

Polychlorinated biphenyl congeners (PCBs) in nursing primiparous and multiparous women

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ABSTRACT

Impact of PCBs was studied as a major source of physiological effects even very low concentration of PCBs transferred to the infants by mother's milk. Milk samples were collected from primipara and multipara women. A significant variation pattern was observed in the level of PCB congeners, as PCBs are lipophilic in nature, another possibility arises that great deals of residues are passed on to infants through mother's milk. A technique was developed to find out polychlorinated biphenyls (PCB) in breast milk. PCBs were extracted by Matrix Solid-Phase Dispersion (MSPD) and analyzed by Gas Chromatography with Electron Capture Detector. The precision (RSD < 10%, n = 125), recovery (85% to 110%) and limit of quantification (between 0.50 and 3.00 $\mu\text{g}\cdot\text{L}^{-1}$), the chi square analysis at $p \leq 0.05$ has shown that the PCB level was higher in multipara as compared to primipara. The analysis of the thirty samples revealed PCB levels above 6.85 $\mu\text{g}\cdot\text{L}^{-1}$ in breast milk samples by analyses of a mixture of PCB congeners. All PCB congeners (28, 52, 101, 123 (+149), 118, 114, 153, 105, 138 (+163), 167, 156 (+171), 157, 180, 170, 189) were found at high level in primiparous and multiparous. Thus, a high correlation between the contamination of breast milk and environmental pollution of PCBs was observed.

Keywords: Poly Chlorinated Biphenyls; Primipara; Multipara; Breast Milk; Contamination; MSPD and ECD; Pakistan

1. INTRODUCTION

The physiological impact of polychlorinated biphenyls (PCBs) has been studied in humans and in animals. The

prime focus in human studies has been on the effects in neonates, women and infants whereas research has also done on adults. According to the National Health and Nutrition Examination Survey (NHANES) concentration of PCBs was examined in serum and lipid content of people who were above twelve years of age.

Polychlorinated biphenyls (PCBs) are lipophilic substance. Humans are exposed to these persistent compounds from different sources. PCBs have been banned for 30 years, but are still found in telephone as dielectrics and pole mounted transformers. Exposure pathways for PCBs, as with most persistent organic pollutants, begin in the outdoor environment [1]. PCBs are characterized by its capacity for bio-accumulation, toxic effects and long distance atmospheric transport. Therefore, they were also present in those places where they have not been used [2], and a number of PCBs congeners have been determined in milk samples [3]. Acute prenatal exposure of PCBs resulted in intrauterine growth retardation, reduced birth weight, delayed developmental milestones, and other abnormalities in infants and children [4,5]. A serious impact of PCBs exposure on human neurodevelopment has been observed [6].

In humans, serum levels of PCBs caused increase in several inflammatory diseases such as infection, sepsis, septic shock and many others [7]. In the similar manner the PCBs are deposited in mother's milk and transferred to their young children. It indicates that the new born may receive the PCB concentration ratio at a greater level as compared to fish and other food items [8].

The current state of knowledge suggests that low-level exposures to PCBs are unlikely to cause any adverse health effects. People at greater risk include Aboriginal peoples, as well as anglers and hunters and their families with the fact that not all of the 209 kinds of PCB have the same effects [9].

Although their use has been restricted in Pakistan decades ago, but PCBs lipophilic nature, these toxic and

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hazards chemicals easily entered into the food chain. As a result mean concentration has been reported in food, blood human tissues and breast milk [10-12]. Recent research in Pakistan has given due importance on the examination and quantity of climatic conditions in major cities populations for determination of impact of industrial pollution.

In Pakistan such studies are scanty and no comprehensive study was conducted therefore the reliable data is not normally available to carry out environmental health and risk assessment. In this study, the residues of PCB were analyzed in women milk samples which were collected through women populace of Karachi. Our object was to measure the concentration level of PCB residues in lactating women those who are indirectly exposed from these organic compounds. Present investigation will provide useful data for upcoming researchers. Wide research has been done globally but in Pakistan no studies have been carried out to create awareness in the society. The usages of PCBs are banned but present findings significantly show that these toxic compounds are still in practice illegally.

