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To cite this Article Saxena, M. C., Seth, T. D. and Mahajan, P. L.(1980) 'Organo Chlorine Pesticides in Human Placenta and Accompanying Fluid', International Journal of Environmental Analytical Chemistry, 7: 3, 245 – 251 To link to this Article: DOI: 10.1080/03067318008071871 URL: http://dx.doi.org/10.1080/03067318008071871

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Intern. J. Environ. Anal. Chem., 1980, Vol. 7, pp. 245–251 0306-7319/80/0703-0245 54.50/0 © Gordon and Breach Science Publishers Inc., 1980 Printed in Great Britain

Organo Chlorine Pesticides in Human Placenta and Accompanying Fluid

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(Received June 25, 1979; in final form September 30, 1979)

Fifty specimens of placenta and accompanying fluid were collected at random from women of general population and analyzed for organo chlorine pesticides by gas liquid chromatograph using electron capture detector. BHC, Lindane, DDT, DDE, DDD and aldrin were detected in the placental tissue and accompanying fluid. Lindane, DDE and aldrin were more frequently detected. All the women were in their twenties but no correlation could be made between the compound and age, race or residence of the women. This is presumably the first attempt in India to detect the presence of pesticides in relation to their placental transfer, without occupational or accidental exposure.

KEY WORDS: Organo chlorine pesticides, human placenta, BHC, Lindane, DDT, DDE, DDD, aldrin, gas-liquid chromatograph.

INTRODUCTION

Residue levels of pesticides in human adipose tissue and blood have been quantitated.¹⁻³ Finnegan *et al.*⁴ have suggested that DDT or its metabolites and dieldrin may pass the placental barrier in the pregnant dog. Still born infants have been found contaminated with organo chlorine pesticides.⁵ Evidence has also been adduced to indicate that pesticides can pass through human placental barrier.⁶

Knowledge of long term trends of organochlorine pesticides in placenta and accompanying fluid is of great significance for the understanding of congenial and abnormal deliveries, mis-carriages etc., especially in the Indian context. This has prompted a survey of such pesticide compounds in the placenta of pregnant women. The present paper embodies the results of our findings on the residual level of pesticides in placental tissue and accompanying fluid.

MATERIALS AND METHODS

Fifty specimens each of placenta and accompanying fluid were collected at term from pregnant women hospitalised in Queen Marry's Hospitals, Lucknow for labor during August to November, 1978. The women subjects investigated here for Organo Chlorine pesticide contamination, were almost identical in their socioeconomic, cultural and dietic habits. On inquiry, it was found that none of the women had an occupational or accidental exposure to any of the reported pesticides.

Placental tissues were collected in acetone washed aluminium foils and kept in deep freezer, until taken out for analysis, normally within 48 hours. Accompanying fluid was collected in glass vials along with 25 mg. of potassium oxalate and stored at 10°C before analysis. Special care was taken for the pesticide contamination of the glass wares and accessaries.

Finely chopped placental tissue (1 gm) was taken in a waring blender and mixed with 3 ml of formic acid and 2 ml of hexane (G.L.C. grade), homogenised 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 an hour at 37°C, in a mechanical shaker. The losses due to evaporation were made good by weighing before and after shaking. The contents were centrifuged for 15 minutes at 2500 R.P.M. and the upper top layer that of hexane was recovered. The extracted samples were further cleaned up following the method of Dale *et* $al.,^7$ which in brief states as follows:

The above recovered hexane was first treated with 1 ml of distilled water in ultra clean test tube in a liquid air-methanol, water bath, to remove the traces of formic acid. The unfrozen phase that of hexane was further treated with conc. H_2SO_4 (1 ml), three times, to remove the fat contents and the cleaned hexane was collected.

In case of fluid samples, 1 ml aliquot was mixed with 3 ml of formic acid and 2 ml of hexane and were directly shaken for an hour at 37°C and rest all the procedure for extraction and clean up process was similar as that of placental tissue.

The cleaned up samples obtained above were analyzed for Organo chlorine pesticides on a varian aerograph series "2400" with $3H^+$ detector. The operating conditions 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:

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200°C, Column temperature: 180°C, Detector temperature: 200°C, Attenuation: 4×10^{-9} , 8×10^{-9} and 16×10^{-9} , Current $10^{-9} \mu$ A, Column: Glass spiral column, length 6 ft., internal dial 8" packed with gas chrome Q(80/100 mesh) coated with 1.5% OV-17+1.95% OV-210 by weight. Sample size: 5 to 10 μ l.

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

RESULTS AND DISCUSSION

Frequency of distribution of Organochlorine pesticides detected in placenta and accompanying fluid is depicted in Table I, Figure 1. Among the



FIGURE 1 Frequency of distribution of organo chlorine pesticides in human placenta and accompanying fluid.

pesticides quantitated were α BHC, β BHC, γ BHC (Lindane), Aldrin, DDT, DDE, DDD and in few cases dieldrin. α , β and γ -isomer (Lindane) were collectively represented as BHC. However, there were some other peaks also which could not be identified, may be of some other compounds and hence could not be accounted for. Range of observations and their geometric mean for the above pesticide compounds in the specimens of placenta and accompanying fluid are listed in Table II and III, respectively, while their ratio is cited in Table IV.

