INNSPUB

Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 9, No. 2, p. 212-221, 2016
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Polychlorinated biphenyl residues and etiology of cancer: a case control study in the residents of Karachi city

Mohammad Attaullah[*1], Masarrat J. Yousuf[2], Sohail Shaukat[3], Alia B. Munshi[4], Sobia Khawaja[5], Syed Ishtiaq Anjum[6]

[1]*Department of Zoology, University of Karachi, Pakistan*

[2]*Department of Zoology, University of Karachi, Pakistan.*

[3]*Center for Environmental Studies, Pakistan Council of Scientific and Industrial Research (PCSIR) Laboratories Complex, Karachi, Pakistan.*

[4]*Center for Environmental Studies, PCSIR Laboratories Complex, Karachi.*

[5]*Department of Zoology, Federal Urdu University of Arts, Science and Technology, Karachi, Pakistan.*

[6]*Department of Zoology, Kohat University of Science and Technology, Pakistan.*

Article published on August 31, 2016

Key words: Polychlorinated biphenyls, Gas Chromatography, Electron Capture Detector, Cancer, Environmental chemicals.

Abstract

Residues of Polychlorinated Biphenyls (PCBs) were comparatively evaluated in the serum samples of cancer patients and healthy residents of Karachi City. This was a preliminary work on the role of PCBs in the etiology of cancer in Pakistan. A random collection of fasting blood samples from diagnosed cancer patients having various malignancies and healthy humans was carried out with informed consent of the donors at various hospitals of Karachi. Serum was separated within 2 hours of collection and then analyzed for residues of the seven PCBs (PCB 28, PCB 52, PCB 101, PCB 138, PCB 153, PCB 180 and PCB 209) on Gas Chromatograph coupled with Electron Capture Detector (GC-ECD). PCBs were detected in 93.98% of the cancer cases and 93.75 % of the normal subjects. Mean level of total PCBs (ΣPCBs) was found significantly elevated in the cancer group (2.711 mg/kg) compared with the control group (0.536 mg/kg). PCB 52 was the most prevalent PCB congener with a mean level of 2.044 mg/kg in the cancer group and 0.134 mg/kg in the control group. It was observed that concentrations of PCBs increased linearly with the increasing age of the cancer patients. Highest mean concentration of ΣPCBs was found in the cases of Chronic Myeloid Leukemia as 11.962 mg/kg. In light of the obtained results, it has concluded that PCBs are positively associated with the etiology of cancer. Further research is recommended to know the exact role and mechanism of PCBs in the field of oncology.

***Corresponding Author:** Mohammad Attaullah ✉ attaullah.ms@gmail.com

Introduction

Polychlorinated biphenyls (PCBs) are a class of synthetic chlorinated hydrocarbons having two benzene rings linked by a single carbon-carbon bond. PCBs consist of 209 isomers and are widely used as dielectric in high performance capacitors, as coolant and insulators in high performance transformers, in paper and paint industry and as plasticizers in synthetic materials. They are very persistent in the environment, highly soluble in fats and so concentrate in the fatty tissues of animals. This potential of bioaccumulation and biomagnification of PCBs has caused serious health hazards including cancer in animals and humans. On the basis of enough evidence of carcinogenicity in experimental animals and humans, PCBs are classified as Group I carcinogens by the International Agency for Research on Cancer (ATSDR, 2014). Approximately 1,000 tons of PCBs are estimated currently cycling through the atmosphere over the US. (HSDB, 2003). PCBs are reported as complete carcinogens acting as general cancer promoters via the generation of reactive oxygen species and the induction of a variety of genes to enhance the effects of other carcinogenic substances (Tharappel *et al.,* 2002). A number of studies to date have reported the potential association between polychlorinated biphenyls exposure and risk of various cancers, e.g. breast cancer (Clark and Snedeker, 2005), pancreas, liver and prostate cancer (Dharmani and Jaga, 2005) and cancers of the brain, kidney, leukemia and lymphoma (Infante-Rivard and Weichenthal, 2007).

The use of PCBs in Pakistan in transformer oil was estimated to be 1500 metric tons per annum with a demand of 4000 metric tons per annum (Khawaja, 2003). Approximately, 750,000 tons of PCBs are still present in the biosphere, which are responsible for the production of neurological, endocrinal and reproductive disorders in humans (James *et al.,* 2001). PCBs become more concentrated as they move upward through the food chain and results in an estimated one million times higher levels in the aquatic organisms than the aquatic environment (ATSDR, 2000). This result in chronic levels of PCBs at the high trophic levels of food chain like humans, and possibly enhances the chances of cancer risk in the target individual.

