Organochlorine pesticides and preterm labour in human beings

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In this study, we compare organochlorine pesticides, viz. aldrin, isomers of HCH, metabolites of DDT and heptachlor in the circulating blood, cord blood and placenta of pregnant women undergoing full term normal delivery with those in premature labour. The data and the statistical outcome of the study show that the mean concentrations of organochlorine pesticides in maternal blood of cases of premature labour were not found to be significantly different from those of controls (full term normal delivery). Similar results were obtained from the residue levels of cord blood and placental tissue. An attempt has also been made to correlate the high concentration of chlorinated hydrocarbon pesticides with the initiation of premature labour on the basis of existing evidences.

THE widespread use of organochlorine compounds as insecticides during the past few decades has led to their ubiquitous presence in the environment. These compounds are highly lipid soluble and are resistant to environmental degradation.

In human beings these compounds are stored in fat-rich tissues and are resistant to metabolism. These substances are present in women and the foetus is exposed during *in utero* development by transplacental transfer. Exposure of the foetus is related to the maternal body burden. Several studies have reported values of organochlorine pesticides in maternal blood and cord blood[1–8]. Relatively high residue levels of organochlorine pesticides have been reported in women with premature delivery[9–11]. Also DDT residue levels were higher in California sea lions which gave birth prematurely than in those with full term pups[12]. Organochlorine pesticides may disturb the hormonal balance of pregnancy and perhaps precipitate labour. Some DDT analogs (p-p′-DDT and o-p′-DDT) are reported to have oestrogenic effect[13,14]. It is also reported that oestrogen and progesterone metabolism is greatly enhanced by the hepatic microsomal xenobiotic metabolizing enzymes stimulated by chlorinated hydrocarbons[11]. Thus, an elevated level could theoretically cause premature labour by decreasing the amount of available progesterone which maintains the homeostasis of pregnancy. The report further states that DDT and p-p′-DDE were found in all cases of abortion but no correlation was found between the accumulation of organochlorine pesticides and incidence of labour[11]. In the present study aldrin, HCH and its isomers, metabolites of DDT and heptachlor in the maternal blood, cord blood and placenta of pregnant women undergoing full term normal delivery are compared with those in premature labour.

The women subjects, 19 to 34 years of age admitted into Mahila Chikitsalya, attached to Department of Obstetrics and Gynaecology, SMS Medical College, Jaipur were used in the present study. Eight women went into labour during 24–32 weeks of gestation and were considered as cases of premature birth. The remaining 22 women had normal full term labour and were used as controls. In general they had no history of any occupational or accidental exposure to pesticides. However, they were asked to fill up a questionnaire incorporating

Table 1. Levels of organochlorine pesticides (ppb) (mean, S. E. and range) in maternal blood of women undergoing full term labour and women who had premature labour

Organochlorine pesticide compounds	FTND (n = 22)			PL (n = 8)			Statistical significance
	Mean ± S.E.	Range and no. of positive samples		Mean ± S.E.	Range and no. of positive samples		
α-HCH	124.7 ± 19.31	(14.0–280.0)	(n = 20)	107.1 ± 39.49	(18.0–286.0)	(n = 7)	NS
γ-HCH	62.4 ± 23.94	(9.0–491.0)	(n = 20)	22.0 ± 5.43	(14.0–38.0)	(n = 4)	NS
β-HCH	126.6 ± 60.11	(18.0–954.0)	(n = 15)	96.2 ± 21.28	(47.0–151.0)	(n = 4)	NS
Heptachlor	1582.8 ± 315.36	(103.0–2563.40)	(n = 15)	1031.8 ± 320.99	(154.0–2555.0)	(n = 7)	NS
Aldrin	182.4 ± 26.35	(2.0–352.0)	(n = 20)	95.8 ± 46.67	(4.6–351.0)	(n = 7)	NS
Heptachlor epoxide	860.2 ± 224.97	(46.0–3653.0)	(n = 22)	564.6 ± 452.55	(46.0–2362.0)	(n = 5)	NS
p-p′-DDE	83.5 ± 14.60	(7.0–222.0)	(n = 21)	81.8 ± 26.88	(11.0–194.0)	(n = 7)	NS
p-p′-DDD	16.9 ± 4.69	(0.001–47.0)	(n = 12)	30.5 ± 15.54	(15.0–46.0)	(n = 2)	NS
p-p′-DDT	35.0 ± 8.68	(1.0–112.0)	(n = 16)	32.1 ± 18.667	(1.0–102.0)	(n = 6)	NS
Σ HCH	245.6 ± 56.12	(69.0–1077.0)	(n = 22)	125.5 ± 33.39	(30.0–312.0)	(n = 8)	NS
Σ Heptachlor	1911.9 ± 425.71	(46.0–8875.0)	(n = 22)	842.7 ± 304.64	(45.0–2825.0)	(n = 8)	NS
Σ DDT	120.2 ± 19.03	(10.0–330.0)	(n = 21)	88.5 ± 15.87	(27.0–145.0)	(n = 8)	NS
Σ OCP	2420.2 ± 465.73	(10.0–9698.0)	(n = 22)	1083.0 ± 310.18	(332.0–3108.0)	(n = 8)	NS

