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The pesticides are poisons, of course else they would not be useful in the control of insects

Dr. Mamta
Associate Professor,
Raj Rishi Government (Autonomous) College, Alwar

ABSTRACT:

Challenge for producing more food for the ever-increasing population of the globe by proper management of crop pests and control of vector borne diseases necessitated the application of more pesticides on the earth. The pesticides are poisons, of course else they would not be useful in the control of insects, rodents, and other undesirable animals and plants. In other words, pesticide research is a continuous process for the humanity, for better living and improved future. The historical development and projected future underscores identification and isolation of newer molecules and formulations in the pesticide industry. It is now a well-accepted fact that only organochlorine pesticides (OCPs) especially DDT, HCH and to some extent Aldrin / dieldrin and heptachlor play a vital role in the tissue accumulation of pesticides.It has been well established that pesticides, particularly the chlorinated hydrocarbons directly affect the fetuses and neonates as they get transferred through placenta and mother milk respectively. This has been confirmed in all the mammalian species that have been examined including humans.Taking above points into consideration, a continued surveillance on the levels of pesticide pollutants in human conception is an important task to ensure the well-being of the human pregnancy. It was, therefore, planned to conduct such as a study in Jaipur, the capital of Rajasthan and the pink city of India. Findings of this research work may provide base line data of the extent of pesticide contamination/exposure in women body, fetus and offspring. The data obtained from the above work may also provide some clues, possible reasons for abortions, premature deliveries, still births, some infant diseases andmortalitiess.The study is mainly concerned with the pesticides burden in the pregnant women and its transfer to Prenates, so it will require the analysis of pesticide residues in the (1) blood of pregnant

women(2) in placenta and cord bloodusing gas liquid chromatography. The results revealed the presence of isomers of HCH, heptachlor, DDT and its metabolites and Aldrin in mostly all the samples analyzed. Efforts werealso made to find out the Ratio of Maternal/Cordblood which may put some light on possible map of the distribution and accumulation pattern of pesticides in the mother and thereby on the degree of their transfer to the fetus. The OCPs residues present in the maternal blood indicate the pesticide burden in the human population, which in turn is a risk to human health. Secondly, this may be considered as an indication of the transfer of these chemicals from maternal to fetal circulation across the placenta which may pose various problems of management of prenatal health.

KEY WORDS:
Mother's blood, Organochlorine pesticides, Contamination, Residues, Gas Chromatograph, Placenta, Cord blood

INTRODUCTION

Pesticides occupy a rather unique position among the many chemicals that man encounters daily, in that they are deliberately added to the environment for the purpose of killing or injuring some form of life. Ideally, their injurious action would be highly specific for undesirable target organisms and non-injurious to desirable non-target organisms. In fact, however, most of the chemicals that are used as pesticides are not highly selective but are generally toxic to many non-target species including man, and other desirable forms of life that co-inhibit the environment. Therefore, lacking highly selective pesticidal action, the application of pesticides must often be predicted on selecting quantities and manners of usage that will minimize the possibility of exposure of non-target organisms to injurious quantities of these useful chemical. As human beings are placed at the top of most food chains [1], it is, therefore, not surprising that human adipose fat and milk fat usually have more than ten times higher levels of persistent chlorinated pesticides and PCBs as compared to milk fat from cows [2-4].Nothing can cause greater fear in an expectant mother than the prospect of her unborn child being defective or intoxicated and nothing touches a man or woman more closely or personally than their reproductive

capacity. In recent years, the public has become alarmed that inadvertent or careless exposure to chemicals in the environment might result in unwanted accumulation of chemicals not only in foodresidue but also in human tissues, and cause various types of harms to them. A wide scale employment of organochlorine pesticides has resulted in the incidental exposure of many non-target species, including man. The new born which are not simply adults as they differ morphologically, physiologically and biochemically from adults, may also get exposed to pesticides via three routes, the two most important of which require exposure of the mother. Thus, a very important mode of exposure occurs, while the neonate is in utero, by the transplacental passage of the pesticides from maternal to fetal circulation. Transplacental migration of toxic chemical to the growing fetus causes a prenatal exposure, and subsequent burden of maternally carried chemicals. Confirmation of this has been done in all the mammalian species that have been examined including man. Also important is the oral exposure to the pesticide residues secreted in the mother's milk. Third is the direct or accidental exposure of the new born to the toxic chemical which is very rare. First ever report as per our knowledge related to transplacental movement of pesticides came in 1968 by Rappolt and his colleague Hale [5] who carried out a survey study in California, U.S.A in which they determined the residue levels of p, p'-DDE and p, p' DDT in the human placenta and cord blood to assess the qualitative and quantitative deposits in the population. They concluded that the use of human obstetrical material as a source of detectable hydrocarbon residues appears to be greatly limited. Limitation was caused at least in part by the low-fat content of the tissue resulting in pesticide levels generally below the level of sensitivity of the gas chromatograph. Only in the case of placenta reportable levels of p, p' DDE were found, with the mean level of 4.8 mg/g.