Prolonged and repeated exposure to carcinogens is one of the greatest risk factor in cancer development. Exposure to carcinogens, when combined with the effects of aging, causes an increase in the number of free radicals causing damage to DNA. Worldwide frequency of cancer in 2012 rose to an estimated 14 million new cases and it is predicted to rise to 22 million per year within the next two decades (Stewart and Wild, 2014). Reports on cancer incidence in Pakistan to-date has been evaluated only frequencies of various cancers, gender and age comparisons and types of cancers (Aziz *et al.,* 2003; Bhurgri, 2004; Bhurgri *et al.,* 2005, 2006; Ali and Baig, 2006; Hanif *et al.,* 2009; Imam *et al.,* 2009). Urban areas are more affected than rural areas. One reason behind this can be the higher exposure of citizens in cities to increased levels of chemicals and environmental pollutants. The mean ages of Pakistanis affected by cancers ranged between 40 and 50 years showing early onset of cancer compared with the developed countries.

The present study was aimed to ascertain the levels of PCBs in the cancer patients and healthy residents of Karachi City and to evaluate a possible association of PCBs in the development of various malignancies in humans. In light of the literature available outside Pakistan on the association of PCBs and risk of cancer, it was necessary to investigate the subject in Pakistan where these chemicals are in use for a long time. The studied cohort in the present work consisted of non-occupational subjects which give a true picture of the concentrations of these chemicals in human bodies, environmental status and the impaired health risks of these chemicals to the biodiversity. Enhancing public awareness of the lethal effects of PCBs is beneficial thereby increasing PCBs management, clean-up of contaminated sites and materials and proper disposal and dumping of these chemicals according to international guidelines.

The present work reaffirms the possible role of these chemicals in the etiology of various cancers as has been ascertained in a number of previous studies. Future work is recommended to know the exact mechanism of carcinogenicity and determination of threshold levels beyond which carcinogenicity can be caused in humans.

Materials and methods

Subjects and sample collection

Fasting blood samples were collected randomly in gel clot activator tubes from 83 diagnosed cancer patients and 32 healthy residents at various hospitals and localities of Karachi City. The studied cohort consisted of non-occupational subjects with no past history of exposure to polychlorinated biphenyls. Serum was separated within 2 hours of blood collection through centrifugation and the serum samples were stored at -20°C for subsequent analysis.

Analytical methods

Aliquot of 2ml serum was first equilibrated at room temperature and then extracted with organic solvents Methanol, n-Hexane and Diethyl Ether. The final 1ml extract of each sample was then mixed with H_2SO_4. All the chemicals used were of GC grade.

The cleaned-up samples were analyzed for pesticide residues through GC-ECD at PCSIR Laboratories Complex, Karachi. Extraction, cleanup and quantification of PCBs in the serum samples with the help of Gas Chromatography coupled with Electron Capture Detector (GC-ECD) was carried out in light of the previously described methods by Atuma and Aune, (1999) and De Caprio *et al.,* (2000). Guidelines of the USEPA methods were followed for the analysis of PCBs (USEPA Method 8082A), Florisil clean up (USEPA Method 3620C); Sulfuric Acid clean up (USEPA Method 3665A).

Standard operating procedure

Standard Mixtures of PCBs each of 0.1 % concentration and 1 µl volume were first processed through GC 17A, CLAURUS and Ni63 ECD consisting of a built-in Auto sampler. Helium (He) was used as the carrier gas in DB-5 column (30 m×0.32 mm ×0.25 µm).

GC was operated in Split mode, with temperature program of the Injector 250°C and Detector 325°C. Total Run Time was 58.67 min with Initial Temp of 120°C and Maximum Temp of 350°C. Standard chromatograms were obtained under standard operating conditions which were used as a reference for comparison of the samples. Sample extracts of 1µl each were processed through the same procedure under same operating conditions.

The time taken by the analyte from entrance into the column and reaching the detector is its retention time, which was used for the identification of the component chemicals in the analyzed samples. The obtained peak areas and peak heights in the chromatograms of each component of the analyte were used to quantify each individual chemical in the organic extract of each sample.

