisms of action of these toxicants from the discovery of receptors that bind specific classes of xenobiotics. Dioxins and polychlorinated biphenyls bind to the aryl hydrocarbon receptor, phenobarbital binds to the constitutive androstane receptor and peroxisome proliferators act via the activated receptor alpha. These three receptors have ligand-dependent transcription activities and therefore mediate changes in gene expression in response to toxicant exposure. The development of transgenic mouse strains where the genes for these receptors are disrupted has demonstrated that receptor activity is essential for the toxicity of these carcinogens. This implies that changes in the expression of key target genes control proliferation and apoptosis in the xenobiotic-induced hepatocyte phenotype (Oliver and Roberts 2002).

Endocrine and Reproductive Toxicity

Polychlorinated biphenyls (PCBs) are considered potential endocrine disruptors due to their ability to act as estrogens, antiestrogens and goitrogens. Studies on the effect of PCB on sperm function and hormonal effects on rats revealed that testis weights were significantly increased whereas sperm count, motility, total motile sperm count, curvilinear velocity, average path velocity, straight-line velocity, and beat-cross frequency for motile sperm were significantly decreased. There was a significant increase in thyroid-stimulating hormone level however; no changes were seen in serum testosterone, thyroid hormones, or prolactin concentrations. These results suggest that the sperm functions may be more susceptible to endocrine disruption caused by dioxin-like PCB congeners . Study on two hundred adolescents in Flanders (Belgium) who had accidental moderate exposure to polychlorinated aromatic hydrocarbons (PCAHs), showed that the pubertal development of boys and girls were delayed. In one such suburb near two waste incinerators, fewer boys reached the adult stages of genital development (62% vs. 92%) and pubic hair growth (48% vs. 77%). Also, in the same suburb, fewer girls had reached the adult stage of breast

development (67% vs. 90%). Left plus right testicular volume was lower in both polluted areas than in the control area (42.4 mL vs. 47.3 mL) but was not related to the current exposure of the adolescents to PCAHs. Through endocrine disruption, environmental exposure to PCAHs may interfere with sexual maturation and in the long-run adversely affect human reproduction. (Den Hond et al. 2002)

The effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on thyroid receptor (TR)-mediated gene expression in the HeLaTR cells showed that triiodothyronin (T3) induced the expression of the reporter gene in a thyroid hormone responsible element (TRE) dependent manner. When the cells were cultured in the presence of T3, the addition of TCDD to the culture media further enhanced the T3-induced expression of the reporter gene. RT-PCR revealed that mRNA levels of 4-1BB, fmfc, PSCA, PSG7, RANTES, and TRAF1, which were highly increased by T3, were further elevated in cells exposed to T3 and TCDD. Also, the mRNA level of BMP6, which was decreased by T3, further declined in the cells exposed to both T3 and TCDD. In contrast to the effect of TCDD, PCB-OH suppressed the modulation of these gene expressions by T3. Neither TCDD nor PCB-OH alone affected the expression of 4-1BB, fmfc, PSCA, PSG7, RANTES, TRAF1, or BMP6. These results indicate that TCDD augments the cellular responses to T3 by hyperactivating TR-mediated gene expression, whereas PCB-OH suppresses cellular responses to T3 by negatively regulating it (Yamada-Okabe et al. 2004).

Developmental Effects

The neurodevelopmental effects of PCB have already being discussed. Studies in several species have shown that exposure to PCBs and their hydroxylated metabolites reduces fecundity and decreases circulating concentrations of thyroid hormones, causing serious reproductive and developmental defects. Thyroid hormones modulate both follicular development and steroidogenesis, and affect estrogen metabolism and the regulation of estrogen receptor. The study on the effects of PCB mixture (Aroclor 1016) on follicle maturation in the Long-Evans hooded rat indicated that Aroclor significantly reduced the number of preantral follicles <50000μ^2 and the total number of antral follicles in the 50-100000 and >100000 μ^2 size classes. T_4 circumvented the Aroclor effect on the number of preantral follicles; however, a significant reduction in the antral follicle number persisted. In addition, there was a significant increase in atresia in the Aroclor-treated ovaries (Baldridge et al. 2003).

Moreover, study on the long-term exposure to polychlorinated biphenyls (PCBs) on developing dental enamel of 8- to 14-year-old children who were pre- and post-natally exposed to PCBs in the contaminated region of Bela Krajina, Slovenia revealed developmental defects of enamel in permanent teeth. About 71.3% of exposed children, compared to 49.5% in the control group have enamel defects, mostly as demarcated opacities and hypoplasia. No significant correlations were found between PCB exposure and developmental defects in deciduous teeth (Jan and Vrbic 2000).

