EFFECT OF HEXACHLOROCYCLOHEXANE ON PREGNANT GOATS

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Received : 04-02-2011   Accepted : 22-05-2012

ABSTRACT

Eighteen goats with one month pregnancy were randomly divided into control, untreated and treated groups. HCH residues in the cabbage leaves prior to treatment were in the range of 0.1261 to 0.1895 mg kg⁻¹. These levels were lower than the maximum residual limit values (MRL). In treated cabbage leaves with HCH dusting the levels were in the range of 19.8317 to 21.7749 mg kg⁻¹. The blood samples from control, untreated and treated animals at 0 month the HCH residues were in the range of 0.0002 to 0.0003 mg kg⁻¹ and these values are non significant within the groups. The average levels of HCH residues in blood samples from untreated group progressively increased from 0.0003 at 0 month to 0.0018 mg kg⁻¹ at the end of 3rd month. Similarly the HCH values from treated group showed a significant increase from 0 to end of 3rd month with an average value of 0.0002 to 0.0527 mg kg⁻¹. Four animals of treated group were aborted in the fourth month of the experiment and the average concentration of HCH residues in placenta was 0.0122 mg kg⁻¹. The accumulation of this insecticide in placenta was about 22.2 per cent of maternal blood. Among the organs, the accumulations of HCH residues were higher in fat followed by liver with 0.0548 and 0.0515 mg kg⁻¹, respectively. The consumption levels of HCH in the treated group at 4.04 mg/kg/day was much higher than the MRL and affected gestation and also induced mortality.

Key words: Pregnant does, HCH toxicity, HCH residual effect.

INTRODUCTION

Goat is generally considered as poor man's cow, a befitting description of their immense contribution to poor people's economy. In metropolitan cities, the goats are reared by landless economically poor families in their back yards. Goats roam more widely than other herbivores and meet their requirements from browsing on weeds neglected by others. The farmers generally feed them with the vegetable wastes such as exfoliated leaves of cabbage, cauliflower etc., obtained from the vegetable market yards. In modern agriculture, pesticides have been recognized as an important factor in the production of crops. Indiscriminate use of pesticides in vegetable crops, their mishandling and negligence to follow proper waiting period, make marketed vegetables often contaminated with pesticides. Surveys carried out earlier in various parts of India showed higher incidence of pesticide residues in food commodities (Noronha et al., 1980). Many samples were found to be contaminated with organochloride insecticides such as hexachlorocyclohexane (HCH), dichloro diphenyl trichloro ethane (DDT), aldrin etc. Bedi et al. (2003) reported that goat meat samples were found contaminated with DDT and HCH residues and maximum concentration of residues were observed in goat adipose tissue followed by muscle, liver and kidney. Seasonal variation of pesticide residues was also reported. Among these the HCH concentration was highest. Regular consumption of the contaminated vegetable leaves, even though with lower concentration than maximum permitted levels, may lead to large bioaccumulation of the pesticides and thus pose a potential health hazard in animals. Among other side effects of organochlorine compounds, there are few reports to show that these compounds adversely affect the reproductive performance. However cooking of meat caused the reduction of DDT, HCH and their metabolities with concomittent increase in the level of DDD residue (Ahmed et al., 2000). Research with chlorinated hydrocarbons demonstrated that these compounds can pass through the placenta and thus can cause abortion, stillbirths, birth defects etc. (Rumsey et al., 1973; Siddiqui et al., 1981). The literature on the effects of the consumption of HCH contaminated feeds on reproductive system of goats is very scanty. Considering the increased use of vegetable wastes for
feeding goats in cities, it is appropriate to investigate the side effects, if any, of the pesticide residues contamination particularly on gestation.