Results

Mean level of total PCBs in the normal samples was 0.536 mg/kg whereas in the cancer samples, the value was 2.711 mg/kg which is very high as compared with the normal samples. Concentration of PCB 180 (0.239 mg/kg) was the highest and PCB 209 (0.006 mg/kg) was the lowest in the normal samples (Table 1, Fig. 1).

PCB 52 was detected frequently in the normal samples (71.88%) while PCB 180 was detected with least frequency (6.25%). In cancer samples, PCB 52 was detected with highest mean concentration (2.044 mg/kg) and frequency of 84.33% while PCB 138 was the lowest detected congener (0.001 mg/kg) having frequency of 3.61% (Table 1, Fig. 1). The overall frequency of the total PCB congeners in cancer samples was 93.98 % which was comparable to 93.75%, found in the normal samples (Table 1).

Concentrations of PCB 52 and PCB 180 were significantly higher in the cancer cases compared with the control group. (Table 1, Fig. 1).

Table 1. Detected levels of PCBs in the normal and cancer samples.

	PCB Congener	Samples Tested	Positive Samples	Positive Test %	ΣX (mg/kg)	Mean (mg/kg)	SD	SE
PCBs in Normal Samples	PCB-28	32	17	53.12%	0.457	0.014	0.025	0.004
	PCB-52	-do-	23	71.88%	4.315	0.134	0.448	0.079
	PCB-101	-do-	13	40.62%	1.878	0.059	0.113	0.020
	PCB-138	-do-	3	9.38%	1.372	0.042	0.157	0.028
	PCB-153	-do-	5	15.62%	1.309	0.040	0.101	0.018
	PCB-180	-do-	2	6.25%	7.638	0.239	1.325	0.234
	PCB-209	-do-	8	25.00%	0.212	0.006	0.016	2.828
	ΣPCBs	32	30	93.75%	17.171	0.536	1.388	0.245
PCBs in Cancer Samples	PCB-28	83	64	77.10%	7.101	0.086	0.162	0.017
	PCB-52	-do-	70	84.33%	169.702	2.044	3.512	0.385
	PCB-101	-do-	16	19.28%	5.845	0.070	0.231	0.025
	PCB-138	-do-	03	03.61%	0.088	0.001	0.007	0.0007
	PCB-153	-do-	05	06.02%	1.095	0.013	0.053	0.005
	PCB-180	-do-	37	44.58%	40.155	0.483	1.613	0.177
	PCB-209	-do-	35	42.17%	1.039	0.012	0.028	0.003
	ΣPCBs	83	78	93.98%	225.095	2.711	3.709	0.407

Σ PCBs: Total sum of the seven tested PCB congeners; ΣX: Total sum of a particular PCB congener; SD: Standard Deviation; SE: Standard Error.

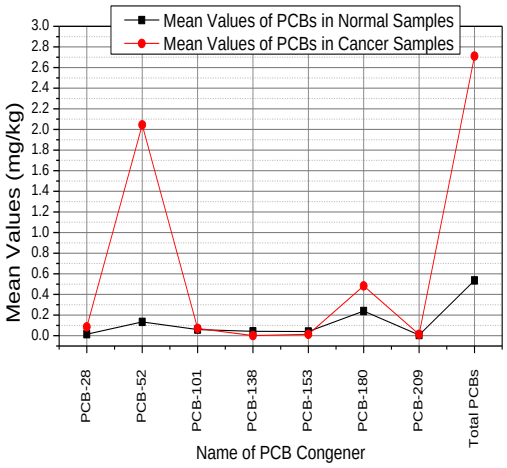

Fig. 1. Comparison of the Mean Values (mg/kg) of Σ PCBs in Normal and Cancer Samples.

The studied cohort was divided into five age groups: 15-28, 29-42, 43-56, 57-70 and 71-84 years. Order of the age groups in respect of the detected mean concentrations of total PCBs was: 29-42 > 43-56 > 71-84 > 57-70 > 15-28 years (Table 2, Fig. 2).

No linear correlation was observed between concentration of PCBs and increasing age in the normal samples. Individuals between 29 years and 56 years were having higher mean levels of total PCBs which show maximum bio-concentration of PCBs in this period of life in the normal subjects. Order of the age groups in respect of the mean concentrations of total PCBs in the cancer samples was 71-84 > 43-56 > 29-42 > 15-28 > 57-70 (Table 2, Fig. 2).