Cardiovascular Effects

There is evidence that exposure to certain PCBs can lead to development of cardiovascular diseases such as atherosclerosis. Though very little is known about the mechanisms underlying this toxicity, endothelial cell dysfunction is a critical event in the initiation and acceleration of atherosclerosis. In one study, porcine pulmonary artery-derived endothelial cells were exposed to three PCBs (PCB 77, PCB 114 or PCB 153) having different binding avidities with the aryl hydrocarbon receptor (AhR) and differences in their induction of cytochrome P450 for up to 24 hours. PCB 77 and PCB 114 significantly disrupted the endothelial barrier function in a dose-dependent manner, by allowing an increase in albumin transfer across endothelial monolayer. These PCBs also contributed markedly to cellular oxidative stress, as measured by 2,7-dichlorofluorescin (DCF) fluorescence and lipid hydroperoxides, and caused a significant increase in intracellular calcium levels. Enhanced oxidative stress and Ca^{2+} in PCB 77- and PCB 114-treated cells were accompanied by increased activity and content of cytochrome P450 1A and by a decrease in the vitamin E content in the culture medium. In contrast to the effects of PCB 77 and PCB 114, cell exposure to PCB 153 had no effect on cellular oxidation, Ca^{2+}, or endothelial barrier function. These results suggest that certain PCBs may play a role in the development of atherosclerosis by causing endothelial cell

dysfunction and a decrease in the barrier function of the vascular endothelium. It is possible that interaction of PCBs with the Ah- receptor and activation of the cytochrome P450 1A subfamily are involved in this pathology (Toborek et al. 1995).

A significant and marked decrease in cell viability was observed in human microvascular endothelial cells (HMEC-1) treated with 2,2',4,6,6'-pentachlorobiphenyl (PCB 104) in a time- and dose-dependent manner. Exposure of HMEC-1 to PCB 104 also dramatically induced inter-nucleosomal DNA fragmentation. However, the caspase inhibitor zVAD-fmk significantly reversed the PCB 104-induced DNA fragmentation in HMEC-1, suggesting that endothelial cell death induced by PCB 104 exposure is, at least in part, due to caspase-dependent apoptotic pathways. Several transcription factors, such as cAMP responsive element-binding protein (CREB), activator protein-1 (AP-1), nuclear factor-kappaB (NF-kappaB), and signal transducers and activators of transcription (STAT1), have been known to play a pivotal role in the molecular signaling cascades for the induction of apoptosis. A series of electrophoretic mobility shift assay showed that PCB 104 specifically increased only CREB DNA-binding activity in a dose-dependent manner. AP-1, NF-kappaB, and STAT1, however, were not activated. In addition, zVAD-fmk significantly and dose-dependently blocked the CREB activation enhanced by PCB 104 exposure. These results suggest that PCB-induced death of human microvascular endothelial cells is mediated, at least in part, via the caspase-dependent apoptotic pathways and that the selective activation of CREB is involved in this process (Lee et al. 2003).

Immunomodulation

PCBs have been found to alter the immune system in rodents, guinea pigs, rabbits and chicken as well as non-human primates. (Tryphonas 1995; Tryphonas et al. 1991). These studies also indicated that higher chlorinated forms of PCB mixtures are more immunotoxic than the lower chlorinated Arochlors. Following exposure to PCB there is a reduction in the antibody production (Tryphonas 1995; Tryphonas et al. 1991) however there are variable changes in respect to thymus and spleen. There is reduction of thymus size in rats and rabbits (Vos and Beems 1971) but no change in mice (Silkworth and Loose 1978). Very little data is available regarding effect of PCB on humans. Alterations in the immune system have been observed in the Japanese and Taiwanese populations accidentally exposed to PCBs through contaminated rice oil. There was significant effect on both cellular and humoral immunity (Lu and Wu 1985; Wu et al. 1984). As PCB can cross placenta and secreted in mothers milk severe adverse effect was found on the newborns (Lan et al. 1990; Yu et al. 1998). Laboratory experiments exhibited reduction of antibody forming response to T-dependent antigen of sheep red blood cells, reduction of primary activation of T cells by mixed lymphocyte response, and reduction of lymphocyte proliferation induced by various mitogens. These immunosuppressant effects of PCB were related with the loss of lymphocyte viability which was determined by the propidium iodide method. DNA fragmentation detected by the diphenylamine method and agarose gel electrophoresis proved the role of apoptosis in the lymphocyte death. The degree of DNA fragmentation was increased in a dose- and time-dependent manner in PCB-treated splenocytes. This proved that probably apoptosis was attributable to the immunotoxicity of PCBs in murine splenocytes (Yoo et al. 1997).