FTND, Full term normal delivery; PL, preterm labour; Σ HCH, Total HCH equivalent; Σ Heptachlor, Total Heptachlor equivalent; Σ DDT, Total DDT equivalent; Σ OCP, Total organochlorine pesticides equivalent; NS, Not significant.

Table 2. Levels of organochlorine pesticides (ppb) (mean, S. E. and range) in cord blood of women undergoing full term labour and women who had premature labour

Organochlorine pesticide compounds	FTND (n = 22)			PL (n = 8)			Statistical significance
	Mean ± S.E.	Range and no. of positive samples		Mean ± S.E.	Range and no. of positive samples		
α-HCH	150.8 ± 19.39	(5.0–508.0)	(n = 21)	189.2 ± 50.92	(52.0–454.0)	(n = 7)	NS
γ-HCH	38.6 ± 7.57	(1.0–95.0)	(n = 27)	40.0 ± 5.78	(22.0–56.0)	(n = 5)	NS
β-HCH	167.2 ± 119.02	(2.0–1945.0)	(n = 16)	98.1 ± 23.57	(20.0–187.0)	(n = 6)	NS
Heptachlor	1420.9 ± 193.62	(90.0–2586.0)	(n = 15)	1328.1 ± 677.47	(176.0–5484.0)	(n = 7)	NS
Aldrin	129.2 ± 35.44	(3.0–481.0)	(n = 21)	95.7 ± 28.04	(6.0–236.0)	(n = 7)	NS
Heptachlor epoxide	864.8 ± 219.14	(15–3169.0)	(n = 16)	650.1 ± 158.42	(201.0–1188.0)	(n = 6)	NS
p-p′-DDE	64.6 ± 15.11	(6.0–211.0)	(n = 20)	81.3 ± 18.77	(16.0–195.0)	(n = 8)	NS
p-p′-DDD	57.6 ± 48.83	(0.001–300.0)	(n = 6)	23.3 ± 6.94	(10.0–34.0)	(n = 3)	NS
p-p′-DDT	44.8 ± 22.00	(0.001–288.0)	(n = 16)	8.2 ± 2.86	(0.001–17.07)	(n = 5)	NS
Σ HCH	305.5 ± 112.54	(7.0–2453.0)	(n = 21)	264.2 ± 71.65	(52.0–697.0)	(n = 8)	NS
Σ Heptachlor	1734.4 ± 27.09	(15.0–4001.0)	(n = 20)	1749.8 ± 737.34	(176.0–6327.0)	(n = 8)	NS
Σ DDT	98.8 ± 34.53	(0.001–721.0)	(n = 21)	94.8 ± 21.93	(33.0–229.0)	(n = 8)	NS
Σ OCP	2101.3 ± 367.21	(15.0–6317.0)	(n = 22)	2175.8 ± 772.39	(4.0–6934.0)	(n = 8)	NS

FTND, Full term normal delivery; PL, preterm labour; Σ HCH, Total HCH equivalent; Σ Heptachlor, Total Heptachlor equivalent; Σ DDT, Total DDT equivalent; Σ OCP, Total organochlorine pesticides equivalent; NS, Not significant.

