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Polychlorinated Biphenyls in Human Amniotic Fluid

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Polychlorinated biphenyls (PCBs) are a group of chlorinated synthetic compounds that have had wide spread industrial application especially in the electrical industry (Miller 1983). Evidence of its formation in the environment by the photooxidation of organochlorine pesticides into lower chlorinated PCBs is available (Maugh 1973). Wasserman et al. (1982) reported high concentration of PCBs in the plasma of women who had premature delivery. High concentration of PCBs have also been reported in women with repeated abortions (Bercovici et al. 1982). Evidences are available from the studies conducted on the Yusho patients that the PCBs are transferred to the foetus through the placenta (Yamashita and Hayashi 1985). This will pose a grave danger for the developing embryo inside the mothers womb. Since there are not many reports available on the analysis of PCBs in the amniotic fluid, it was thought to analyse some samples of amniotic fluid obtained from random population who do not have record of any previous exposure to PCBs.

This paper reports the levels of PCBs in the 26 samples of amniotic fluids samples obtained from maternity hospitals from the women during normal delivery.

MATERIALS AND METHODS

Hexane-double distilled (Ranbaxy), anhydrous sodium sulfate, aluminium oxide, sulphuric acid, KOH pellets from Sarabhai chemicals (A.R. grade), ethyl alcohol-double distilled, silica gel (Acme chemicals, A.R. grade), Pyrex glass columns (30 cm X 10 mm).

10 ml of amniotic fluid samples from each women was collected in screw cap glass tubes.

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For the extraction of PCBs, the method of Heeshane et al (1983) was follwed with slight modifications. Each sample was treated with 5 gm of KOH pellets and 3 mL of absolute alcohol and cyclomixed until the KOH pellets dissolved. The contents were kept in the waterbath at 90°C for one hour with periodic shaking to enhance the evaporation of ethanol. At the end of the saponification the tubes were allowed to cool and 10 ml of analytical grade hexane was added to each tube and cyclomixed. The organic layer was then allowed to separate.

The hexane layer was then washed twice with 15 mL of distilled water in a separating funnel of 150 mL by vigorous shaking. The two layers were allowed to separate, which was aided by addition of a drop or two of concentrated sulfuric acid and gentle shaking. The hexane layer was separated out and to which 3 mL of concentrated sulfuric acid was added and cyclomixed. Finally the hexane extract obtained was washed with 10 mL distilled water and concentrated in a dry testtube to 1 mL by the gentle flow of nitrogen.

The concentrated hexane extract was subjected to clean-up on silica gel-alumina column. 4.5 gm of activated silica gel was packed into a column of 30 cm X 10 mm dimension. The silica gel was topped with 2 gm of aluminium oxide and 3 cm layer of anhydrous sodium sulfate. The packed column was washed with 15 mL hexane. After washing the hexane layer was allowed to reach the top of the anhydrous sodium sulfate when the sample was loaded and allowed to get absorbed into the sodium sulfate layer. Elution was next started with hexane and the first 10 mL eluant was discarded. The next 50 ml eluant was collected. The eluant was concentrated to 1 mL in rotary flash evaporator. The concentrated extract was analysed by gas chromatography equipped with Ni^{63} electron capture detector.

The gas chromatograph used was Sigma 3B model of Perkin-Elmer fitted with 6 ft X 2.0 mm glass column packed with 3% SE-30 on 80-100 chromosorb WAW. The carrier gas used was nitrogen with a flow rate of 35 mL/min. Temperature parameters were 210°C(column), 250°C (injector) and 300°C(detector).

Quantification of PCB residues was done by comparing respective total areas of PCB peaks in the sample with the total area of chromatogram of Aroclor 1260.

RESULTS AND DISCUSSION

Table 1. Range and Mean PCB concentration in 26 human amniotic fluid samples.

No. of Samples with PCB	PCB conc. range in ppm	Mean ± S.D ppm	Percent incidence
26	0.001 - 1.162	0.131 ± 0.026	100

From Table 1 it is quite evident that the highest concentration was found to be 1.162 ppm and lowest was 0.001 ppm with a mean concentration of 0.131 ± 0.026 ppm.

Table 2. Percent incidence of PCB contamination of 26 human amniotic fluid under specific concentration range.

PCB range in ppm	No. of Samples	Average Mean ± S.D ppm	Percent incidence
0.001 - 0.3	24	0.134 ± 0.027	92.30
0.3 - 0.5	1	0.477	3.84
1.0 - 1.5	1	1.162	3.84

From Table 2 it can be seen that 92% of the amniotic fluid samples had PCB levels between 0.001-0.3 ppm with a mean value of 0.134 ± 0.027 ppm, while 3.84% each of other samples had between 0.3-0.5 ppm and 1.0-1.5 ppm PCB respectively, suggesting very high levels in the fluid surrounding the foetus.

The only study of the levels of PCBs in amniotic fluids is that of Genji et al (1979). They reported a mean concentration of 0.0006 ppm PCB in amniotic fluids of women from Nagasaki city of Japan. In comparison to this mean concentration of PCB in the amniotic fluid samples of the study is over 200 times higher than those found in the Nagasaki women. Genji et al (1979) argued that, eventhough the concentration of PCB found in the amniotic fluid samples is quite low, intrauterine exposure may be significant for several reasons, such as small size of the embryo and large volume of the fluid. Finally, intrauterine exposure occurs during a period when the embryo is small and has been found to be vulnerable to a variety of teratogens and lacks protective barriers that are found postnatally such as the fat deposits that might absorb the larger portion of these residues.

High levels of PCBs in amniotic fluids of pregnant mothers strikes a warning note to the expectant mothers whose fetuses may be affected by these chemicals.

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