This shows a quite different pattern of PCBs deposition as compared with the normal samples. The age group 71-84 years has the highest mean values while the age group of 57-70 years has the lowest mean value of total PCBs. Except the age group 57-70 years, the remaining age groups have shown a linear ascending correlation with concentration of total PCBs.

Table 2. Comparison of Σ PCBs in the age groups of normal and cancer samples.

	Age Statistics		Total PCBs (ΣPCBs)		
	Age Groups (Yrs)	Mean Age (Yrs)	ΣX (mg/kg)	Mean (mg/kg)	SD
Normal Samples	15-28	22	3.606	0.212	0.328
	29-42	33.5	9.032	1.129	2.528
	43-56	47.3	3.261	1.087	0.982
	57-70	62	0.618	0.309	0.101
	71-84	79	0.654	0.327	0.208
	15-84	33.4	17.171	0.536	1.388
Cancer Samples	15-28*	19.8	53.627	2.438	2.981
	29-42	35.6	80.030	2.964	3.190
	43-56	47.7	66.445	3.322	5.470
	57-70	63.6	13.753	1.250	1.147
	71-84	76.3	11.240	3.747	2.668
	14-83	39.5	225.095	2.711	3.709

ΣX: Total detected PCBs in all samples of a particular age group.

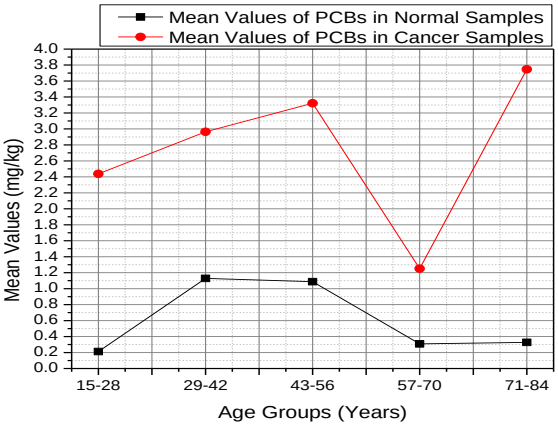

Fig. 2. Comparison of the Mean Values (mg/kg) of Total PCBs in the Age Groups of Normal and Cancer Samples.

The highest mean level of total PCBs was found in cancers of the female genital system (5.440 mg/kg) and the lowest in the brain tumor (Glioblastoma Grade-IV) as (0.470 mg/kg).

Mean levels of total PCBs in all of the ten analyzed major cancer sites were higher than the mean level detected in the normal group (0.536 mg/kg) except the brain tumor (Table 3, Fig. 3).

The 10 major cancer sites were further categorized into 25 sub sites. Highest concentration of mean total PCBs amongst the sub sites of cancers was found in CML (11.962 mg/kg) and lowest was found in the pharyngeal cancer as 0.005 mg/kg (Table 3, Fig. 3).

Table 3. Evaluation of ΣPCBs in the major and sub sites of cancer cases.

Major Cancer Site	Sub Site	Total PCBs		
		ΣX (mg/kg)	Mean (mg/kg)	SD
Oral Cavity & Pharynx	Tongue	10.475	3.491	4.895
	Pharynx	0.005	0.005	0
Total		10.480	2.620	4.500
Digestive System	Oesophagus	38.315	5.473	6.110
	Stomach	5.972	2.986	2.338
	Colon	15.066	2.511	3.558
	Rectum	19.851	4.962	2.268
	Bile Duct	1.220	1.220	0
	Pancreas	3.832	1.916	0.272
	Appendix	0.063	0.063	0
Total		84.319	3.666	4.337
Respiratory System	Larynx	5.964	1.192	1.422
	Lung	8.830	2.943	2.159
Total		14.794	1.849	1.931

Major Cancer Site	Sub Site	Total PCBs		
		ΣX (mg/kg)	Mean (mg/kg)	SD
Breast	Breast	10.641	1.064	0.749
Skin	Neck	0.950	0.236	0.342
	Cheek	12.053	2.410	2.410
	Abdomen	2.411	2.411	0
Total		15.414	1.541	2.021
Female Genital System	Cervix	15.496	5.165	1.889
	Ovary	7.945	7.945	0
	Vagina	3.762	3.762	0
Total		27.203	5.440	2.001
Brain	Glioblastoma	0.470	0.470	0
Bone	Ewing's Sarcoma	5.222	5.222	0
Blood	ALL	4.509	1.127	1.281
	AML	22.376	2.034	1.947
	CML	23.925	11.962	9.034
Total		50.810	2.989	4.829
Lymphatic System	HL	0.306	0.153	0.139
	NHL	5.436	2.718	1.595
Total		5.745	1.436	1.710