**MATERIALS AND METHODS**

**Experimental animals**

Eighteen non-descript local goats of one month pregnancy were selected. The animals were randomly divided into three groups consisting of six animals each. Group I served as control group and were fed with standard feed consisting of concentrated mixture and green fodder. Group II served as untreated group in which the animals were similarly fed with the concentrates as in control, however the fodder was replaced with exfoliated cabbage leaves obtained from the local vegetable market of Hyderabad city. Group III animals served as treated groups fed with cabbage leaves similar to group II, however those were dusted with HCH (10% dust) at the rate of 10 g/kg of leaves using tiny hand duster prior to feeding ad libitum. The dusting of cabbage leaves with HCH at the rate of 10 g/kg was based on the treatment of HCH as insecticide to the cabbage flower heads in field condition. The animals were constantly observed for any signs of toxicity during the experimental period.

**Estimation of HCH residues in cabbage leaves**

The cabbage leaves obtained from the vegetable market were randomly divided into three groups. From one group 50 g of cabbage leaves per replication were collected in stratified random technique for analysis as untreated sample. Similarly 50 g of cabbage leaves were taken after dusting the HCH at the rate of 10 g/kg and served as treated. For recovery study of insecticides from the last group 50 g of untreated cabbage leaves were cleaned with ordinary water to eliminate residues and then placed in a wide mouth reagent bottle (500 ml capacity). A known amount of standard solution (i.e., 100 µg) prepared from technical grade HCH containing α+β+γ+δ isomers were added. To this 150 ml of hexane solvent is added. The contents of the reagent bottle was then shaken four to six times and left for over night to facilitate absorption of the insecticide by the leaves. Then the sample was subjected to extraction as per the procedure followed by Kapoor et al. (1981).

**Extraction of residue from leaves**

The samples of leaves were transferred to a blender with 100 ml distilled acetonitrile and macerated for 5 minutes and then transferred to Buchner funnel fitted with a filter paper and suction filter. The pulp of the leaves on the filter paper in Buchner funnel was again macerated with another 50 ml distilled acetonitrile and filtered into the same flask. This was repeated for the third time and the filtrate was collected. The quantity was measured and transferred to the 500 ml separating funnel adding 50 ml of distilled hexane and the hexane layer was collected where the insecticide was dissolved and allowed to pass through the funnel containing cotton wool and sodium sulphate in to the bottle. This was repeated for second time adding 25 ml of hexane to the separating funnel and collected the hexane layer in previously collected hexane and total quantity measured. The hexane layer extract was transferred to the Kuderna Danah evaporator and concentrated to 10 ml by evaporating the solvent (Kapoor et al., 1981).

**Estimation of HCH residues from blood and other organs**

The plasma was collected at 0 month, end of the first month, second month and third month from each animal from all three groups and stored at -18°C till the estimation. The tissue samples of different organs namely heart, liver, fat, stomach, lungs and kidney were collected from the animals soon after the death in petridishes containing normal saline and then transferred immediately to the laboratory. Recovery studies were made with blood and organs obtained from healthy goat sacrificed for the purpose. The procedure for recovery studies was similar to that for cabbage leaves.

**Extraction of residues from blood and organs**

Extraction of residues from the blood and other organs was done as described above; however instead of distilled acetonitrile used for cabbage leaves, ethyl alcohol was used for blood and organs.

**Clean by digestion method**

The concentrated hexane extract was transferred to a 500 ml separating funnel. To the above 500 ml separating funnel a 100 ml capacity separating funnel containing 25 ml of sulphuric acid was attached. From this funnel sulphuric acid is released drop by drop into 500 ml separating funnel where hexane extract was present. The acid layer from the 500 ml separating funnel was separated and added 30 ml of distilled water in 500 ml of
separating funnel and swirled for two to three minutes. The hexane layer was collected through sodium sulphate into the bottle and used for gas chromatography estimation. For organs and blood, cleanup digestion was done using 100 ml of sulphuric acid for digestion purpose instead of 25 ml of sulphuric acid used in cabbage leaves and the same procedure was followed as for cabbage leaves for organs and blood samples.