Other Toxic Effects

In a population-based cross-sectional study of the effects of 15 polychlorinated biphenyl (PCB) congeners and three organochlorine pesticides in residents of two districts in eastern Slovakia, one with extensive environmental contamination from a former PCB production site (Michalovce) and the other matched on geography but with low (background) levels (Svidnik) cancer incidence from 1985 through 1994 was calculated as standardized incidence ratios (SIRs) and 95% confidence intervals for each district using indirect standardization and rates from eastern Slovakia. Among males from Michalovce (exposed) there was an excess of cancer of the tongue (SIR=1.46; 1.06-1.96), stomach (SIR=1.15; 1.00-1.32), lung (SIR=1.14; 1.04-1.24), testis (SIR=1.40; 0.97-1.97), and kidney (SIR=1.23; 0.98-1.52), and lower than expected incidence of prostate cancer (SIR=0.83; 0.69-0.97); in contrast, there was an excess of peritoneal (SIR=3.05; 1.11-6.63) and laryngeal

cancer (SIR=1.43; 0.99-1.98) in Svidnik (not exposed). Among females from Michalovce there was an excess of cancer of the lip (SIR=2.54; 1.53-3.96), stomach (SIR=1.22; 1.02-1.44), and lung (SIR=1.17; 0.94-1.45); in contrast, there was an excess of kidney (SIR=1.61; 1.03-2.40) and thyroid (SIR=1.97; 1.12-3.20) cancer in Svidnik. Taken together, these results raise the possibility that high environmental exposure to organochlorines in the Michalovce district may be associated with higher rates of certain cancers, particularly stomach and lung cancer (Pavuk et al. 2004). Also bladder cancer is reported due to exposure to PAH (Boffetta et al. 1997).

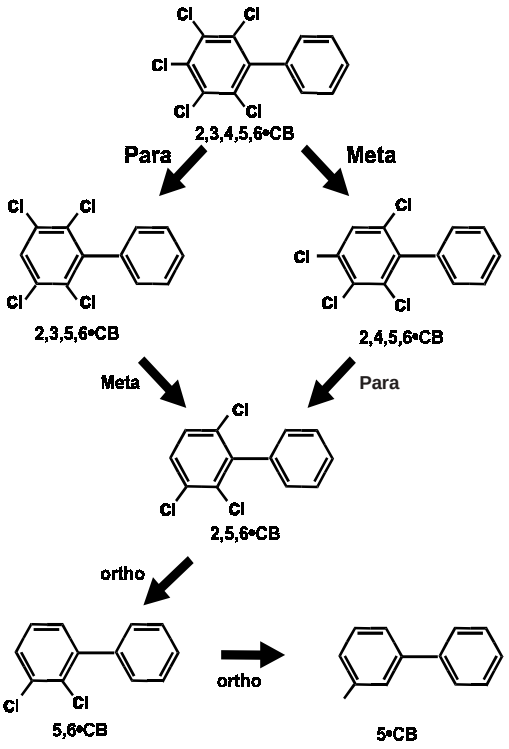

Fig. 2. This figure shows the dechlorination pathway of PCB by anaerobic organisms. These organisms replace the chlorine molecules with hydrogen atoms (reduction reaction). As the exact mechanism is not known, the enzymes responsible for this dechlorination are not shown in this figure. The final product is still a biphenyl compound. Removal of chlorine atoms leads to increased water solubility and the rate of absorption thereby probably increasing the toxic effects. The end products act as substrate for further metabolism by aerobic organisms. (Berkaw et al. 1996)

Bioremediation

Even though PCBs were banned in USA in 1977, but they still persist in the environment and many PCB contaminated sites have been identified (www.epa.gov). The cost of removing these PCBs by incineration or by ionizing radiation (Schmelling et al. 1998) is tremendous. During incineration PCBs generates other notorious products such as dioxin which is deadlier than the parent PCB molecule. Therefore, this is not a feasible technique. As a consortium of several bacteria like *Pseudomonas, Clostridium, Nocardia* or fungus like *Phanerochaete chrysosporium* can degrade PCB, in-situ bioremediation has been proposed as a relatively low cost and ecologically friendly procedure and it appears that microbial degradation can be an important endpoint for PCBs. The rate PCB degradation depends on microbe type, nutrients like salts, type of PCB including the chlorination and position of the chlorine atoms on the biphenyl ring. The final degradation product is CO_2 (Freudenberg and Neish 1968).

