Studies on Pesticide Residues in Market Milk and their Public Health Significance

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ABSTRACT

The present study was undertaken to ascertain the levels of organochlorine pesticide (OCP’s) residues in market milk. The detectable levels of pesticides were found in all the samples of market milk collected from different areas of Punjab. HCH was found to be the most the predominant pesticide detected comprising an average of 53% of total of OCP residues in milk; DDT, and dieldrin comprised of 43% and 2%, respectively, while heptachlor and aldrin each comprised one per cent. Average level of total HCH residues in market milk was 0.042 mg kg\(^{-1}\). In market milk \(\alpha\), \(\beta\), \(\gamma\) and \(\delta\) - HCH residues were found in 79.7%, 60.9%, 100% and 31.3% samples, respectively. Average level of total DDT residue in market milk was 0.0334 mg kg\(^{-1}\). Various metabolites of DDT in milk were found in the form of p,p’-TDE, p,p’-DDE and p,p’-DDT in 93.7%, 82.8% and 45.3% samples, respectively. Persistent OCP’s such as DDT and its derivatives, isomers of HCH poses toxicological and ecological impact due to their lipophilic nature and persistence in biotic and abiotic components of an environment.

Keywords: DDT, HCH, milk, organochlorine, OCPs, pesticide residues

Introduction

In modern agriculture, role of various pesticides has become increasingly important in production of efficient and cheap food commodities to meet worldwide food demands. These chemicals have greatly increased agricultural yields and saved million of lives from insect borne diseases, but indiscriminate use of pesticides has resulted in pest resistance, environmental contamination and adverse effects on beneficial and non-target organisms. Prolonged and uncontrolled use of organochlorine pesticides (OCP’s) with poor enforcement of regulations, their lipophilic nature, low chemical and biological degradation rates have contributed to the prevalence of residues of these pesticides in food commodities in India (Mukherjee and Gopal, 1993, Kalra et al., 1999, John et al., 2001, Bedi et al., 2004). Major contribution of pesticides has been attributed to animal foods such as dairy products and meat (Noren, 1983).

Animal feed containing pesticides is the main source for accumulation of these residues in tissues of animals (Gill et al., 2001) although inhalation of polluted air and absorption through intact skin may also take place. As their concentration exceeds a certain threshold level, pesticides are translocated to mammary glands and are secreted in milk as residues. The indiscriminate use of pesticides pose a considerable risk due to presence of the residues in agricultural and animal husbandry practices, thus awareness and need for regular screening of various animal products such as milk is imperative in the interest of trade and consumers. Therefore, present study was undertaken to
ascertain the magnitude of contamination of market milk with organochlorine pesticide residues from various areas of Punjab to assess their public health significance.

**Materials and Methods**

Sixty four samples of market milk supply were collected from various areas of Punjab. Each sample consisted of 500 ml of milk. The samples were analyzed as soon as possible and where it was not possible to analyze the samples immediately, 2.5 ml of potassium dichromate saturated aqueous solution containing 1% amyl alcohol was added. Milk samples were kept in a water bath at 40°C, so as to melt the fat before analysis. All the chemicals of analytical reagent (AR) quality were obtained from E. Merck (India) Ltd. and all the solvents were glass distilled before use. The suitability of the reagents/solvents was checked by running blanks. Elution column was prepared. A cotton swab was placed at the bottom of a chromatographic column and it was rinsed with petroleum benzene. After thorough rinsing 100 ml petroleum benzene was added into the elution column. Deactivated silica gel weighing 25 g was added to the column while tapping the column to avoid formation of air bubbles.

Ten g of milk sample was weighed in 100 ml beaker, activated silica gel weighing 25 g was added to the beaker containing milk and stirred with glass rod till free flowing powder of silica gel was formed. This powder was poured into the column while tapping the column. Petroleum benzene measuring 300 ml was added into elution column after giving washings to the beaker thus a total volume of 400 ml of elution solvent was used. The extract was collected in a round bottom flask and concentrated in rotary vacuum evaporator and flask was rinsed 4 times using 3 ml petroleum benzene each time. All rinsings were collected in a 15 ml glass stopper centrifuge tube and stored for gas liquid chromatography (GLC) estimations.

