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Placental Transfer of Pesticides in Humans

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Abstract. Transfer of organochlorine pesticides from mother to fetus has been studied in 100 women. The concentrations of organochlorine pesticides were examined in maternal blood, placenta, and umbilical cord blood of the same mother/child pair. Residue levels of dichlorodiphenyl trichloro ethane (DDT) and its metabolites, isomers of benzene hexachloride (BHC) and aldrin were detected in all the samples analyzed, indicating their transfer from mother to the fetus. A correlation was found to exist between the pesticide concentration and age, dietetic habits and area of residence of pregnant women.

Key words: Placenta – Umbilical cord – Organochlorine pesticides – DDT – BHC – Aldrin – Gas-liquid chromatograph

Introduction

In the process of agricultural modernisation, synthetic organic chemicals have obtained a key and indespensable position. Emphasis has always been in the extensive use of pesticides to curb parasitic infections and other vector borne diseases. Among the pesticides, organochlorine compounds (OCP's) are well known for their long persistence in the environment (Vettorazzi 1975) and in living organisms (Gowdar et al. 1976); thus posing a challenge to the ecologists and toxicologists. For this reason, some advance countries which can afford even costlier chemicals have banned the use of DDT, Aldrin etc. and have introduced safer and easily degradable pesticides as a measure to check unwarranted environmental pollution. Despite this the consumption of organochlorine pesticides particularly DDT and endrin have been continued in India and may be intensified during the course of years (Proceedings of Indo – US workshop on

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biodegradable pesticides held at ITRC, Lucknow, April 16–19, 1979). Such a situation is indeed critical because OCP's are lipophilic in nature (Moakes et al. 1965) and are stored in to body tissues (Bindra et al. 1973), because of a low rate of biodegradation (Anonymous 1978). Further, such compounds are reported to be carcinogens (Peraino et al. 1975), mutagens (Fishbein 1978), and enzyme inhibitor (Folmar 1978). The presence of such pesticides in body tissues of the adult is well documented (Dejonckheere et al. 1977; Brown et al. 1975). Placental transfer of these toxicants, therefore, may be of significance and needs to be discussed in detail.

In our earlier paper (Saxena et al. 1980) we have reported the detection of organochlorine pesticides in placenta and accompanying fluid of 50 pregnant women. We have now undertaken a detailed and systematic study of the transfer of such compounds from mother to the fetus in 100 pregnant women of different ages, dietetic habits, and area of residence.

Materials and Methods

One hundred pregnant women, admitted to Queen Mary's Hospital attached to the Department of Obstetrics and Gynaecology, King George's Medical College, Lucknow, are included in the present study. Women between the age of 18–34 years were from the urban and rural population around Lucknow (India). Detailed history of women were recorded including the results of normal biochemical tests like haemoglobin, albumin etc. There were cases of premature labor and abortions also. None of the women on enquiry reported accidental or occupational exposure to any of the pesticides studied.

Sixty women were in the age group of 18–25 years while 40 were between 26–34 years of age. There were two pregnant women of 37 years of age also. Enquiry revealed that 42 pregnant women were vegetarian while the remaining 58 women used to take non-vegetarian meals frequently. Case histories showed that 43 women were residing in rural areas around Lucknow while the rest (57 women) were from the urban population.

To study the placental transfer of organochlorine pesticides, placental tissues as well as maternal and cord blood of 100 pregnant women were analysed.

Maternal blood was collected in heparinized vials 8–12 h before parturition and stored at $10 \pm 2°$ C until analysed. Placental tissue was collected in acetone washed aluminium foil at the time of delivery and also stored at the same temperature. Umbilical cord blood was collected by milking the cord into heparinized vials. Stored samples were analysed within 48 h. Extraction and clean-up of the samples was carried out by a modified method of Dale et al. (1970) as reported in our earlier paper (Saxena et al. 1980). Briefly the method includes the following steps:

Finely chopped placental tissue (1 g) was homogenised in a waring blender with 3 ml of formic acid and 2 ml of hexane (GLC Grade), and the contents were quantitatively transferred into a 25 ml conical flask by further addition of formic acid (2 ml) and hexane (1 ml). The contents were shaken for 1 h at 37° C, in a mechanical shaker (Systronic – 2400). Losses due to evaporation were corrected by weighing before and after shaking. The contents were centrifuged for 15 min at 2,500 rpm and the upper layer (hexane) was recovered. The hexane was first treated with 1 ml of distilled water in a liquid air-methanol bath, to remove the traces of formic acid which might have been coextracted along with the pesticide compounds. The unfrozen hexane phase was further washed with concentrated H_2SO_4 (1 ml), three times, to remove the fat contents and the cleaned hexane was collected.