Table 3. Levels of organochlorine pesticides (ppb) (mean, S. E. and range) in placenta of women undergoing full term labour and women who had premature labour

Organochlorine pesticide compound	FTND (n = 22)			PL (n = 8)			Statistical significance
	Mean ± S.E.	Range and no. of positive samples		Mean ± S.E.	Range and no. of positive samples		
α-HCH	107.0 ± 26.0	(9.0–369.0)	(n = 21)	112.0 ± 22.45	(68.0–210.0)	(n = 6)	NS
γ-HCH	22.6 ± 4.87	(1.0–76.0)	(n = 15)	39.2 ± 14.37	(3.0–107.0)	(n = 8)	NS
β-HCH	202.7 ± 141.16	(15.0–1879.0)	(n = 13)	71.7 ± 21.88	(13.0–171.0)	(n = 8)	NS
Heptachlor	1297.5 ± 242.15	(232.0–2808.0)	(n = 14)	835.2 ± 250.00	(406.0–1876.0)	(n = 4)	NS
Aldrin	247.3 ± 35.45	(5.0–494.0)	(n = 18)	364.0 ± 12.14	(20.0–84.0)	(n = 5)	NS
Heptachlor epoxide	917.6 ± 225.9	(15.0–2789.0)	(n = 15)	307.7 ± 153.34	(9.0–688.0)	(n = 4)	NS
p-p′-DDE	276.3 ± 185.45	(1.0–3766.0)	(n = 20)	40.2 ± 8.58	(11.0–75.0)	(n = 8)	NS
p-p′-DDD	22.6 ± 13.23	(2.0–73.0)	(n = 5)	132.5 ± 123.86	(9.0–256.0)	(n = 2)	NS
p-p′-DDT	93.9 ± 31.70	(4.0–415.0)	(n = 13)	13.2 ± 5.35	(0.001–29.0)	(n = 5)	NS
Σ HCH	237.1 ± 89.11	(11.0–981.0)	(n = 22)	195.7 ± 50.49	(16.0–488.0)	(n = 8)	NS
Σ Heptachlor	1595.2 ± 296.40	(15.0–5024.0)	(n = 20)	914.4 ± 309.18	(9.0–2293.0)	(n = 5)	NS
Σ DDT	321.9 ± 195.64	(1.0–4181.0)	(n = 21)	82.0 ± 32.68	(19.0–298.0)	(n = 8)	NS
Σ OCP	2195.5 ± 389.42	(25.0–5734.0)	(n = 22)	868.3 ± 288.76	(184.0–2635.0)	(n = 8)	NS

FTND, Full term normal delivery; PL, preterm labour; Σ HCH, Total HCH equivalent; Σ Heptachlor, Total Heptachlor equivalent; Σ DDT, Total DDT equivalent; Σ OCP, Total organochlorine pesticides equivalent; NS, Not significant.

factors relevant to pesticide residue accumulation such as social status, dietary habits, area of residence, weight, parity, age, habits of pesticides use, etc.

Five ml of maternal blood from each case was collected by venipuncture in preheparinized vials 4–8 hours before parturition. A fraction of placental tissue was collected in acetone-washed aluminium foil at the time of delivery. Umbilical cord blood was collected by squeezing the cord into preheparinized vials. All the samples were stored at –10°C in deep freeze and analysed within 48 hours of their storage.

Pesticides were extracted and separated from samples by liquid partition and column chromatography so they may be analysed by GLC and TLC procedures.

Pesticides from maternal blood/cord blood extracted by a method given by Bush and his coworkers in 1984 with little modifications[15]. To 2 ml of maternal blood or neonatal blood, methanol (5 ml) and 1:1 diethyl ether/hexane (8 ml) were added and the contents were shaken for 3 minutes by hand. Loss of solvent due to evaporation was assessed by weighing the tube before and after shaking. The contents were then centrifuged for 10 minutes at 3000 rpm. It was re-extracted by adding 8 ml 1:1 diethyl ether/hexane. Pooled solvent so obtained contains the lipophilic organochlorine pesticides.