It seems that bioremediation needs two steps – dechlorination, which is usually mediated by anaerobic microorganism (Fig. 2) (Berkaw et al. 1996) followed by oxidative ring cleavage by aerobic organisms (Fig. 3) (Commandeur and Parsons 1990; Copley 1997; Morris et al. 1992). Our earth curst contains mainly aerobic organisms; dechlorination mediated by anaerobes is therefore the rate limiting step for PCB degradation.

It is well documented that the white-rot fungus (*Phanerochaete chrysosporium*) can degrade PCB in both lignolytic and non-lignolytic conditions (Dutta et al. 1998; Jackson et al. 1999). It has also been shown that the manganese peroxide enzyme activity increases with the addition of manganese (Brown et al. 1991; Brown et al. 1990). Though the best method to study PCB degradation is by using GC/Mass Spectroscopy (Dutta et al. 1998; Juan et al. 1999), the PCB breakdown products can also be studied by nuclear magnetic resonance (NMR) or by infra-red spectroscopy (IR-spectroscopy) (Pellizzari et al. 1985; Umana et al. 1985) where the different functional groups formed during the PCB degradation can be identified. High Pressure Liquid chromatography (HPLC) has been used for a long time to see the breakdown of PCBs

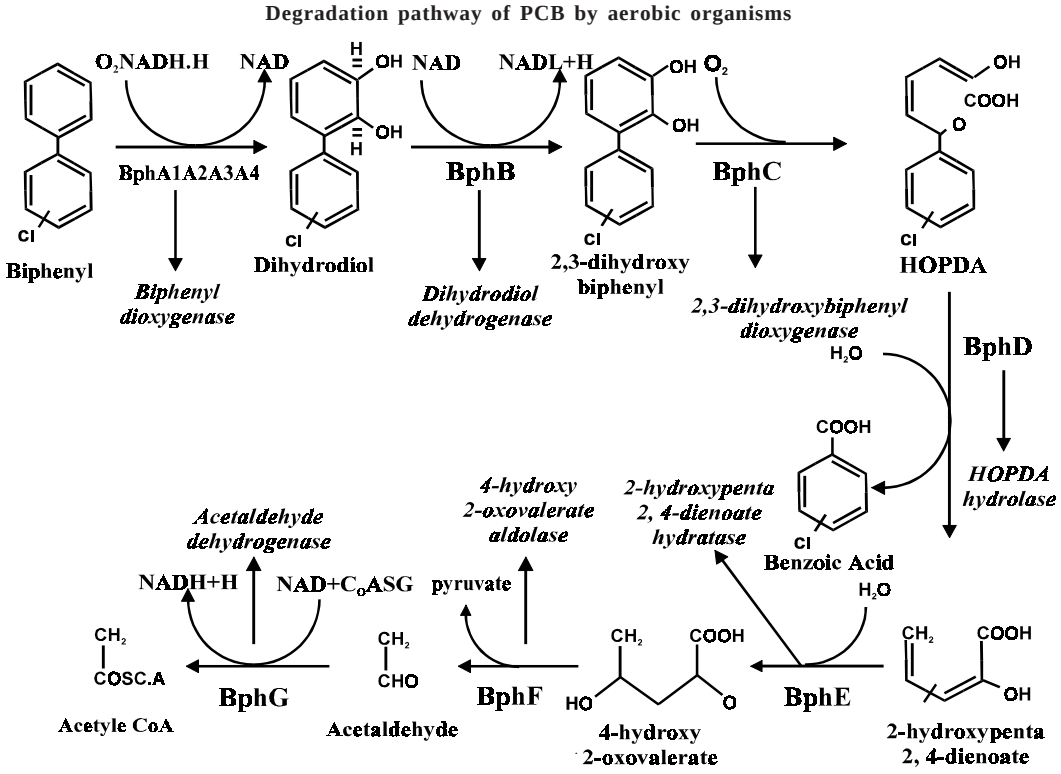

Fig. 3. This shows the degradation pathway of PCB by aerobic organisms. HOPDA stands for 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoic acid. BphA has four subunits A1, A2, A3 and A4 and converts bi[phenyl to dihydrodiol with addition of wo oxygen atoms (Fukuda et al. 1994). HOPDA is formed by meta cleavage of 2,3-dihydroxy biphenyl (Kimbara et al. 1989). BphD converts HOPDA inot two molecules: benzoic acid and 2-hydroxypenta-2,4-dienoate (Kimbara et al. 1989). Acetyl CoA is then used in Krbs cycle and CO_2 is produced (Freudenberg and Neish 1968). [Modified from (Ohtsubo et al. 2000) with addition of different enzymes.]