Objective

To assess level of Polychlorinatedbiphenyl (PCBs) and their effects transferred to infants through mother milk were studied associations with their health hazards. This type of study has never done in Pakistan previously. This integration of data from different sources with a mathematical model allows us to assess infant's disease levels, immunological effects and overall uncertainty in predicted risks. The uncertainties resulting from the assessments of exposure and pollution status of PCBs are propagated through the risk characterization process.

2. MATERIALS AND METHODS

2.1. Study Population and Sampling

2.1.1. Milk Donors

The pregnant women who came to register their names for delivery in Karachi Gynae hospitals were divided into two groups multiparous and primiparous. They had been asked about their consent for the milk sample collection. They had been provided a questionnaire for this purpose. After a brief history taken from these women they were followed for their expected days of delivery. They usually showed hesitation in providing the milk sample but the staff nurse helped in this purpose. The breast pump had been provided to the women and lady nurse collected their samples in sterilized vials of 10 ml.

2.1.2. Analytical Method

Breast milk samples were brought to lab and stored at -30°C till further analysis.

1) Extraction

Milk sample was extracted through matrix solid-phase dispersion (MSPD) in manifold assembly. The 1-mL breast milk sample was pipette into atared 10-mL conical centrifuge tube and the weight was recorded. The sample was spiked with labeled internal standard to give a concentration of 200 pg/g in the milk and mixed allowing enough time for the internal standards to reach equilibrium with the milk components [13].

2) Instrumentation

Analyses were performed by gas chromatography using Perkin Elmer GC-Calrus 500. The standard PCB congeners were purchased from Supelco Company. This standard mixture of PCB was injected into GC (ECD) to get the standard mixture peaks for comparison. Concentration level of PCBs were determined analyzed using Perkin Elmer Gas chromatograph Clarus 500 equipped with electron capture detector (ECD), 300°C , mode: constant column + makeup flow, combined flow: 60 ml/min, make up gas type: nitrogen; inlet: operated in split less mode, initial temp: 200°C , pressure: 17.38 psi, purge flow: 15 ml/min, total flow: 19.2 ml/min; Oven: initial temp: 100°C , hold time: 5 min, ramp at $4.0^{\circ}\text{C}/\text{min}$ to 220°C , two capillary columns were used for initial injections (calculations) and for confirmation injections (validate initial injection of pesticides).

a) Initial injections: RTX5 w/Integra Guard, $30\text{ m} \times 0.25\text{ mm} \times 250\text{ }\mu\text{m ID} \times 0.25\text{ }\mu\text{m}$ film thickness, constant pressure @ 17.38 psi, nominal initial flow: 33 cm/sec.

b) Confirmation injection: RTX35 w/Integra Guard, $30\text{ m} \times 0.25\text{ mm} \times 250\text{ }\mu\text{m ID} \times 0.25\text{ }\mu\text{m}$ film thickness constant pressure @ 17.38 psi, nominal initial flow: 33 cm/sec.

It is general practice in pesticide Labs. of CES to use different chromatographic columns for quality assurance. As far as the procedure was carried out for each batch of 6 samples calibration and its authenticity were regularly observed during the start and end. To check the quality control methods the use of reagent blanks surrogate and matrix spike recovery was taken into consideration. For each sample batch there was one procedural blank, one laboratory control sample and a duplicate of sample. The procedural blank was spiked with the solvent and a surrogate internal standard PCBs congeners 198 and 194. All samples were spiked with the surrogate compound to determine efficiency. Results are calculated on the basis of the mean value of samples. Internal spiking and reagent blank determined the recovery values. The recovery values were in the range 92% - 157% for PCBs. The limit of quantification was calculated on the basis of % RSD and it was 0.001 - 0.01 $\mu\text{g}/\mu\text{l}$ and limit of reportable amount was obtained by multiplying with 3 and it was 0.003 $\mu\text{g}/\mu\text{l}$ or 0.3 $\text{ng}/\mu\text{l}$ PCB congeners.

3) Statistical Analyses

A descriptive analysis was conducted for all PCBs. Arithmetic means and standard deviation (SD) were calculated for normally distributed variables while geometric means and 95% confidence interval are presented for log transformed variables. The chi square test was applied for comparison between primipara and multipara women. A $p < 0.5$ was taken statistically significant. The simple relationship between PCBs load and milk was studied using analysis of variance while the analysis of covariance (ANOVA) was applied in order to adjust for confounders. The analyses were carried out using the SAS software (version 9.1; SAS Institute Inc., Cary, NC, USA).