Specimens of maternal blood and cord blood were processed as follows: 1 ml of mother's blood and umbilical cord blood was extracted by addition of 5 ml formic acid and 2 ml hexane in a 25 ml conical flask and the contents were shaken at 37° C for 30 min in a mechanical shaker. The contents were centrifuged for 10 min at 2,000 rpm. The rest of the procedure was similar as described for placental tissue.

Purified samples were analysed for organochlorine pesticides by gas-liquid chromatography in a Varian Aerograph Series "2400" equipped with Electron Capture Detector (3 H^+). The operating conditions of the instrument were as follows:

Carrier gas:	Nitrogen purified by passing through silica gel and molecular sieve
Gas pressure:	65 p.s.i
Flow rate:	40 ml/min
Injector temperature:	200° C
Column temperature:	180° C
Detector temperature:	200° C
Attenuation:	4×10^{-9}, 8×10^{-9}, and 16×10^{-9}
Current:	10^{-9} μA
Column:	Glass spiral colum, length 6 ft. internal dial 1/8'' packed with Gas Chrome Q (80/100 mesh) coated with 1.5% OV−17 + 1.95% Ov−210 by weight
Sample size:	5−10 μl

Peak areas and retention time of the detected pesticides in the samples were compared with those of known pesticide standards for each specimen. The presence of residues detected was further confirmed by thin layer chromatography technique.

Results and Discussions

The results of the analysis of maternal blood, placental tissue, and umbilical cord blood of 100 mother/child pairs are listed in Tables 1−4. Significant differences

Table 1. Concentration of organochlorine pesticides in placenta, maternal blood, and cord blood of 100 pregnant women (ppb)

Organochlorine pesticide compounds	Placenta (range) mean ± SD	Maternal blood (range) mean ± SD	Cord blood (range) mean ± SD	Ratio: maternal/cord mean ± SD
Total BHC	(4.2 − 390.7) 45.4 ± 21.0	(8.5 − 850.0) 49.9 ± 25.6	(2.0 − 507.8) 43.1 ± 26.4	1.9 ± 1.7
Lindane	(0.7 − 95.6) 17.1 ± 15.8	(2.4 − 135.0) 19.0 ± 12.4	(1.2 − 175.7) 11.8 ± 5.3	2.3 ± 2.1
Aldrin	(n.d. − 83.3) 12.7 ± 20.4	(n.d. − 143.5) 21.1 ± 30.4	(n.d. − 253.3) 19.8 ± 34.3	5.5 ± 20.2
p,p'−DDE	(n.d. − 234.7) 22.0 ± 31.0	(2.0 − 324.8) 18.8 ± 19.6	(1.0 − 850) 15.8 ± 31.5	2.4 ± 2.5
p,p'−DDD	(n.d. − 93.3) 8.3 ± 9.9	(n.d. − 432.7) 5.7 ± 7.7	(n.d. − 48.2) 7.1 ± 16.1	1.5 ± 2.2
p,p'−DDT	(n.d. − 93.3) 18.1 − 20.8)	(n.d. − 433.6) 17.3 ± 37.8	(n.d. − 140.0) 12.1 ± 17.1	1.5 ± 1.6
Σ DDT	(n.d. − 298.7) 56.2 ± 51.6	(7.7 − 1257.3) 47.2 ± 52.2	(2.7 − 1029.8) 50.7 ± 117.0	1.5 ± 1.3

n.d.: Not detected
Σ DDT: Total DDt equivalent (sum of p,p'−DDE, p,p'−DDD, and p,p'−DDT as equivalent to DDT)

Table 2. Concentration of organochlorine pesticides in maternal blood, placenta, and umbilical cord blood of 100 pregnant women of different age group (ppb)

Pesticides detected	Maternal blood		Placenta		Umbilical cord blood	
	18–25 Years (60 cases) mean ± SD	26–34 Years (40 cases) mean ± SD	18–25 Years (60 cases) mean ± SD	26–34 Years (40 cases) mean ± SD	18–25 Years (56 cases) mean ± SD	26–34 Years (34 cases) mean ± SD
Total BHC	43.0 ± 46.2	63.7 ± 60.5	41.8 ± 7.0[d]	50.9 ± 29.3[d]	32.9 ± 125.2	45.7 ± 30.6
Lindane	14.7 ± 19.2[a]	27.4 ± 28.8[a]	12.9 ± 7.7[e]	23.6 ± 21.4[e]	10.2 ± 16.1[h]	14.9 ± 6.3[h]
Aldrin	20.6 ± 30.7	22.1 ± 28.6	13.7 ± 22.7	11.2 ± 15.9	18.5 ± 105.0	22.6 ± 25.4
p,p′-DDE	14.4 ± 19.2[b]	27.6 ± 38.5[b]	16.0 ± 24.9[f]	31.3 ± 30.2[f]	12.3 ± 8.8	23.1 ± 24.6
p,p′-DDD	4.9 ± 7.7[c]	7.4 ± 8.2[c]	9.0 ± 11.3	7.1 ± 7.5	5.8 ± 9.2	8.0 ± 14.8
p,p′-DDT	15.3 ± 42.4	21.5 ± 28.8	14.6 ± 22.2[g]	23.4 ± 38.5[g]	7.3 ± 17.2[i]	22.1 ± 12.3[i]
Σ DDT	42.1 ± 38.5	56.1 ± 86.3	51.4 ± 48.3	63.8 ± 56.9	49.5 ± 173.7	51.1 ± 44.2