The residues in cleaned up extracts were quantified using gas liquid chromatography (GLC) and electron capture detector (ECD) was used for organochlorines. Instrumental parameters and operating conditions were as follows:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gas</td>
<td>Nucon 5700</td>
</tr>
<tr>
<td>Chromatograph</td>
<td>ECD, Ni 63</td>
</tr>
<tr>
<td>Detector</td>
<td>o.d. 1/8&quot;, i.d.3 mm, L - 4&quot;, Support : Chromosorb W.H.P. Mesh size: 100-120</td>
</tr>
<tr>
<td>Liquid phase</td>
<td>7.5% OV-210</td>
</tr>
</tbody>
</table>

The clean up extract measuring 2-8 µg was injected in GLC. Winacid 5.1 software on PC was used for integration and computation of signals. The compounds were identified and quantified by comparison of the retention time and peak heights/area of the sample chromatographs with those of standards run under the same operating conditions. The standards were run before and after all analyses. The results were confirmed by using alternate columns.

**Results and Discussion**

The detectable levels of pesticides were found in all the 64 milk samples collected from different areas of Punjab. HCH was found to be the most predominant pesticide detected comprising an average of 53% of total of OCP residues in milk; DDT, and dieldrin comprised 43% and 2%, respectively, while heptachlor and aldrin each comprised 1%.

Average level of total HCH residues in market milk was 0.042 mg kg⁻¹. In market milk α-, β-, γ- and δ- HCH residues were found in 79.7%, 60.9%, 100% and 31.3% samples, respectively. Mean level of α-HCH was 0.0124 mg kg⁻¹ with a range of BDL-0.028 mg kg⁻¹, β-HCH was 0.0065 mg kg⁻¹ with a range of BDL-0.022 mg kg⁻¹, of γ-HCH was 0.0207 mg kg⁻¹ with range of 0.007-0.048 mg kg⁻¹ and of δ-HCH was 0.00225 mg kg⁻¹ with range of BDL-0.0011 mg kg⁻¹ (Fig. 1). The proportions of various
HCH isomers in market milk were \( \alpha \)-HCH (30%), \( \beta \)-HCH (16%), \( \gamma \)-HCH (49%) and \( \delta \)-HCH (5%).

In the present study, concentration of total HCH residues in milk were found to be less than the earlier studies conducted in India (Deka, 1995; Neelama and Nath, 1996; Gupta et al., 1997), but in contrast Ayyaduari et al. (1999) reported below detectability levels of DDT and DDE residues in milk from Chennai. Variations might be due to certain factors, such as magnitude and frequency of pesticide application, efficacy of absorption and excretion, age and nutritional as well as socio-economic status. The most important factor for this appears to be the complete ban on the use of technical HCH and some restrictions on use of DDT and \( \gamma \)-HCH. The earlier studies carried out in other countries revealed high levels of OCP’s, but in recent studies, a sharp decline in DDT and HCH residues in milk is reported.

The substantial change in the isomeric composition of HCH in milk was found in the present study as compared to earlier reports. Levels of \( \gamma \)-HCH residues were highest, followed by \( \alpha \)-HCH, whereas levels of \( \beta \)- and \( \delta \)-HCH were very less. In the present study the proportion of various HCH isomers in market milk were \( \alpha \)-HCH-30%, \( \beta \)-HCH-16%, \( \gamma \)-HCH-49% and \( \delta \)-HCH-5%. In most of the earlier published works in India among various HCH isomers, \( \beta \)-HCH levels were reported to be highest, followed by \( \alpha \)- and \( \gamma \)-HCH (Kalra and Chawla, 1985; Kannan et al., 1992; Battu et al., 1996; Neelama and Nath, 1996; Kalra et al., 1999).