**Estimation of HCH residues**

The HCH residues were estimated for the above samples by gas chromatography (Packard model No.437) as per the procedure followed by Kapoor et al. (1981).

**RESULTS AND DISCUSSION**

Goats roam more widely than other herbivores and meet their feed requirements from browsing on weeds neglected by others. It is a common site that many goats seen picking vegetable wastes in the vegetable market yards. The vegetable crops are often infested by many pests during different stages of crop right from the day of sowing to harvest. The farmers are advised to control these pests by application of insecticides. Usually the farmers use pesticides indiscriminately on vegetable crops and their mishandling and negligence to follow proper waiting period make marketed vegetables often contaminated with varied concentrations of pesticide residues. Agnihotri et al. (1974) who obtained vegetable samples from different markets of Delhi and found that 10 samples have shown as high as 50 parts per million (ppm) or more of BHC residues and further observed that about 25 per cent of the samples analysed showed residues of DDT or BHC were either close to or much above the maximum residue limits (MRL) prescribed by FAO/WHO. Among the vegetables, cabbage and cauliflower showed higher levels of BHC. Khandekar et al. (1982) found 92 out of 311 samples to contain insecticide residues above the MRL in Mumbai vegetable markets. Similar studies were also made by Rup Lal et al. (1989) and observed in some vegetables, Lindane (Isomer of HCH) levels to be above the maximum residue limits.

In the present study, the cabbage leaves obtained from a vegetable market yard were analysed for HCH residues once every month during three months of experimentation (Table 2). The HCH residues were in the range of 0.1261 to 0.1895 mg kg⁻¹ with an average of 0.1600 mg kg⁻¹ (0.16 ppm). The present values are less than the permitted maximum residue limits of 0.25 ppm or 0.25 mg kg⁻¹ (Narsimha Rao, 1994). The MRL values are mostly recommended in order to ensure safety of human health. However, there seems to be no published literature concerning the MRL values for safety of animal health.

The study is aimed to investigate the effect of insecticide contaminated feeds on reproductive system of goats. Since these treated cabbage leaves were fed to the group (treated animals), it is difficult to assess the individual intake of insecticides daily.

**TABLE 1: Recovery percentage of HCH in the different samples**

<table>
<thead>
<tr>
<th>Organs</th>
<th>α</th>
<th>β</th>
<th>γ</th>
<th>δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cabbage leaves</td>
<td>94.5</td>
<td>99.5</td>
<td>97.2</td>
<td>87.5</td>
</tr>
<tr>
<td>Blood</td>
<td>85.5</td>
<td>92.3</td>
<td>98.5</td>
<td>94.5</td>
</tr>
<tr>
<td>Heart</td>
<td>89.5</td>
<td>88.5</td>
<td>91.2</td>
<td>89.0</td>
</tr>
<tr>
<td>Lungs</td>
<td>87.6</td>
<td>83.2</td>
<td>93.5</td>
<td>81.5</td>
</tr>
<tr>
<td>Stomach</td>
<td>78.5</td>
<td>88.5</td>
<td>90.5</td>
<td>89.0</td>
</tr>
<tr>
<td>Placenta</td>
<td>85.5</td>
<td>92.0</td>
<td>98.5</td>
<td>95.0</td>
</tr>
<tr>
<td>Fat</td>
<td>86.5</td>
<td>99.5</td>
<td>98.0</td>
<td>95.0</td>
</tr>
<tr>
<td>Liver</td>
<td>82.5</td>
<td>90.5</td>
<td>89.2</td>
<td>81.5</td>
</tr>
<tr>
<td>Kidney</td>
<td>81.5</td>
<td>82.5</td>
<td>89.2</td>
<td>78.5</td>
</tr>
</tbody>
</table>