[a, b, c] Statistically significant ($p < 0.005$, 0.001, and 0.05, respectively)
[d, e, f, g] Statistically significant ($p < 0.01$, 0.0005, 0.005, and 0.05, respectively)
[h, i] Statistically significant ($p < 0.05$ and 0.0005, respectively)

Table 3. Concentration of organochlorine pesticides in maternal blood, placenta, and umbilical cord blood of 100 pregnant women of different dietetic habits (ppb)

Pesticides detected	Maternal blood		Placenta		Umbilical cord blood	
	Vegetarian (42 cases) mean ± SD	Non-vegetarian (58 cases) mean ± SD	Vegetarian (42 cases) mean ± SD	Non-vegetarian (58 cases) mean ± SD	Vegetarian (36 cases) mean ± SD	Non-vegetarian (54 cases) mean ± SD
Total BHC	52.4 ± 28.2	61.3 ± 117.5	38.5 ± 65.4[b]	52.7 ± 34.9[b]	38.3 ± 43.7	35.6 ± 20.2
Lindane	19.1 ± 11.8	21.7 ± 21.5	13.7 ± 24.7	16.5 ± 15.7	12.4 ± 2.0	11.4 ± 7.12
Aldrin	17.9 ± 25.5	20.4 ± 26.5	12.1 ± 27.5	13.8 ± 22.5	34.9 ± 32.8[d]	17.3 ± 30.7[d]
p,p'-DDE	14.3 ± 13.9[a]	28.2 ± 50.7[a]	27.5 ± 46.4	32.9 ± 39.6	35.3 ± 139.5	20.5 ± 28.4
p,p'-DDD	4.8 ± 6.7	15.7 ± 62.1	7.1 ± 9.8[b]	10.5 ± 15.9[b]	6.5 ± 11.1	8.4 ± 11.6
p,p'-DDT	21.7 ± 39.7	23.7 ± 14.3	18.8 ± 18.0	20.7 ± 22.1	14.8 ± 18.3	17.0 ± 23.0
Σ DDT	41.6 ± 44.2	72.1 ± 183.4	57.7 ± 59.4	73.6 ± 73.7	62.2 ± 51.0	50.0 ± 50.4

[a] Statistically significant ($p < 0.05$) [b, c] Statistically significant ($p < 0.05$ and 0.05, respectively) [d] Statistically significant ($p < 0.005$)

Table 4. Concentration of organochlorine pesticides in maternal blood, placenta, and umbilical cord blood of 100 pregnant women of different area of living (ppb)

Pesticides detected	Maternal blood		Placenta		Umbilical cord blood	
	Rural (43 cases) mean ± SD	Urban (57 cases) mean ± SD	Rural (43 cases) mean ± SD	Urban (57 cases) mean ± SD	Rural (42 cases) mean ± SD	Urban (48 cases) mean ± SD
Total BHC	55.0 ± 91.0	60.5 ± 32.6	48.0 ± 27.8	57.7 ± 61.9	27.0 ± 13.6[e]	47.3 ± 92.0[e]
Lindane	18.4 ± 22.9[a]	12.2 ± 25.2[a]	17.5 ± 16.9	18.9 ± 17.6	8.8 ± 9.6[f]	16.9 ± 27.7[f]
Aldrin	23.9 ± 30.3	20.8 ± 26.5	15.3 ± 29.0	12.3 ± 18.7	14.9 ± 17.6[g]	22.8 ± 46.7[g]
p,p'-DDE	23.6 ± 56.9	24.7 ± 34.4	30.6 ± 42.5	29.3 ± 42.3	15.4 ± 25.9	22.8 ± 46.7
p,p'-DDD	18.9 ± 74.3	8.2 ± 9.5	12.7 ± 17.2[c]	6.9 ± 8.3[c]	6.2 ± 8.4	7.3 ± 25.7
p,p'-DDT	42.2 ± 86.9[b]	24.4 ± 34.4[b]	23.0 ± 20.2	20.9 ± 34.1	17.0 ± 26.2	13.7 ± 14.5
Σ DDT	87.2 ± 217.8	55.4 ± 52.4	75.7 ± 70.7	58.4 ± 42.4	40.6 ± 53.1	41.6 ± 72.1