The change in the pattern of HCH residues can be due to ban imposed on use of technical HCH w.e.f. 1.4.1997 and only \( \gamma \)-HCH can be used for field application on crops. In technical HCH, \( \alpha \)-HCH is 60-65%, \( \beta \)-HCH is 5-6%, \( \gamma \)-HCH is 13% and \( \delta \)-HCH is 2-3%. Among various HCH isomers only \( \gamma \)-HCH has the insecticidal property and is the only isomer of HCH which is permitted for use in agriculture. Higher levels of \( \gamma \)-HCH residues detected in the present study as compared to earlier reports may be due to the use of \( \gamma \)-HCH in agriculture as compared to earlier use of technical HCH both in agriculture and public health. The dusting of HCH is recommended for protection of paddy crops from insect pests and consequently, fodder crops are likely to be contaminated. \( \gamma \)-HCH is recommended for controlling stem borer and ear head bug (Parmar and Dureja, 1990). HCH is used by farmers as a cheap and commonly available option in rice pest control and for termite control. In some states of India rice straw is predominant fodder for milch and work animals.

Average level of total DDT residue in market milk was 0.0334 mg kg\(^{-1}\) in the present study. DDT residues in samples of market milk were found in the form of p,p’-TDE, p,p’-DDE and p,p’-DDT in 93.7%, 82.8% and 45.3% samples, respectively. Mean level of p,p’- TDE was 0.0176 with range of BDL-0.033 mg kg\(^{-1}\); of p,p’-DDE was 0.130 mg kg\(^{-1}\) with range of BDL-0.024 mg kg\(^{-1}\).
and of p,p’-DDT was 0.0028 mg kg⁻¹ with range of BDL-0.019 mg kg⁻¹ (Fig. 2). The proportions of various DDT metabolites in market milk was p,p’-DDT (8%), (53%) and p,p’-DDE (39%). The presence of p,p’-DDE and p,p’-TDE mainly indicates past exposure to this insecticide, while presence of p,p’-DDT reflects current exposure (Peterson, 1979). It is observed that the levels of residues appearing in milk are related to the use of fodder contaminated directly (by use of pest control) (Gill et al., 2001) or indirectly (drift from dust sprayed on other major crops, translocation from soils and water) with the insecticides.

Under the Prevention of Food Adulteration Act in India, the MRL values prescribed for α-, β-, γ- and δ-HCH in whole milk are 0.05, 0.02, 0.01 and 0.02 mg kg⁻¹. In present study, market milk samples’ residue levels of α- and β- and δ-HCH did not exceed the MRL, but residues levels for γ-HCH exceeded the MRL value of 0.05 mg kg⁻¹ in 60 (93.75%) samples of market milk. Levels of total DDT residues exceeded MRL in 11 (17.18%) market milk samples. Battu et al. (2006) found all liquid milk samples analysed were above MRL for lindane in Punjab.

The use of OCP’s in large quantities and remarkable biological persistence in environment causes their wide spread presence in all elements of food chain. Because human are at top of the food chain, obviously human adipose tissues contain relatively high concentration of these bioaccumulating chemicals. Pesticides have carcinogenic, teratogenic, mutagenic, allergic and neurotoxic effects on human health and decreased fertility (Ekbom et al., 1996; Au et al., 1999; Straube et al., 1999). DDT and HCH are widely recognized as neurotoxic substances affecting the peripheral and central nervous system, respectively and causing hyper excitability of nerves and muscles (Hassal, 1983). The chemicals such as DDT, PCBs, HCH and HCB as well as some other inducers of drug metabolizing enzymes are also promoters in experimental hepatocarcinogenesis (Pilot and Sinica, 1980). p,p’DDE, a metabolite of DDT, was found in brain samples from persons affected by neurological disorders (Fleming et al., 1994).

Use of technical HCH has been completely banned in 1997 and use of DDT is restricted to public health programmes. Residues are still encountered in human and bovine milk and it takes many years for substantially decrease the residues. However, continuous monitoring of milk and milk products and human load will have to be carried out so as to obtain clear picture of residue level prevailing in different matrices. Contamination of an environment, livestock and food of animal origin viz., milk and meat can be avoided by better management and judicious use of pesticides, banning of persistent organochlorine pesticides with strict compliance and bringing more awareness about residues among public.

References