More scary studies have indicated that we have largely over looked the darker side of these chemicals as OCPs are reported to be carcinogenic [6], [7] mutagenic [7],[8] teratogenic [8],[9] immunosuppressive [10],[11] create endocrine dysfunction such as hypothyroidism or high estrogenic activity [12],[13] disturb reproductive processes [14],[15] growth depressants [16],[17] induces several psychogenic and neurogenic abnormalities in adult stages [18],[19], and are

associated with abortions, premature deliveries, still births and infants with low birth weights [20]-[23]. OCPs have been in use in India nearly for a half century now. Even after having clear cut evidence suggesting that these chemicals have the ability to eliminate entire species from the planet, the annual consumption of pesticides in India is about 85,000 tons of which OCPs comprise the bulk [24]. Therefore, today OCPs are perhaps the most ubiquitous of the potentially harmful chemicals encountered in the environment and are still widely detected in humans despite the considerable decline in environmental concentrations [25-30].

The exposure of the general population to pesticide may occur through several environmental media, including food, water and air and to a lesser extent soil. There is evidence that greatest exposure to pesticides for the general population takes place as a result of ingesting food and water containing small residues of pesticides. This type of incidental exposure of human beings may result in the accumulation of OCPs in their blood in quite high concentrations. Presence of pesticides in the blood is the indication of environmental pollution by these toxins or the body burden of these pesticides which in turn is a risk to the human health.

Shattering of the concept of “Placenta “being the barrier

For years the term “placental barrier" typified a concept that the main function of the placenta was to protect the fetus against passage of noxious substances from mother to fetus. However, the placenta has other functions, it provides nutrition for the conceptus, exchanges maternal and fetal blood gases, disposes off fetal excretory material, and maintains pregnancy by a variety of hormonal mechanisms. Most of the vital nutrients necessary for the development of the fetus are transported by energy coupled specific active transport systems. For example, vitamins, amino acid, essential sugars, and ions such as calcium and iron are transported from mother to fetus against a concentration gradient [31, 32]. In contrast most toxic materials pass the placenta by simple diffusion, except for a few antimetabolites that are structurally similar to the endogenous purines and pyrimidines that are normally actively transported from maternal to fetal circulation. Many foreign substances can cross the placenta. In addition to

chemicals, viruses (e.g. rubella and HIV), cellular pathogens (e.g. syphilis spirochete), antibody globulins, and even erythrocytes traverse the placenta [33]. Anatomically, the placental barrier is a number of layers of cells interposed between fetal and maternal circulation. The number of layers varies with the species and the state of gestation, and this probably affects the permeability of the placenta. Placentas in which maternal endothelial and epithelial layers are absent so that the chorionic villi bathe in the maternal blood they are called haemochorial. This condition is found in monkey and man. Within a single species, the placenta may also change its histologic classification during gestation [34]. The relationship of the number of layers of the placenta to its permeability has not been thoroughly investigated, but presently is not thought to be of primary importance in determining the distribution of chemicals to the fetus. Except for cases where chemical agents are selectively toxic to one type of placenta over another (i. e. trypan blue), few correlations have been made between anatomic classification of the placenta and transfer of chemicals between mother and fetus [35].

The placenta should be viewed as a lipid membrane that permits bidirectional transfer of substances between maternal and fetal compartments, rather than as a "barrier". The transfer depends on three major elements the type of placentation, the physiochemical properties of the compound, and placental biotransformation. Blood flow constitutes the major rate limiting factor in placental transfer of the more lipid soluble compounds. Placental blood flow progressively increases throughout pregnancy at a rate that is proportional to fetal size even though placental mass, relative to fetal mass, is reduced [36].
Since there is no such data is available from Jaipur, pink city of India and capital of Rajasthan, therefore it was planned to carry out systematic study in which (1) blood of pregnant women(2) in placenta and (3)cord blood was used for biological monitoring of pesticides which besides indicating quantitative and qualitative trends in residue deposits and distribution in pregnant women of the general population, also gives an assessment of the vulnerability of the progeny to these environmental toxins. We here report the results of the chemical analysis of 1) blood of pregnant women(2) in placenta and (3)cord blood from 101 women by using very sensitive and well controlled chemical analysis

technique Gas Liquid Chromatography (GLC). The results revealed the presence of isomers of HCH, heptachlor, DDT and its metabolites and Aldrin in mostly all the samples analyzed. Efforts were also made to find out the Ratio of Maternal/Cord blood which may put some light on possible map of the distribution and accumulation pattern of pesticides in the mother and thereby on the degree of their transfer to the fetus. The OCPs residues present in the maternal blood indicate the pesticide burden in the human population, which in turn is a risk to human health. Secondly, this may be considered as an indication of the transfer of these chemicals from maternal to fetal circulation across the placenta which may pose various problems of management of prenatal health.