3. RESULTS

The detection of PCB congeners in all the samples analyzed indicates the spatial distribution in Karachi women. Multiparous women were found to have more PCB concentration than primiparous women. The results indicated the remarkable difference between the two groups of women. PCB 28 was entirely absent in primiparous women on the other hand PCB 138 was not detected in any of the milk samples from multiparous women. By looking into the individual concentration of each PCB the primiparous milk samples were found to have the concentration of PCB congeners as N.A, 0.058 ng/ml, 0.017 ng/ml, 0.006, 0.008 ng/ml, 0.083 ng/ml and N.A for PCB 28, 52, 101, 138, 153, 180 and 209 respectively (**Table 1**). On the other hand mean values for the same PCB congeners in multiparous milk samples were detected as 0.006 ng/ml, 0.025 ng/ml, 0.001 ng/ml, N.A, 0.003 ng/ml, 0.244 ng/ml and 0.166 ng/ml (**Table 2**).

The sum of mean values *i.e.*, Σ PCB in multiparous women was found to be 0.445 ng/ml where as the Σ PCB in primiparous women samples was 0.172 ng/ml. The one PCB congener *i.e.* PCB 209 make the difference as this PCB concentration was entirely absent in primipara samples whereas it was detected in the highest concentration in multiparous women milk samples as 0.166 ng/ml.

4. DISCUSSION

To best of our knowledge this study was the first to elucidate the concentration status of PCB congeners in women subject of Karachi. From the standpoint of Asian countries the reports are available from Philippines [14] and Japan [15].

According to [14] polychlorinated diphenyl ethers and hexabromocyclododecanes had no significant relationship between primiparous and multiparous women with reference to its status of concentration. They have covered a range of POPs accumulation in breast milk of

women in addition to PCB congeners. The primiparous mothers had significantly higher organochlorinated insecticides such as DDTs, CHLs and HCHs than multiparous women and no such trend was reported with reference to PCB congeners determined a range of PCBs in blood and milk samples of mothers [16]. They reported the mean levels of many PCB congeners as 7.1, 2.7, 5.3 and 0.4 pg in primiparous mothers. The present investigation concerning with the milk samples only as the multiparous women samples were found to have mean values as 0.445 ng/ml (Σ PCB) and primiparous women milk samples indicated Σ PCB value as 0.172 ng/ml. Our investigation indicate that multiparous women remain at high risk of PCB transfer from their breast milk to the infants than primiparous women was based on findings from 30 mothers which includes 15 primiparous and 15 multiparous mothers (**Figure 1**).

It is known that the level of PCB congeners reflects the mother's exposure to these chemicals during their life time. Mean concentrations of the sum of PCBs in the analyzed milk samples seems to be relatively low as 114.8 ng/g in primipara and 101.8 ng/g in secundipara [17]. Mean concentration of these chemicals shows an incline trend of the persistent organic pollutants in multiparous women residing in Karachi since the time when chemicals like PCB were ban and restricted.

The concentrations of the PCBs [138, 153 and 180] in first-time pregnant female and multiparous women reported to be higher than those for pregnant serum females. A research report conducted by showed the PCB congeners in women in the following order as PCB 138 (11.2 ng/g), PCB 153 (14.7 ng/g) and PCB 180 (8.3 ng/g) for primiparous women and PCB 138 (6.1 ng/g), PCB 153 (5.3 ng/g) and PCB 180 (4.5 ng/g) for multiparous women [18].

The previous results clearly indicate that PCB-153 was higher than PCB-28 in primiparous [19] same as present investigation.

Present results indicate that residues of PCBs in breast milk of women do not reliably decrease over the number of child or course of lactation. This is contrast with a more recent study on depuration of PCBs in milk [20].

It could be assumed from the above results that in the developed countries rate of persistency of POP's are relatively reduced in human biological materials which may be due to the awareness of hazardous effects of PCBs or strict implementation of law in terms of use of such banned chemicals.