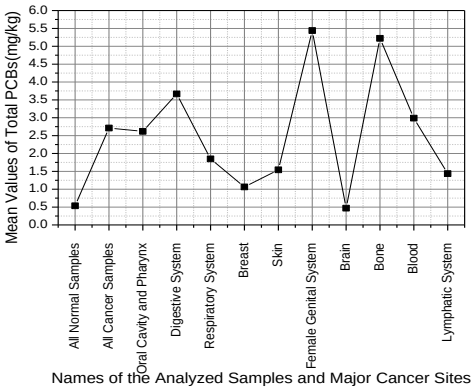

Fig. 3. Fig. 3. Comparison of the mean values (mg/kg) of total PCBs in all normal samples, all cancer samples and the major cancer sites.

PCB-28 and PCB 101 were detected in moderate concentrations while PCB 138 and PCB 153 were the least detected PCB congeners. PCB 52 was the most prevalent and ubiquitous congener while PCB 180 was the second highest congener in respect of the detected mean concentration in the cancer cases. Highest mean concentration and frequency of PCB 209 was found in cases of the female genital system.

Table 4. Mean values of individual PCB congeners in the major cancer sites

Type of Major Cancer Site	Mean values of individual PCB Congeners (mg/kg)						
	PCB-28	PCB-52	PCB-101	PCB-138	PCB-153	PCB-180	PCB-209
Oral Cavity and Pharynx	0.058	2.590	ND	ND	ND	0.005	0.014
Digestive System	0.083	2.387	0.128	0.003	0.023	1.030	0.011
Respiratory System	0.029	1.155	0.007	ND	ND	0.650	0.008
Skin	0.024	1.240	0.021	ND	ND	0.243	0.010
Female Genital System	0.128	4.678	0.051	ND	ND	0.559	0.024
Breast Cancer	0.171	0.882	0.004	ND	ND	0.003	0.002
Blood	0.076	2.648	0.134	0.0004	0.017	0.102	0.009
Lymphatic System	0.033	0.320	0.007	ND	0.063	1.007	0.004

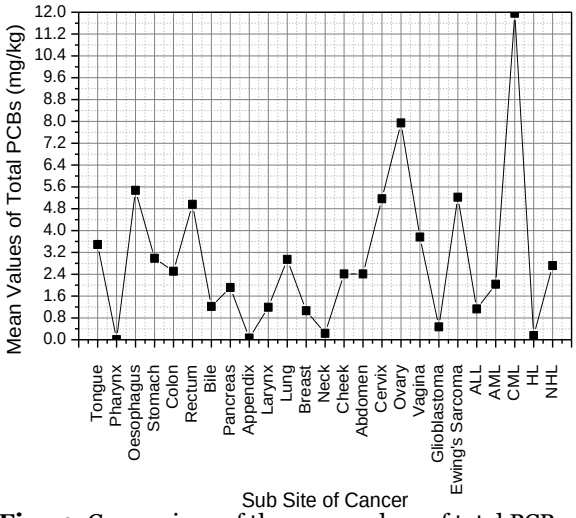

Fig. 4. Comparison of the mean values of total PCBs in the sub sites of cancer samples.

Discussion

The cancer types were categorized according to the categorization of the American Cancer Society, based on the affected major sites (systems) and sub sites (organs and tissues) of the body (Peterson, 2008). The studied cancer sites consisted of ten major cancer sites and twenty five sub sites (Table 3, Fig. 3).

Elevated concentrations of the tested PCB congeners were detected in the cancer cases as compared with the normal subjects. Mean level of total PCBs in the cancer versus normal group was 2.711 vs. 0.536 mg/kg. The overall detection of PCBs in the cancer and normal samples was 93.98 % and 93.75 % respectively. In a previous study, Hoppin *et al.* (2000) has also reported elevated concentrations of total PCBs in pancreatic cancer cases compared with controls (330 ng/g lipid *versus* 220 ng/g lipid). They have reported significant dose response relationship between PCBs and pancreatic cancer risk (*P* for trend < 0.001). In the present findings, mean level of PCB 153 was lower in both the cancer and control subjects and has shown a lower risk. Concentration of PCB 180 (0.483 mg/kg serum) was high in the cancer cases compared with the normal samples (0.239 mg/kg) and shows a correlation with the finding of Hoppin *et al.* (2000).