(Tayal et al. 1999). Recent HPLC and spectrophotometric studies in our lab showed that microbes can dechlorinate PCBs even in the presence of oxygen provided the nitrate reductase enzyme is induced (De 2003). This hypothesis is tested using white-rot fungi (De 2003) and further confirmed *in vitro* using pure nitrate reductase enzyme (unpublished).

Reductive Dechlorination

Nitrate reductase enzymes are responsible for reducing the nitrate groups to ammonia. Earlier studies have shown that *Phanerochaete chrysosporium* can reduce nitrates and thereby breaks down nitrogenous toxic chemicals like 2,4 DNT (Jackson et al. 1999). Nitrate reductase was also found to play an important role in the PCB degradation, as PCBs are first degraded by reductive dechlorination. The ring cleavage probably occurs through production of non-specific peroxides by the white rot fungus. The importance of nitrate reductase enzyme on dechlorination has been also documented by several authors (Kuritz et al. 1997; Reddy et al. 1998; Song et al. 2000). We have also shown using HPLC that addition of molybdenum which acts as cofactor for nitrate reductase increases PCB breakdown whereas tungsten which inhibits this enzyme decreases its breakdown (Fig. 4) (De 2003).

CONCLUSION

In conclusion we can say that PCB degradation can be accelerated even in the

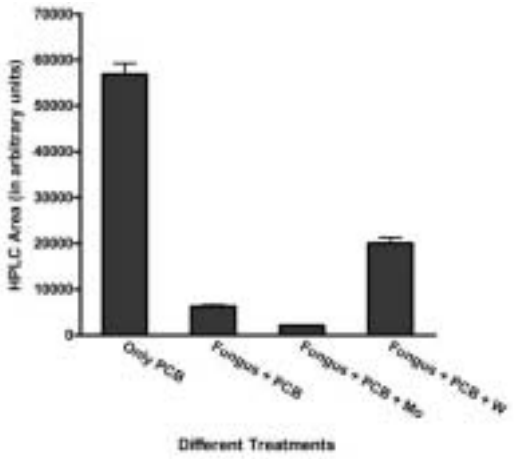

Fig. 4. Result of HPLC studies showing different degrees of PCB breakdown in presence of different salts. *Phanerochaete chrysosporium* was grown in ME medium. (n=3) Modified from (De 2003)

aerobic condition simply by inducing the nitrate reductase enzyme. In future these methods can probably be used for bioremediation of the contaminated sites, thereby preventing the exposure of human and animals to its toxic effects.

REFERENCES

Allen JR, Abrahamson LJ 1973. Morphological and biochemical changes in the liver of rats fed polychlorinated biphenyls. *Arch Environ Contam Toxicol,* **1:** 265-280.

Amend LJ, Lederman PB 1992. Critical Evaluation of PCB Remediation Technologies. *Environmental Progress,* **11:** 173-177.

Baldridge MG, Stahl RL, Gerstenberger SL, Tripoli V, Hutz RJ 2003. Modulation of ovarian follicle maturation in Long-Evans rats exposed to polychlorinated biphenyls (PCBs) in-utero and lactationally. *Reprod Toxicol,* **17:** 567-73.

Berkaw M, Sowers KR, May HD 1996. Anaerobic ortho-dechlorination of polychlorinated biphenyls by estuarine sediments from Baltimore Harbor. *Appl Environ Microbiol,* **62:** 2534-2539.

Berridge MJ 1998. Neuronal calcium signaling. *Neuron,* **21:** 13-26.

Berridge MJ, Lipp P, Bootman MD 2000. Signal transduction. The calcium entry pas de deux. *Science,* **287:** 1604-5.

Boffetta P, Jourenkova N, Gustavsson P 1997. Cancer risk from occupational and environmental exposure to polycyclic aromatic hydrocarbons. *Cancer Causes Control,* **8:** 444-72.