MATERIALS AND METHODS

101 pregnant women admitted to Zanana Hospital and Mahila Chikitsalya: attached to the Deptt. of obstetrics & Gynecology. S.M.S. Medical College, Jaipur (India) and two private hospitals "Sanjeevani Hospital" and " Meera Hospital", Bani Park, Jaipur are Included in the present study. In general, they had no history of any occupational or accidental exposure to pesticides. However, they were asked to fill up a questionnaire giving information about their health and relevant to the pesticide residue accumulation such as age, dietary habits, area of residence, parity, social status, accidental or occupational exposure to pesticides etc. according to WHO methodology [37]by interviewing the subjects at the time of collection of samples.

Sample Collection

Maternal blood, placenta and cord blood:

Five ml of maternal blood from each case was collected by venipuncture in pre-heparinzedvials 4-8 hours before parturition and stored at -10°C in a deep freeze until analysed. Afraction of placental tissue was collected in acetone washed aluminium foil at the time of delivery and stored at the same temperature. Umbilical cord blood was collected bysqueezing the cord into pre-heparinzed vials All the stored samples were analysed within 48hours of their storage.

Extraction of Pesticide from Samples

Pesticides were extracted and separated from samples by liquid partition and column chromatography so that they could be analyzed by Gas Liquid Chromatography (GLC) and Thin Layer Chromatography (TLC) procedures. All reagents and chemicals used were of analytical grade and checked for any pesticide contamination. Specimens of maternal bloodblood, placenta and cord blood were extracted and then cleaned by florosil column as per the methodology given by Bush and his coworkers with little modifications according to the prevailing laboratory conditions [38].

Quantitative Estimation

Quantitative estimation of pesticide residues in all the extracts was done by HP 5890 series II gas chromatograph (GC) equipped with Ni 63 Electron capture detector (ECD) coupled to HP 3396A integrator. Glass coiled column (1.43 m x 4 mm L x I. D) was packed with Solid Support, Chromosorb 100/120 mesh size along with the Liquid phase: 1.5% OV -14±1.95% OV-210 .Puri□ed nitrogen (IOLAR-1) gas was used as the carrier gas and a known volume of sample was injected in the column with the help of the 10 µl Hamilton syringe. Different peaks of the samples were identi□ed by comparing their retention times with those of standards. Quantitation of the samples were done by the data obtained from the integrator and were based on peak areas. Standards were obtained from Environmental Protection agency (EPA) U.S.A.

Recovery Analysis and Confirmation of Pesticide residues

Recovery analysis was done by forti□cation experiments and the percentage recovery was 95-98%.TLC was used for confirming the identity of the OCPs already detected by the GC. The pesticides for which the GC was standardized and were estimated were Aldrin, isomers of HCH (□, □ & □), metabolites of heptachlor (Heptachlor & Heptachlor epoxide) and DDT (DDE, DDD and DDT).

Statistical Analysis

The calculations are based on biological statistics and values are expressed as mean± standard error (S.E.). The difference in the pesticide residue levels between different groups was analyzed with the help of student t test. Significance between the residue levels of different groups was judged at 5 % and 1% levels.

DISCUSSION

There is a strong tendency of our present civilization to resort to the use of chemicals to control various unwanted forms of plant and animal life. Substantial gains from the use of pesticides in agriculture as well as in health programmes have been acknowledged by all. Nonetheless, the harmful residues that remain on crops, especially the edible portion, have been a cause of great concern to everyone. Placenta serving as a barrier between mother and baby for large number of endogenous and exogenous substances contains appreciable content of fat and hence can serve as a carrier for lipophilic xenobiotics, including organochlorine pesticides. Placenta has already been reported to carry the organochlorine pesticides and there is are many report in Indian context from Lucknow [39,26]. In the present study obstetrical material - placenta, umbilical cord blood, and also the blood of mother was used for biological monitoring of pesticides which besides indicating quantitative and qualitative trends in residue deposits and distribution in pregnant women of the general population, also gives an assessment of the vulnerability of the progeny to these environmental toxins.Results of the present study on accumulation and distribution of organochlorine pesticides in pregnant women are incorporated in table 1. Analysis of the specimens shows the presence of isomers of HCH, heptachlor and its metabolites, DDT and its metabolites as well as Aldrin. A number of OCPs used in different national programmes seem to be accessible to human organisms through different routes of exposure, with digestive tract being the main pathway. After the absorption of organochlorine pesticides, they are circulated in the blood and then distributed to different organs and tissues where they get accumulated in accordance with neutral fat content of the tissue. Since, placenta contains appreciable amount of fat, the pesticides and their metabolites