The present study reveals that the human placenta stores a number of pesticides. It is noteworthy that lindane, the γ isomer and the only potent insecticide among the BHC isomers was detected in all the specimens and its mean level was 389.64 ppb, in placenta and 27.12 ppb in accompanying

 TABLE I

 Placenta and accompanying fluid specimens from 50 women containing specified pesticide

| Fifty | placenta specir | Fifty accompanying flux | | luids | | |
|-------------------------|------------------|---------------------------------------|---------------|---------------|--------------------|---------|
| Pesticide detected | Placenta only | Placenta and accompanying fluid | Total + ve | Fluid only | Fluid and placenta | To + |
| внс | _ | 50 | 50 (100 %) | | 50 | 50 (10 |
| Lindane | | 48 | 48 (96%) | 2 | 48 | 50 (1 |
| 0,p'-DDT | 6 | 14 | 20 (40%) | 1 | 14 | 15 (|
| p, p' - DDT | 23 | _ | 23 (46%) | | | |
| p,p'-DDE | 2 | 29 | 31 (62%) | 11 | 29 | 40 (|
| p,p'-DDD | | 13 | 13 (26%) | 27 | 13 | 40 (|
| DDT Total equivalent | 2 | 43 | 45 (90%) | | 43 | 43 (|
| Aldrin | 2 | 44 | 46 (92%) | 4 | 44 | 48 (|
| Dieldrin | 2 | _ | 2 (4%) | 2 | | 2 |

| Pesticide | No. of positive individuals | Geometric mean (ppb) | Range of observations (ppb) |
|-------------------------|-----------------------------|----------------------------|-----------------------------------|
| ВНС | 50 | 1360.48 | 317-3200 |
| Lindane | 48 | 389.64 | 62-1631 |
| D, p' - DDT | 20 | 49.63 | 9–63 |
| p, p'-DDT | 23 | 52.77 | 29-170 |
| p,p'-DDE | 31 | 50.54 | 34-79 |
| p,p'-DDD | 13 | 38.21 | 80-1031 |
| DDT Total equivalent | 45 | 200.91 | 9–1134 |
| Aldrin | 46 | 158.7 | 2-1587 |
| Dieldrin | 2 | 3.93 | 1–6 |

TABLE II

Concentration of organo chlorine pesticides in 50 placental specimens.

TABLE III

Concentration of organo chlorine pesticides detected in 50 accompanying fluid specimens

| Pesticides | No. of positive individuals | Geometric mean (ppb) | Range of observations (ppb) |
|-------------------------|-----------------------------------|----------------------------|-----------------------------------|
| внс | 50 | 88.40 | 16-135 |
| Lindane | 50 | 27.12 | 9-61 |
| O,p'-DDT | 15 | 1.06 | 0.5-11 |
| p,p'-DDE | 40 | 10.51 | 2–21 |
| p,p'-DDD | 40 | 9.99 | 1-24 |
| DDT Total equivalent | 43 | 23.83 | 0.5-26.4 |
| Aldrin | 48 | 20.76 | 1-152 |
| Dieldrin | 2 | 2.42 | 1–3 |

fluid. The same was the case with aldrin, which was generally detected in both the specimens. But we could not lay emphasis on its findings for the reason, that aldrin is associated with dieldrin, an epoxide of aldrin, which could not be quantitated. On exploration, it was found that use of H_2SO_4 in the clean up process, precludes the determination of dieldrin.⁸ Therefore it would be reasonable to call the study of aldrin as semione.

Among the specimens studied, pp'DDE and pp'DDD were more frequently detected than other compounds. Amount of O'p' and pp'DDT

TABLE IV

| Pesticide compounds | Ratio placenta/accompanying fluid | | |
|---------------------------|-----------------------------------|--|--|
| BHC | 15.4 | | |
| Lindane | 14.4 | | |
| <i>O</i> , <i>p</i> '-DDT | 46.8 | | |
| p,p'-DDE | 4.8 | | |
| <i>p</i> , <i>p</i> ′-DDD | 3.8 | | |
| DDT | | | |
| Total equivalent | 8.4 | | |
| Aldrin | 7.6 | | |
| Dieldrin | 1.6 | | |

Ratio of the concentration of organo chlorine pesticides in 50 placental and accompanying fluid specimens

detected were relatively small. The concentration of DDE and DDD were much higher than that of DDT. The higher level of the metabolites suggests long term exposure to DDT.⁹

It may not be out of context to mention here O'Leary,¹⁰ who found higher level of pp'DDE in premature babies than in full term babies. The absence of DDA in all these specimens can be explained on the basis that degradation of DDE to DDA does not occur.⁶

A comparative look can be had from Table IV, which indicates that placenta stores higher amounts of pesticides than the accompanying fluid. Differences in the rates and amount of pesticides accumulated in the tissues based upon their chemical action, susceptibility to biotransformation have been discussed by Hayes.¹¹

The significance of these findings vis a vis miscarriages, still birth, deformities etc. in the neonates needs more extensive study.

Acknowledgement

Authors wish to express their deep sense of gratitude to Dr. C. R. Krishna Murti, Director, ITRC for the initiation of these studies and providing necessary facilities and to Mr. S. Q. Mehdi for providing technical assistance.

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