Bowman RE, Heironimus MP, Barsotti DA 1981. Locomotor hyperactivity in PCB-exposed rhesus monkeys. *Neurotoxicology,* **2:** 251-68.

Brown JA, Alic M, Gold MH 1991. Manganese peroxidase gene transcription in *Phanerochaete chrysosporium*: activation by manganese. *J Bacteriol,* **173:** 4101-4106.

Brown JA, Glenn JK, Gold MH 1990. Manganese regulates expression of manganese peroxidase by *Phanerochaete chrysosporium*. *J Bacteriol,* **172:** 3125-3130.

Commandeur LC, Parsons JR 1990. Degradation of halogenated aromatic compounds. *Biodegradation,* **1:** 207-20.

Copley SD 1997. Diverse mechanistic approaches to difficult chemical transformations: microbial dehalogenation of chlorinated aromatic compounds. *Chem Biol,* **4:** 169-74.

De S 2003. *Degradation of PCB by Phanerochaete chrysosporium: Reductive Dechlorination and Gene Induction.* Master Thesis Howard University.

Dean CE, Jr., Benjamin SA, Chubb LS, Tessari JD, Keefe TJ 2002. Nonadditive hepatic tumor promoting effects by a mixture of two structurally different polychlorinated biphenyls in female rat livers. *Toxicol Sci,* **66:** 54-61.

Den Hond E, Roels HA, Hoppenbrouwers K, Nawrot T, Thijs L, Vandermeulen C, Winneke G, Vanderschueren D, Staessen JA 2002. Sexual maturation in relation to polychlorinated aromatic hydrocarbons: Sharpe and Skakkebaek's hypothesis revisited. *Environ Health Perspect,* **110:** 771-6.

Dutta SK, Jackson MM, Hou L-H, Powell D, Tatem HE 1998. Non-lignolytic TNT mineralization in contaminated soil by *Phanerochaete chrysosporium*. *J Bioremediation,* **2:** 97-103.

Faroon O, Jones D, de Rosa C 2001a. Effects of polychlorinated biphenyls on the nervous system. *Toxicol Ind Health,* **16:** 305-33.

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

Faroon OM, Keith S, Jones D, de Rosa C 2001c. Effects of polychlorinated biphenyls on development and reproduction. *Toxicol Ind Health,* **17:** 63-93.

Freudenberg K, Neish AC 1968. *Constitution and Biosynthesis of Lignin.* Berlin: Springer-Verlag.

Fukuda M, Yasukochi Y, Kikuchi Y, Nagata Y, Kimbara K, Horiuchi H, Takagi M, Yano K 1994. Identification of the bphA and bphB genes of Pseudomonas sp. strains KKS102 involved in degradation of biphenyl and polychlorinated biphenyls. *Biochem Biophys Res Commun,* **202:** 850-6.

Hsu PC, Guo YL, Li MH 2004. Effects of acute postnatal exposure to 3,3',4,4'-tetrachlorobiphenyl on sperm function and hormone levels in adult rats. *Chemosphere,* **54:** 611-8.

Inglefield JR, Mundy WR, Meacham CA, Shafer TJ 2002. Identification of calcium-dependent and -independent signaling pathways involved in polychlorinated biphenyl-induced cyclic AMP-responsive element-binding protein phosphorylation in developing cortical neurons. *Neuroscience,* **115:** 559-73.

Jackson MM, Hou L, Banerjee HN, Sridhar R, Dutta SK 1999. Disappearance of 2,4-dinitrotoluene and 2-Amino,4,6-dinitrotoluene by Phanerochaete

chrysosporium under non-ligninolytic conditions. *Bull Environ Contam Toxicol* **62:** 390-6.

Jan J, Vrbic V 2000. Polychlorinated biphenyls cause developmental enamel defects in children. *Caries Res* **34:** 469-73.

Juan CY, Thomas GO, Semple KT, Jones KC 1999. Methods for the analysis of PCBs in human food, faeces and serum. *Chemosphere* **39:** 1467-76.

Kimbara K, Hashimoto T, Fukuda M, Koana T, Takagi M, Oishi M, Yano K 1989. Cloning and sequencing of two tandem genes involved in degradation of 2,3-dihydroxybiphenyl to benzoic acid in the polychlorinated biphenyl-degrading soil bacterium Pseudomonas sp. strain KKS102. *J Bacteriol*, **171:** 2740-7.

Kodavanti PR, Tilson HA 1997. Structure-activity relationships of potentially neurotoxic PCB congeners in the rat. *Neurotoxicology*, **18:** 425-41.