detected in the maternal blood in the present study gets deposited in them and ultimately find an opportunity to reach the growing fetus via placental transfer.Since, the developing fetus has no means to get rid of any endogenous or exogenous substances except the placenta; the presence of lipophilic insecticide residues in the circulating blood of fetus clearly indicates that they are being transferred from the mother through placenta. Because case histories revealed no accidental or occupational exposure to any of the detected pesticides, subjects were definitely got exposed through the food chain and the environment. Placental transfer is undoubtedly responsible for the presence of these toxicants in new born babies. It is obvious from the table: 1 that human cord blood, stores certain detectable chlorinated hydrocarbon pesticides including isomers of HCH, metabolites of DDT and heptachlor and Aldrin. In the cord blood, a-HCH, Aldrin and DDE were detected quite frequently whereas DDD was detected only in quite a few samples. Concentration of total DDT and total HCH were found to be 75.4 and 142.1 ppb respectively. The frequency of distribution of the pesticides detected in the placenta of 101 women are incorporated in the table.1. In the present study concentration of total DDT and total HCH came out to be 99.9 and 170.6 ppb respectively.

The magnitude of transfer of different pesticides to the developing one can be had from the ratio of their concentration in mother's blood and umbilical cord blood as given in table.

CONCLUSION

It is quite clear from the foregoing discussion, that in all over the world Indian mothers have got the significant and may be highest body burden of OCPs. This is because in the third world countries such as India, because of the cost - benefit ratio OCPs are still the major pesticides used in agriculture and public health sector. This is in accordance with the findings of Dale and his coworkers (1965) that the Indians have got the highest body burden of OCPs [45]. The present study directly reflects the national scene of magnitude of pesticide pollution which signifies the distribution and accumulation of non-biodegradable lipophilic pesticides in pregnant women on one side and subsequently the vulnerability of the successive generation from its very

inception in the womb of the mother on other side. The so called “placental barrier" which separates the maternal compartment from the fetal compartment must be regarded as an epithelial membrane of lipoid character, i.e. a lipid sieve through which lipophilic non-polar substances are able to penetrate according to their concentration gradient and degree of lipophilicity. A physiological state like pregnancy, no doubt helps mother in fighting against the increasing burden of environmental pollutants like persistent organochlorine pesticides. During the state as evident from the present investigation, the mothers appreciably reduce their body burden of pesticides at the expense of intoxication of their own developing baby in the womb through cord blood during the gestational period. Starvation stress caused an increase in the cord blood concentrations of DDT and its metabolites, calls for a strict regulation in time to take meals and a balance between calorific intake and energy requirement of the mother during pregnancy to avoid mobilization of fat depots resulting into mobilization of stored residues of OCPs [46].

It can be concluded that the magnitude of pesticide pollution is quite high to contaminate the food and environment and as a result toxicant reach the human body through various sources mainly through the absorption from the gastrointestinal tract via contaminated food chain. From there, they are further circulated in maternal blood, cord blood stored in milk and placental tissue of the women [28,30]. Since, the pesticides are reported to be carcinogenic, mutagenic, teratogenic, immunosuppressive, induces endocrine dysfunction and high estrogenic activity, disturb the reproductive processes, growth depressants, induces several psychogenic and neurogenic abnormalities in adult stages and are also reported to be associated with abortions, premature deliveries, still births, low birth weight consequences are obvious on the mother and the developing baby. It poses various problems of management of neonatal nutrition and health. It calls for suggestions like special care in nutrition and in the environment of mother throughout the life and especially during pregnancy and lactation. It would be advisable for a woman to avoid the consumption of fatty food stuffs and heavily polluted working environment. 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. Present findings on obstetric

toxicology of pesticides particularly in relation to distribution of pesticidal pollutants in pregnant women may finally lead to a better understanding of the influence of chemicals on fetal development and provide grounds for further studies on placental toxicology as related to pesticide pollution in India. In the end, it must be emphasized that there is a rising protest that pesticides are destroying harmless wild life and endangering the health of man himself. The battle against the harmful insects would be much less costly and more efficient, and the problem of contamination of the environment by toxic materials would be vastly reduced, if insect activities are controlled by natural means. The use of pest-specific predators; parasites or pathogens; sterilization of insects with the help of radiations; trapping insects using insect attractants like pheromones; use of juvenile hormones or hormone inhibitors may therefore be suggested as alternate ways of pest control.

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