**TABLE 2: HCH residues (mg kg⁻¹) in cabbage leaves.**

<table>
<thead>
<tr>
<th>Group</th>
<th>1st month</th>
<th>2nd month</th>
<th>3rd month</th>
<th>Average ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>0.1261</td>
<td>0.1645</td>
<td>0.1895</td>
<td>0.1600±0.0184</td>
</tr>
<tr>
<td>Treated</td>
<td>19.7317</td>
<td>20.1426</td>
<td>21.7748</td>
<td>20.5497±0.6239**</td>
</tr>
</tbody>
</table>

** Significant (P<0.01).**

Each value is an average of replicate sample.
However, considering the total consumption of cabbage leaves per day by the six animals in the group which is averaged as 14.4 kg. It can be assumed that each animal consume 2.4 kg of cabbage leaves. The dusted cabbage leaves are known to contain HCH levels on an average of 20.5497 mg kg\(^{-1}\) as observed from residual analysis (Table 2) prior to consumption. Thus, the daily intake of HCH by each animal is about 49.3 mg. On body weight basis (4.04 mg/kg/day) of insecticide. There are no studies to indicate LD50 values for HCH to the goats. The present consumption is lower by the goats when compared to the reported lethal dose of laboratory animals and dogs. For instance, Frank and Braun (1984) calculated a lethal dose of 13-16 mg kg\(^{-1}\) of BHC for calves that died in 50 hours. While Herbst et al. (1975) found that dosage at the rate of 6 - 10 mg/kg/day produced no effect in mice. In the present experiment the goats sustained much larger dose of HCH than the MRL of 0.25 mg kg\(^{-1}\) in feed and also for longer period. This may be due to the fact that the goats being ruminants, the pesticide ingested by them is well diluted with the rumen fluid and may significantly be modified due to the reduced pH of the rumen liquor. In most cases in the rumen the insecticides may have detoxified or altered in such a manner that may result in delayed absorption (Oehme and Barrett, 1986).

The animals were observed for signs of toxicity during the experimentation. The signs of toxicity such as increased respiratory rate, restlessness, frequent micturation were observed in treated group animals. Two of the dead animals showed convulsions before the death. The remaining four animals aborted and were recumbent, showed signs of dehydration with loss of coordination of movements. Upon termination of the experiment, they were treated symptomatically and eventually recovered from toxicity. Lindane is found to inhibit adrenal and gonadal steroidogenesis in animals. Besides this, \(\alpha\), \(\beta\) and \(\gamma\) HCH are found to inhibit progesterone production in the studies conducted by Lance and Douglas (2000). While, Anna Maria Fausta et al. (2001) reported that Lindane toxicity affected the ultra structure of spermatozoa in rabbits. The other two groups showed no clinical signs of pesticide toxicity except one animal in untreated group showed intermittent muscular spasms prior to the parturition.

Recovery percentages of pesticide from cabbage leaves, blood and other organs were comparable with other scientists (Barquet et al., 1981) who used gas chromatography (Table 1). HCH residues in blood samples were analysed from all the three groups (Table 3&4). Similarly both experimental groups i.e., untreated and treated groups showed HCH values at the 0 month comparable to control animals. The HCH residues in blood samples from untreated groups showed a significant rise from 0 month to third month of experimental period. The progressive increases in HCH residues indicate accumulation of the insecticides in the storage organs. Similar to our studies, Siddiqui et al. (1981) estimated HCH residues from large number of Indian women and found on an average the blood samples contained.

| Table 3: Accumulation of HCH residues (mg kg\(^{-1}\)) in blood samples from different group. |
|---|---|---|---|
| Group | 0 month | 1\(^{st}\) month | 2\(^{nd}\) month | 3\(^{rd}\) month |
| Control | 0.0002±0.00003 | 0.0002±0.00002 | 0.0003±0.00003 | 0.0002±0.00003 |
| Untreated | 0.0003±0.00003 | 0.0013±0.00005 | 0.0016±0.00009 | 0.0018±0.00009 |
| Treated | 0.0002±0.00003 | 0.0174±0.0001 | 0.0459±0.0001 | 0.0527±0.0001 |