Kuritz T, Bocanera LV, Rivera NS 1997. Dechlorination of lindane by the cyanobacterium Anabaena sp. strain PCC7120 depends on the function of the nir operon. *J Bacteriol*, **179:** 3368-70.

Laden F, Ishibe N, Hankinson SE, Wolff MS, Gertig DM, Hunter DJ, Kelsey KT 2002. Polychlorinated biphenyls, cytochrome P450 1A1, and breast cancer risk in the Nurses' Health Study. *Cancer Epidemiol Biomarkers Prev*, **11:** 1560-5.

Lan SJ, Yen Y, Yin., Lan JL 1990. Immunity of PCB transplacental Yu-cheng children in Taiwan. *Bull Environ Contam Toxicol*, **44:** 224-229.

Lee YW, Park HJ, Son KW, Hennig B, Robertson LW, Toborek M 2003. 2,2',4,6,6'-pentachlorobiphenyl (PCB 104) induces apoptosis of human microvascular endothelial cells through the caspase-dependent activation of CREB. *Toxicol Appl Pharmacol*, **189:** 1-10.

Levin ED, Schantz SL, Bowman RE 1988. Delayed spatial alternation deficits resulting from perinatal PCB exposure in monkeys. *Arch Toxicol*, **62:** 267-73.

Loch-Caruso R 2002. Uterine muscle as a potential target of polychlorinated biphenyls during pregnancy. *Int J Hyg Environ Health*, **205:** 121-30.

Lu YC, Wu YC 1985. Clinical findings and immunological abnormalities in Yu-Cheng patients. *Environ Health Perspect*, **59:** 17-29.

Morris PJ, Mohn WW, Quensen JF, 3rd, Tiedje JM, Boyd SA 1992. Establishment of polychlorinated biphenyl-degrading enrichment culture with predominantly meta dechlorination. *Appl Environ Microbiol*, **58:** 3088-94.

Ohtsubo Y, Nagata Y, Kimbara K, Takagi M, Ohta A 2000. Expression of the bph genes involved in biphenyl/PCB degradation in Pseudomonas sp. KKS102 induced by the biphenyl degradation intermediate, 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoic acid. *Gene*, **256:** 223-8.

Oliver JD, Roberts RA 2002. Receptor-mediated hepatocarcinogenesis: role of hepatocyte proliferation and apoptosis. *Pharmacol Toxicol*, **91:** 1-7.

Pavuk M, Cerhan JR, Lynch CF, Schecter A, Petrik J, Chovancova J, Kocan A 2004. Environmental exposure to PCBs and cancer incidence in eastern Slovakia. *Chemosphere*, **54:** 1509-20.

Pellizzari ED, Moseley MA, Cooper SD 1985. Recent advances in the analysis of polychlorinated biphenyls in environmental and biological media. *J Chromatogr*, **334:** 277-314.

Portigal CL, Cowell SP, Fedoruk MN, Butler CM, Rennie PS, Nelson CC 2002. Polychlorinated biphenyls interfere with androgen-induced transcriptional activation and hormone binding. *Toxicol Appl Pharmacol*, **179:** 185-94.

Reddy GV, Gelpke MD, Gold MH 1998. Degradation of 2,4,6-trichlorophenol by Phanerochaete chrysosporium: involvement of reductive dechlorination. *J Bacteriol*, **180:** 5159-64.

Rice DC 1999. Behavioral impairment produced by low-level postnatal PCB exposure in monkeys. *Environ Res*, **80:** S113-S121.

Rice DC, Hayward S 1997. Effects of postnatal exposure to a PCB mixture in monkeys on nonspatial discrimination reversal and delayed alternation performance. *Neurotoxicology*, **18:** 479-94.

Rumsby PC, Evans JG, Phillimore HE, Carthew P, Smith AG 1992. Search for Ha-ras codon 61 mutations in liver tumours caused by hexachlorobenzene and Aroclor 1254 in C57BL/10ScSn mice with iron overload. *Carcinogenesis*, **13:** 1917-20.

Schantz SL, Seo BW, Wong PW, Pessah IN 1997. Long-term effects of developmental exposure to 2,2',3,5',6-pentachlorobiphenyl (PCB 95) on locomotor activity, spatial learning and memory and brain ryanodine binding. *Neurotoxicology*, **18:** 457-67.