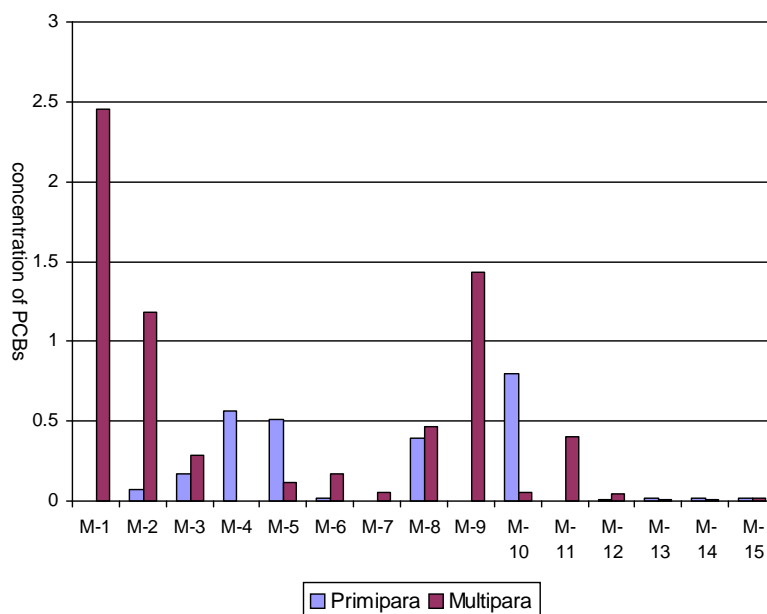
Present investigation shows that human milk samples were highly contaminated with PCBs. Their concentration was found to be comparatively higher than previous finding. The use of such industrial chemicals should be strictly monitored, as their deposition may increase the environmental contamination. The disposal of PCB con-

Table 1. Statistical analysis of PCB residues (ng/ml) in milk samples of primipara women.

Name of PCBs	Positive samples	Positive test (%)	Mean	SD	SE	Range min - mix
PCB 28	00	00	00	00	00	00
PCB 52	5	33	0.058	0.236	0.063	0.484 - 11.507
PCB 101	5	33	0.017	0.128	0.034	1.599 - 4.943
PCB 138	2	13	0.006	0.077	0.020	1.387 - 2.530
PCB 153	5	33	0.008	0.089	0.023	1.516 - 3.036
PCB 180	6	40	0.083	0.0213	0.057	2.464 - 3.306
PCB 209	00	00	00	00	00	00
Σ PCB			0.172			

Table 2. Statistical analysis of PCB residues (ng/ml) in milk samples of multipara women.

Name of PCBs	Positive samples	Positive test (%)	Mean	SD	SE	Range min - mix
PCB 28	4	27	0.006	0.081	0.021	1.425 - 2.565
PCB 52	7	46	0.025	0.157	0.040	1.425 - 6.175
PCB 101	4	27	0.001	0.041	0.010	0.855 - 1.045
PCB 138	00	00	00	00	00	00
PCB 153	4	27	0.003	0.059	0.015	1.140 - 1.710
PCB 180	9	60	0.244	0.219	0.056	17.86 - 28.50
PCB 209	4	27	0.166	0.328	0.084	7.790 - 23.75
Σ PCB			0.445			

**Figure 1.** Bar graph showing total PCB concentration in 15 primipara and 15 multipara.

centration as a waste product from some industries may increase the risk of its entry in human body.

In the city of Karachi there are large area used as a waste source. Municipal wastes are burned under low temperature. It leads to the formation of PCBs under low temperature. The pollution near the wastage sites are

likely to be more infected with the PCB concentrations as compared to those living away from such sites. Further, the PCBs come from electric appliances like tube lights and also in materials like carbon paper and plastic products which were dumped in considerable quantities daily.

It can be anticipated that if the same situation/condition continues in future then the pollution may rise more and hence the concentration levels in human biological materials may also high because the release of these contaminants are not at all controlled even now.

5. CONCLUSION

Our PCB contamination is a public health crisis which has been ignored far too long. All samples were found to be contaminated with PCB residues which are no longer used in Pakistan.

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LIST OF ABBREVIATIONS

PCB: polychlorinated biphenyls
SD: standard deviation deviation
GC: gas chromatograph
pg: picogram

ml: milliliter
ng: nonogram
LOQ: limit of quantitation
ECD: electron capture detector