Each value is an average of replicate sample. Values bearing different superscript differ (P<0.05).

| Table 4: HCH residues (mg kg\(^{-1}\)) in blood and placenta of aborted animals. |
|---|---|
| Animal No. | Residues in Blood | Residues in Placenta |
| T\(_1\) | 0.0542 | 0.0118 |
| T\(_3\) | 0.0548 | 0.0125 |
| T\(_4\) | 0.0544 | 0.0122 |
| T\(_6\) | 0.0562 | 0.0123 |
| Average ± S.E. | 0.0549±0.0004 | 0.0122±0.0001 |

Each value is an average of replicate sample.
residues from large number of Indian women and found on an average the blood samples contained 0.022 mg kg\(^{-1}\) due to consumption of agrochemicals.

The effect of HCH residues in untreated group appeared to have no adverse effect on gestation. The prenatal growth is more or less directly related to the transport of various compounds across the placental barrier. It has been reported that uptake of aminoacids is inhibited by the presence of metabolic poisons (Joseph et al., 1968). The report of Courtney (1979) indicated that the long term consumption of food contaminated with pesticides might be associated with the still births.

The HCH residues in blood samples from treated group showed a progressive significant rise from 0 month to third month (Table 3). In the available literature, there is no data on HCH residues from blood samples in animals that were subjected to longer periods of consumption of contaminated feed for comparison. In the present study attempts were made to correlate the HCH levels of the maternal blood to the levels in placenta which resulted in abortion. The correlation between maternal blood and in placenta indicated that on an average about 22.2 per cent of the maternal residues were accumulated in the placenta and resulted in abortion. O'Leary et al. (1970) compared the levels of DDT in maternal blood to umbilical cord blood and reported about 43 per cent of the maternal blood DDT residues was presented in the umbilical cord. Presence of organochlorinated compounds in human placenta of pregnant women have been linked with possible miscarriages, still births (Saxena et al., 1980 and ITRC, 1987). Similarly, several studies have also suggested evidences that organochlorine compounds act as antagonists to pregnancy, through spontaneous abortion, premature or still born deaths (ITRC, 1987). However, no data is available on the levels of HCH residues in blood and or placenta in pregnant animals that are for abortion and thus it is difficult to conclude the minimum HCH levels in placenta to cause abortion.

Among the organs, accumulation of HCH residues was higher in the fat followed by liver and kidney (Table 5). The lowest accumulation was in the heart. Davidow and Frawley (1951) showed the \(\alpha\) and \(\beta\) isomers of BHC are stored in liver 30 times more than other isomers of BHC when administered in equivalent dosage levels. Oshiba and Kawakita (1972), Oshiba (1972) observed BHC storage reaching equilibrium in fat in not less than eight weeks in mice. Liver being the chief organ for BHC metabolism it is obvious that high concentrations of BHC are also found in this organ. However, no data is available on the pesticide accumulation in different organs in animals. Further, it is not known how the storage in different organs influences the circulating levels of HCH residues. The HCH residue levels in blood are almost similar to the levels found in the adipose tissue.

The present study suggest that similar to the reports from other market yards, the exfoliated leaves of the cabbage is also considerably contaminated with the HCH pesticides and prolonged feeding of these contaminated leaves, appears to have deleterious effects on reproduction and this chemical has a tendency to store in different organs. Hence the feeding practices should be carefully planned.

**ACKNOWLEDGEMENT**

The authors are highly thankful to Acharya N.G. Ranga Agricultural University, for extending facilities to work on pesticides. We are highly thankful to Dr. B. Narsimha Rao, Professor & Head, Department of Entomology, Dr. M. Muniraj Naidu, Associate Professor, Department of Animal Nutrition.
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