Schantz SL, Widholm JJ, Rice DC 2003. Effects of PCB exposure on neuropsychological function in children. *Environ Health Perspect*, **111:** 357-576.

Schmelling DC, Poster DL, Chaychian M, Neta P, Silverman J, Al-Sheikhly M 1998. Degradation of Polychlorinated Biphenyls Induced by Ionizing Radiation in Aqueous Micellar Solutions. *Environ Sci Technol*, **32:** 270.

Schwarz M, Buchmann A, Stinchcombe S, Luebeck G, Moolgavkar S, Bock KW 1995. Role of receptors in human and rodent hepatocarcinogenesis. *Mutat Res*, **333:** 69-79.

Seegal RF, Bush B, Shain W 1990. Lightly chlorinated ortho-substituted PCB congeners decrease dopamine in nonhuman primate brain and in tissue culture. *Toxicol Appl Pharmacol*, **106:** 136-44.

Silkworth JB, Loose LD 1978. Cell-mediated immunity in mice fed either Arochlor 1016 or hexachlorobenzene. *Environ Health Perspect*, **45:** 326-332.

Smialowicz RJ 1989. Evaluation of the immunotoxicity of low level PCB exposure in the rat. *Toxicology*, **56:** 197-211.

Song B, Palleroni NJ, Haggblom MM 2000. Isolation and characterization of diverse halobenzoate-degrading denitrifying bacteria from soils and sediments. *Appl Environ Microbiol*, **66:** 3446-53.

Tayal AK, Das L, Kaur I 1999. Biodegradation of pentachlorophenol (PCP) by white rot fungal strains screened from local sources and its estimation by high-performance liquid chromatography. *Biomedical Chromatography*, **13:** 220-224.

Tilson HA, Kodavanti PR 1998. The neurotoxicity of polychlorinated biphenyls. *Neurotoxicology*, **19:** 517-25.

Toborek M, Barger SW, Mattson MP, Espandiari P, Robertson LW, Hennig B 1995. Exposure to

polychlorinated biphenyls causes endothelial cell dysfunction. *J Biochem Toxicol,* **10:** 219-26.

Tryphonas H 1995. The use of non-human primates in the study of PCB immunomodulation. *Hum Exp Toxicol,* **14:** 107-10.

Tryphonas H, Luster MI, Schiffman G, Dawson LL, Hodgen M, Germolec D, Hayward S, Bryce F, Loo JC, Mandy F, et al. 1991. Effect of chronic exposure of PCB (Aroclor 1254) on specific and nonspecific immune parameters in the rhesus (Macaca mulatta) monkey. *Fundam Appl Toxicol,* **16:** 773-86.

Umana M, Moseley A, Pellizzari ED, Gurka DF, Haseth JA 1985. The measurement of on-the-fly fourier transform infrared reference spectra of environmentally important compounds. *Appl Spectroscopy,* **39:** 297-303.

Vos JG, Beems RB 1971. Dermal toxicity studies of technical polychlorinated biphenyls and fractions thereof in rabbits. *Toxicol Appl Pharmacol,* **19:** 617-633.

Whysner J, Wang CX 2001. Hepatocellular iron accumulation and increased cell proliferation in polychlorinated biphenyl-exposed Sprague-Dawley rats and the development of hepatocarcinogenesis. *Toxicol Sci,* **62:** 36-45.

Winneke G, Walkowiak J, Lilienthal H 2002. PCB-induced neurodevelopmental toxicity in human infants and its potential mediation by endocrine dysfunction. *Toxicology,* 181-**182:** 161-5.

Wu YC, Lu YC, Kao HY, Pan CC, Lin RY 1984. Cell-mediated immunity in patients with polychlorinated biphenyl poisoning. *Taiwan Yi Xue Hui Za Zhi,* **83:** 419-29.

Yamada-Okabe T, Aono T, Sakai H, Kashima Y, Yamada-Okabe H 2004. 2,3,7,8-tetrachlorodibenzo-p-dioxin augments the modulation of gene expression mediated by the thyroid hormone receptor. *Toxicol Appl Pharmacol,* **194:** 201-10.

Yoo BS, Jung KH, Hana SB, Kim HM 1997. Apoptosis-mediated immunotoxicity of polychlorinated biphenyls (PCBs) in murine splenocytes. *Toxicol Lett,* **91:** 83-9.

Yu ML, Hsin JW, Hsu CC, Chan WC, Guo YL 1998. The immunologic evaluation of the Yucheng children. *Chemosphere,* **37:** 1855-65.