In another study, Weiderpass *et al.* (2000) has reported higher concentrations of PCBs in the endometrial cancer cases as compared with the normal samples.

Lipid adjusted concentrations of the marker PCBs in the cases versus controls were reported to be: PCB 28 (6.3 ng/g vs. 5.5 ng/g), PCB 52 (2.2 ng/g vs. 2.0 ng/g), PCB 101 (2.0 ng/g vs. 2.1 ng/g), PCB 138 (112.5 ng/g vs. 108.5 ng/g), PCB 153 (237.1 ng/g vs. 236.2 ng/g), PCB 180 (162.2 ng/g vs. 163.4 ng/g).

Although, concentrations of PCBs were comparatively higher in the cancer cases but no marked difference was found in the cases and control subjects. No association has reported by the authors between concentrations of PCBs and endometrial cancer risk. On the other hand, the concentrations of PCB congeners in the present study are much higher and indicate a marked difference between the cancer cases and controls. In this way, they seem to have a role in the risk of various cancers.

Lower chlorinated biphenyls have been associated with oxidative DNA damage in the human breast cancer tissue by producing free radicals (Oakley *et al.,* 1996). The presence of the lower chlorinated PCB congener, PCB 52 in high concentrations in the overall cancer cases indicate the role of PCB 52 in the DNA damage and may cause ultimately various malignancies. Khawaja *et al.* (2010) has reported residues of marker PCBs in the breast milk of healthy lactating women from Karachi. The present study has shown higher concentrations and frequencies of most of the PCB congeners in the studied cohort compared with the previous study of Khawaja *et al.* (2010). This shows that concentrations of PCBs are on the rise in the citizens of Karachi City particularly in the cancer cases.

Mean concentration of total PCBs in the studied age groups of cancer cases was found elevated as compared with the respective age groups of the normal subjects. No consistent pattern of PCBs deposition was found with increasing age in the normal samples. However, the cancer cases have shown a linear increase in concentration with increasing age except the age group 57-70 years. Age is an important confounding factor and has found to be significantly associated with increased deposition of PCBs (Glynn *et al.,* 2003).

In the present study, the increasing pattern in the levels of serum total PCBs in the cancer cases, with increasing age is indicative of the association of PCBs deposition with age and the consequent risk of various cancers in the target individuals.

In another case-control study, Ward *et al.* (2000), has reported increasing levels of PCBs in the breast cancer cases (mean age 41.1 years) and controls (mean age 41.2 years). Increase in concentrations of DDE and PCBs showed somewhat positive relationship in women older than 50 years, and negative relationship in women younger than 50 years. Cases and controls with mean age <50 years were having mean PCBs as 692 vs. 749.8 ng/g lipids. Cases and controls with age ≥ 50 years showed elevated concentrations of PCBs as 905.1 vs. 893.9 ng/g lipids respectively. Elevated concentrations of the tested chemicals in the breast cancer cases compared with the control subjects show acorrelation with our present findings. Mean age of the cancer cases in the present study was 39.9 years which is lower than the cases in the study of Ward *et al.* (2000). The early onset of cancer incidence in the citizens of Karachi reduces the life expectancy, which may be due to the deposition of high body burdens of PCBs in the human population.

An important observation was the presence of higher levels of total PCBs in all of the major cancer sites, except the brain tumor, compared with the normal samples (0.536 mg/kg). Individuals with higher levels of PCBs are at greater risk than those with lower concentrations of PCBs. The characteristic high levels of PCBs in cancer cases, compared with controls, has been reported by many researchers, in breast cancer (Stellman *et al.,* 2000; Laden *et al.,* 2001); NHL (Quintana *et al.,* 2004) etc.

Highest level of mean total PCBs was found in cases of Chronic Myeloid Leukemia (CML), as 11.962 mg/kg while lowest was found in pharyngeal carcinoma. Elevated concentrations of PCBs in the human body cause apoptosis of cells by damaging DNA and results in cancer (Ghosh *et al.,* 2010).

Dihydroxy metabolites of PCBs when oxidized by Cu (II) or peroxidease, result in oxidative DNA damage (Oakley *et al.,* 1996). The elevated levels of PCBs particularly lower chlorinated congener PCB 52 detected in the present study shows an association with the overall cancer risk by oxidative DNA damage and apoptosis of cells as reported in the cited study. PCB 101 and PCB 209 were detected in moderate concentrations while PCB 138 and PCB 153 were the least detected congeners. PCB 180 was the second highest detected congener.