[a, b] Statistically significant ($p < 0.05$ and 0.05, respectively)
[c, d] Statistically significant ($p < 0.01$ and 0.05, respectively)
[e, f, g] Statistically significant ($p < 0.05$, 0.05, and 0.01, respectively)

in the level of OCP's were observed in different age groups, dietetic habits and residence of pregnant women. The ratios of values measured in maternal blood and cord blood as calculated for 100 mother/child pair is given in Table 1.

We could not detect dieldrin, an epoxide of aldrin, while aldrin was frequently present. This may be attributed to the involvement of H_2SO_4, which precluded the determination of dieldrin (Skaftason et al. 1979).

A number of organochlorine pesticides used in different national programmes are accessible to the human organism. Thus, the organochlorine pesticides circulating in human blood, get an opportunity to reach the growing fetus via placental transfer. Residues of OCP's detected in the blood of 100 pregnant women, placentae and umbilical cord blood revealed that such compounds reach the fetus. Our study has shown that the concentration of OCP is high in maternal blood specimens and low in umbilical cord blood. The trend is: maternal blood > placenta > umbilical cord blood.

From the above trend it is obvious that the mother has the highest burden of OCP's and considering steady state conditions, only a partial transfer of such compounds to the fetus takes place. But in few cases, the concentration of OCP's was higher in umbilical cord blood as compared with maternal blood and placenta. A possible explanation for this finding may be that at the time of labor the energy provided by food stuff may not be sufficient and, therefore, body fats may have been consumed. Since organochlorine pesticides are lipophilic and are known to accumulate in fat, it seems possible that they may have been made available from the depots and also reached the fetus at a high concentration.

Mother's age has been found to influence the accumulation of OCP's in circulating blood and its subsequent transfer to the fetus. Pregnant women of ages 18–25 years were found to show a low level of OCP's as compared with those between 26–34 years of age (Table 2). Statistical evaluation of the data have revealed that values for lindane, p,p′-DDE and p,p′-DDD are significantly different in the maternal blood of two groups. In placentae the differences for total BHC, lindane, p,p′-DDE and p,p′-DDT are significant, and in umbilical cord blood only lindane and p,p′-DDT show significant differences for the two age groups. The amount of lindane (γBHC), the most potent isomer of BHC, was significantly different in all the three specimens studied of two age groups. This suggests that a longer life span may cause a greater accumulation of pesticides.

Similarly, dietetic habits of the mothers have been found to play a role on the body burden of OCP's. Interestingly, higher concentrations of OCP's were found in pregnant women with non-vegetarian dietary habits than in those with vegetarian dietary habits (Table 3). Concentrations of BHC, p,p′-DDD and aldrin were found significantly different in placentae and cord blood of two groups. The significant difference of p,p′-DDE in the two groups may result from high DDT values in mutton, eggs, and chicken (Saxena et al. 1981; Lakshminarayana et al. 1972) which are common in non-vegetarian meals.

Another aspect of the study has been considering the area of residence of pregnant mothers. Residue levels of lindane and p,p′-DDT were significantly higher in the maternal blood of pregnant women living in rural areas as

compared to those living in urban areas. p,p'-DDD and ΣDDT are significantly different in placentae of the two groups and significant differences were also found in cord blood for BHC, lindane, and aldrin (Table 4). The cause for this difference may be that those living in rural areas are more exposed to an "polluted" ecology than those living in urban areas, because agricultural fields are the main site of application of pesticides.

From the foregoing discussion it has been made clear that organochlorine pesticides present in the body of human beings are also transported to the fetus crossing the placental barrier and the ratio of their concentration in mother's blood and umbilical cord blood may help to get an estimate of the order of placental transfer for pesticide compounds which is:

p,p'-DDD > p,p'-DDT > ΣDDT > BHC > Lindane > p,p'-DDE > Aldrin.

Such a study is of special significance for the Indian population since Indians have been reported to have the highest body burden of organochlorine pesticides (Dale et al. 1965).

If we consider some possible toxic manifestations of OCP's inhibition of some enzyme reactions and impairment of amino acid uptake might occur (Hort et al. 1971).

In the light of our findings stricter regulations may be discussed and such measures have to be weighed against the benefits of the use of pesticides.

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