The method adopted for the extraction of pesticides from the placental tissue is nearly the same as that described for the blood since the main contents of placenta

are blood and blood capillaries. Two g of placental tissue was homogenized in pestle and mortar with a little sea sand[15]. All reagents and chemicals used were of HPLC and analytical grade and checked for any pesticide contamination.

Florosil column was used for the clean up of extracted samples. It was prepared essentially in the same way as described by Bush and his coworkers[15].

A Hewlett Packard 5890 series II gas chromatograph equipped with ^{63}N foil electron capture detector (ECD) and coupled with an integrator, HP 3396 A was used for the analysis of samples. Purified nitrogen (IOLAR-I) was used as the carrier gas. A coiled glass column (1.43 m × 4 mm L × I.D.) was packed with solid support, chromosorb 100/120 mesh size along with the liquid phase 1.5% OV-17 + 1.95% OV-210. A known volume of the sample was injected into the column with the help of a 10 µl Hamilton syringe. The different peaks in the samples were identified by comparing their retention times with those of the standards. Quantitation of the samples was done by the data obtained from the integrator and were based on peak areas. Standards were obtained from Environmental Protection Agency (EPA), USA.

Detected pesticides were further confirmed by thin layer chromatography. Since, sensitivity range of TLC is much less than GLC, therefore extracted and cleaned samples after their analysis on GLC were concentrated prior to their use in TLC.

The calculations are based on biological statistics and values are expressed as mean ± standard error (S.E.). The difference in the pesticide residue level between different groups was analysed with the help of Student's *t* test. Significance between the residue levels of different groups was judged at 5% and 1% levels.

The concentration of organochlorine pesticides was estimated in the maternal blood, cord blood and placental tissue of each subject undergoing premature labour and normal delivery and the data and statistical outcome of the study are shown in Tables 1–3. The mean concentration of organochlorine pesticides in maternal blood of cases of premature labour was not found to be significantly different from that of controls (full term normal delivery). Similar results were obtained from the residue levels of cord blood and placental tissue.

The data and the statistical outcome of the study show that mean concentrations of organochlorine pesticides in maternal blood, cord blood and placental tissue of premature labour were not found to be significantly different from those of controls (full term normal delivery). Our findings are contradictory to the findings of O'Leary and his coworkers[11] from Florida in which mean and range of DDE in foetal whole blood from healthy white controls were 4.9 ppb (2 to 13 ppb) while the values in the premature group were 22.1 ppb (18.7 to 26.8 ppb). Similar findings were observed in the Negro infants. The Negro premature group had a mean of 6.1 ppb and range of 3 to 12 ppb, while the Negro premature infant had a mean of 19.0 ppb and range 6.6 to 34.4 ppb. The above results suggested the involvement of high levels of DDE in the initiation of premature labour.

Our results do not coincide with the findings of Siddique[16] in which he reported that relatively high levels of organochlorine pesticides, especially DDT and DDE in cases of premature labour as compared to full term normal labour in the specimen of maternal blood and placental tissue, could suggest the involvement of these pesticides in the termination of pregnancy.

Since, we could not find any differences in the organochlorine pesticide accumulation between preterm and full term cases, our findings suggest that there are some factors other than pesticides such as stress, high blood pressure, poor nutrition, excessive physical activity, smoking, infections, teenage pregnancy, anaemia and other medical conditions such as diabetic nephropathy and uterine anomalies which are responsible for the initiation of preterm labour.

Data is not stratified with age, parity, dietary habits, weight, social status, area of residence and pesticide use habits because there were only limited number of cases of premature labour.

Hence, from the present study it is not clear whether the organochlorine pesticides play any role in the initiation of preterm labour (Tables 1–3). Therefore, further studies are to be conducted in order to draw any conclusion about the antagonistic role of organochlorine pesticides in pregnancy.