Although many of the PCBs have been banned for decades, the detected concentrations are nevertheless of interest as high body burdens indicate presence of these chemicals in various environmental compartments and their possible side effects. Instead of occupational exposure, the present study was carried out in the general population, representing the level of risk associated with these chemicals.

A linear trend between increasing levels of PCBs and increasing age was observed in the cancer group, but no such trend was found in the normal subjects. PCBs have shown a positive association with the risk of cancer. The overall elevated concentrations of PCBs in the cancer group as a whole compared with the healthy subjects provide ample proof of a possible association of these chemicals with the risk of various cancers. Concentrations of PCBs seem to have resulted in lower life expectancy and early onset of cancer incidence (mean age 39.9 years) in the human population of Karachi. This was a preliminary work on the subject in Pakistan. With the collaboration of government and medical authorities, further research is recommended to explore the exact role and mechanism of PCBs in the etiology of various cancers.

Acknowledgements

We are thankful to Dr. Alia Bano Munshi, Chief Scientific Officer and Dr. Sohail Shaukat, Scientific Officer, for providing laboratory facilities and analytical support of the samples on GC-ECD at Center for Environmental Studies, PCSIR Laboratories Complex, Karachi.

References

Ali TZ, Baig S. 2006. Evaluation of a Cancer Awareness Campaign: Experience with a Selected Population in Karachi. Asian Pacific Journal of Cancer Prevention **7**, 391-395.

ATSDR. 2000. Toxicological profile for polychlorinated biphenyls (PCBs). Atlanta, GA, US Department of Health and Human Services, Agency for Toxic Substances and Disease Registry 765 pp.

ATSDR. 2014. Case Studies in Environmental Medicine, Polychlorinated Biphenyls Toxicity. Agency for Toxic Substances and Disease Registry, Course: WB 2460.

Atuma SS, Aune M. 1999. Method for the determination of PCB congeners and chlorinated pesticides in human serum. Bulletin of Environmental Contamination and Toxicology **62**,8-15.

Aziz Z, Sana S, Saeed S, Akram M. 2003. Institution based tumor registry from Punjab: Five year data based analysis. Journal of Pakistan Medical Association **53(8).**

Bhurgri Y, Bhurgri A, Pervez S, Bhurgri M, Kayani N, Ahmed R, Usman A, Hasan SH. 2005. Cancer Profile of Hyderabad, Pakistan, 1998-2002. Asian Pacific Journal of Cancer Prevention **6**, 474-480.

Bhurgri Y, Pervez S, Kayani N, Bhurgri A, Usman A, Bashir I, Ahmed R, Hasan, SH,Khurshid M. 2006. Cancer Profile of Larkana, Pakistan (2000-2002).Asian Pacific Journal of Cancer Prevention **7**, 518-521.

Bhurgri Y. 2004. Karachi Cancer Registry Data Implications for the National Cancer Control Program of Pakistan. Asian Pacific Journal of Cancer Prevention **5**, 77-82.

Clark HA, Snedeker SM. 2005. Critical evaluation of the cancer risk of Dibromochloropropane (DBCP). Journal of Environmental Science and Health, Part C, Environmental Carcinogenesis and Ecotoxicology Reviews **23**, 215-260.

DeCaprio AP, Tarbell AM, Bott A, Wagemaker DL, Williams RL, O'Hehir MO. 2000. Routine analysis of 101 polychlorinated biphenyl congeners in human serum by parallel dual-column gas chromatography with electron capture detector. Journal of Analytical Toxicology **24**, 403-420.

Dharmani C, Jaga K. 2005. Epidemiology of acute organophosphate poisoning in hospital emergency room patients. Reviews in Environmental Health **20**, 215-232.

Ghosh S, De S, Chen Y, Sutton DC, Ayorinde FO, Dutta SK. 2010. Polychlorinated biphenyls (PCB 153) and (PCB 77) absorption in human liver (Hep G2) and kidney (HK2) cells in vitro: PCB levels and cell death. Environment International **36**, 893-900.

Glynn AW, Granath F, Aune M, Atuma S, Darnerud PO, Bjerselius R,Vainio H, Weiderpass E. 2003. Organochlorines in Swedish Women: Determinants of serum concentrations. Environmental Health Perspectives **111**, 349-355.

Hanif M, Zaidi P, Kamal S, Hameed A. 2009. Institution-based Cancer Incidence in a Local Population in Pakistan: Nine Year Data Analysis. Asian Pacific Journal of Cancer Prevention **10**, 227-230.

Hoppin JA, Tolbert PE, Holly EA, Brock JW, Korrick SA, Altshul LM, *et al.* 2000. Pancreatic Cancer and Serum Organochlorine Levels. Cancer Epidemiology Biomarkers and Prevention **9**, 199-205.

HSDB. 2003. Polychlorinated Biphenyls. Compiled by the National Library of Medicine. Hazardous Substance Data Bank: http://csi.micromedex. com/ Data/HS/HS3945H.htm.

Imam SZ, Rehman F, Zeeshan MM, Maqsood B, Asrar S, Fatima N, Aslam F, Khawaja MR. 2009. Perceptions and Practices of a Pakistani Population Regarding Cervical Cancer Screening. Asian Pacific Journal of Cancer Prevention **9**, 42-44.

Infante-Rivard C, Weichenthal S. 2007. Pesticides and childhood cancer: An update of Zahm and Ward's 1998 review. Journal of Toxicology and Environmental Health, Part B, Critical Reviews **10**, 81-99.

James MO, Robertson Hansen. 2001. Polychlorinated biphenyls: metabolism and metabolites. The University Press of Kentucky p. 36-46.

Khawaja MA. 2003. Polychlorinated biphenyls (PCBs) problem in Pakistan. SDPI Research News Bulletin (May - June 2).

Khawaja S, Yousuf MJ, Khan AJ. 2010. Polychlorinated Residues in Milk of Lactating Women from Karachi, Pakistan. Journal of Basic and Applied Sciences **6**, 153-157.

Laden F, Collman G, Iwamoto K, Alberg AJ, Berkowitz GS, Freudenheim JL *et al.* 2001.1, 1-Dichloro-2,2-bis (*p*-chlorophenyl) ethylene and Polychlorinated Biphenyls and Breast Cancer: Combined Analysis of Five U.S. Studies. Journal of Natural Cancer Institute **93**, 768-76.

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

Peterson KR. 2008. "Cancer (medicine)." Micros oft® Encarta® 2009 [DVD]. Redmond, W.A., Microsoft Corporation 2008.

Quintana PJE, Delfino RJ, Korrick S, Ziogas A, Kutz FW, Jones EL *et al.* 2004. Adipose Tissue Levels of Organ chlorine Pesticides and Polychlorinated Biphenyls and Risk of Non-Hodgkin's Lymphoma. Environmental Health Perspectives **112**, 854-861.

Stellman SD, Djordjevic MV, Britton JA, Muscat JE, Citron ML, Kemeny M, Busch E, Gong L. 2000. Breast Cancer Risk in Relation to Adipose Concentrations of Organ chlorine Pesticides and Polychlorinated Biphenyls. Cancer Epidemiology Biomarkers and Prevention **9**, 1241-1249.

Stewart BW, Wild CP, editors 2014. World Cancer Report, 3rd February, 2014 by International Agency for Research on Cancer (IARC). Lyon, France.

Tharappel JC, Lee EY, Robertson LW, Spear BT, Glauert HP. 2002. Regulation of cell proliferation, apoptosis and transcription factor activities during the promotion of liver carcinogenesis by polychlorinated biphenyls. Toxicology and Applied Pharmacology **179**, 172-184.

USEPA Method. 3620C 2007. Florisil cleanup. United States Environmental Protection Agency, Revision 3, CD-ROM pp. 1-27.

USEPA Method. 3665A 1996. Sulfuric acid Permanganate cleanup. United States Environmental Protection Agency, Revision 1, CD-ROM. pp 1-5.

USEPA Method. 8082A 2007. Polychlorinated biphenyls (PCBs) by gas chromatography. United States Environmental Protection Agency, Revision 1, CD-ROM pp. 1-56.

Ward EM, Schulte P, Grajewski B. 2000. Serum Organ chlorine Levels and Breast Cancer: A Nested Case-Control Study of Norwegian Women. Cancer Epidemiology Biomarkers and Prevention **19**, 1357-1367.

Weiderpass E, Adami H, Baron JA, Wicklund-Glynn A, Aune M, Atuma S, Ingemar PI. 2000. Organochlorines and Endometrial Cancer Risk. Cancer Epidemiology Biomarkers and Prevention **9**, 487-493.