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Transfer factors of radionuclides ^{137}Cs and ^{65}Zn from soil to pearlmillet and sorghum

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The soil to plant transfer factors (TF) of ^{137}Cs and ^{65}Zn were determined for two crops, sorghum and pearlmillet, under irrigated conditions in greenhouse and under rainfed conditions in field. In the greenhouse experiment, the accumulation of ^{137}Cs was almost doubled when the soil contamination level was doubled. Under field conditions, ^{137}Cs concentration in both pearlmillet and sorghum grains as well as straw was nearly four times more at 148 kBq kg^{-1} level of soil contamination as compared to lower level of 74 kBq kg^{-1} soil. The TF values for ^{65}Zn determined under greenhouse conditions for both the crops were nearly a hundred-fold higher as compared to ^{137}Cs.

AFTER the Chernobyl nuclear station accident in 1986, worldwide concern has grown to evaluate the dose of radiation which may affect man. The *Handbook of Parameter Values* for prediction of radionuclide transfer in the ecosystems published by the International Atomic Energy Agency[1] is based on data entirely for crops, animals and conditions found in temperate climate zones. Very few data are available for radionuclide transfer and uptake in tropical and subtropical ecosystems. In the published literature, data for the uptake of radionuclides from soil for tropical cereals, fruits, herbs, tea and root crops are few in numbers and primarily limited to Cs. Although more data are available for rice, due to the complicated nature of production and the number of varieties, the data are not consistent. Currently it is assumed that transfers to animal and man are similar to those in temperate environments. Whilst this assumption is probably not unreasonable, it has never been validated.

Keeping these points in view, a study has been initiated to assess the transfer of ^{137}Cs and ^{65}Zn from soil artificially contaminated with these radionuclides to pearlmillet and sorghum under rainfed conditions in field and under irrigated conditions in pot culture.

The field experiment was conducted in the Gamma Garden of the Indian Agricultural Research Institute research farm in 1994 kharif season. In field experiments in the main yield plots of 9 m^2 (3 m × 3 m), microplots of 1 m^2 (1 m × 1 m) were made and contaminated with 74 kBq kg^{-1} soil (2 μCi kg^{-1} soil) and 148 kBq kg^{-1} soil (4 μCi kg^{-1} soil) with ^{137}Cs. The entire upper 5 cm soil of the microplots (75–80 kg) was dug out and sprayed with the 500 ml solution of radionuclide containing the required amount of activity. The radionuclides were mixed thoroughly with the entire soil and the soil was then transferred back to the microplots and brought to 50% water saturation level to a depth of 20 cm. Radionuclides were allowed to equilibrate in soil for eight weeks and during this period the soil was kept between 35 and 50 per cent moisture saturation.

After equilibration period, pearlmillet variety M-179 and sorghum variety PC-121 were grown with a row-to-row spacing of 40 cm and plant-to-plant spacing of 20 cm. The crops were fertilized with 80 kg N ha^{-1} through urea applied in two splits, 40 kg P_2O_5 ha^{-1} through single superphosphate and 40 kg K_2O ha^{-1} through muriate of potash applied as basal.

As maturity, the plants were harvested and separated into grain and straw. In grain and straw samples from microplots, ^{137}Cs activity was measured using a 3″ × 3″ NaI (T1) flat type detector for ^{137}Cs, 0.661 MeV peak as per the procedures given in the IAEA Technical Report[2]. The soil samples were also drawn from microplots to a depth of 20 cm. Though the upper 5 cm soil was contaminated with radionuclide, the soil samples were collected to a depth of 20 cm from six locations in each microplots with a Viehmeyer tube and pooled to a composite sample as per the procedure described in IAEA Technical Report[2]. The soil samples were air dried, ground in a wooden pestle mortar and counted in a well type NaI (T1) detector (2.5″ × 2.5″). The counting efficiency for ^{137}Cs was 0.881% for flat type detector and 11.82% for the well type detector.

A similar experiment was conducted under pot culture conditions in the same soil. Eight kg soil was taken in ceramic pots and contaminated with ^{137}Cs and ^{65}Zn at the rate of 148 kBq kg^{-1} soil and 296 kBq kg^{-1} soil, respectively. In equilibrated soils, four seeds of pearlmillet or sorghum were sown and on germination the plants were thinned to two in each pot. Here the results on transfer factors of ^{137}Cs under both field and pot culture conditions and of ^{65}Zn in pot culture condition are presented. The data on soil to plant transfer factors of the