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Organochlorine Pesticide Residues in Bovine Milk

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Abstract Monitoring of bovine milk of different places in Bundelkhand region of India was carried out to evaluate the status of organochlorine pesticide (OCP) residues. Out of a total of 325 samples 206 (63.38%) were contaminated with residues of different OCPs. The average concentration of total HCH was 0.162 mg/kg. Among the different HCH isomers the frequency of occurrence of α-isomer was maximum followed by δ-, γ- and β. Endosulfan (α, β, sulfate) was detected in 89 samples with mean concentration of 0.0492 mg/kg while total DDT comprising of DDT, DDE and DDD was present in 114 samples having mean concentration of 0.1724 mg/kg. Dicofol was positive in 17 samples.

Keywords Organochlorine pesticide (OCP) residues · Bovine milk

Organochlorine pesticides (OCP) have been used in agriculture and livestock for quite a long time to control a variety of crop pests and animal ectoparasites. They have also been employed to combat vectors of malaria and some other deadly diseases of human being. Of late, some of the compounds belonging to OCPs like aldrin, dieldrin, heptachlor, DDT, HCH, etc. which are also important constituents of the toxic group known as persistent organic pollutants (POPs), have been banned or restricted. The OCPs owing to their high persistence nature due to chemical stability and lipophilic character accumulate in different environmental compartments and in food chain thus causing elevated contamination in human body. These residues bio concentrate in lipid rich tissues according to equilibrium pattern of internal transport and lipid tissue content and decline at a very slow rate even after sources of contamination are eliminated.

The intake of contaminated feed and fodder by the milch animals is the main source of entry of pesticides in to the animal body which ultimately results in the contamination of milk, meat, etc. consumed by human being. Thus human body also gets contaminated. There are number of reports available about the occurrence of organochlorine pesticide residues in milk from different parts of India as well as abroad although it has been found that there is a steady decline in the level of those residues in milk and other products during recent years as a result of banning or restricting some of the compounds belonging to the group, introduction of newer molecules and changes in the management practices of insect pests, etc. Milk has a very special position in the diet of infants, children and elderly for whom it is considered as a perfect natural food. Being a fat rich food it is an important source of OCP accumulation and hence one of the convenient food stuff for measuring the persistent OCPs. It is recommended by the United Nations Environment Programme (UNEP) as an indicator of POPs (UNEP 2001) and has been studied in various surveys (ICMR 1993; Mukherjee and Gopal 1993; Kumar and Nath 1996; Waliszewski et al. 1996; Wong and Lee 1997; Kalra et al. 1999; John et al. 2001; Nigam and Siddiqui 2001; Pandit et al. 2002; Vasanthi et al. 2003; Zhong et al. 2003; Pardio et al. 2003; Waliszewski et al. 2003; Battu et al. 2004; Surendranath et al. 2005). The present paper reports here the status of the organochlorine residues in milk intended for human consumption, collected from different places of Bundelkhand region of India

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like Jhansi, Lalitpur, Banda, Gwalior, Jalaun, Rath, Tikamgarh, Chhatarpur, Sagar, Panna and Bhind.

Materials and Methods

Bovine milk samples (325) were collected at random from different places covering UP and MP districts of Bundelkhand region of India like Jhansi, Banda, Gwalior, Jalaun, Tikamgar, Hamirpur, Chattarpur, Panna, Sagar and Bhind. Sample were taken afresh in the morning in glass bottles, kept in ice thermocol box during transport and stored in deep fridge in the laboratory before analysis. The analysis was performed within 24 h after collection of samples.

Single column extraction and clean up according to method suggested by Stijve (1983) with modification was followed. Briefly, an aliquot of 2 g homogenized milk was mixed with pre activated florisil, silica gel and anhydrous sodium sulphate to make it a free flowing powder. A chromatographic column (15 × 300 mm) was then prepared in hexane with 3 g activated florisil in between two layers of anhydrous sodium sulphate. The free flowing powder made earlier was then quantitatively transferred to the column. The column was then eluted with 50 mL of dichloromethane and acetone (9:1 v/v). The eluate was concentrated to dryness. Meticulous care was taken to see that even traces of dichloromethane were removed. Finally the volume was made up in isooctane.

The qualitative and quantitative determination was done in gas chromatography on a Varian CP-3800 equipment fitted with Ni^{63} electron capture detector. The column used was WCOT fused silica capillary having dimension of 30 m × 0.32 mm id × 0.25 μm film thickness (CP-SIL 5 CB) at the temperature programming of 180°C (1 min) to 250°C (5 min) at 3°C/min, injector temperature at 260°C (1:10) and detector temp at 300°C. The carrier gas was nitrogen at a flow rate of 1 mL/min through column and 30 mL/min make up. The identification of peaks and quantification of concentration was done based on the external standard solution injected initially and after every five samples.

Recovery experiment was conducted by spiking the milk samples with all the pesticide standards taken for analysis at 0.5 and 1.0 mg/kg level to see the efficiency of extraction and analytical procedure. Detector linearity was determined by linear regression analysis of concentration versus response curve. Calibration of the instrument was done before sample analysis using standards of the pesticides. The pesticides determined were α-HCH, β-HCH, γ-HCH, δ-HCH, op^I DDE, pp^I DDE, op^I DDD, pp^I DDD, op^I DDT, pp^I DDT, aldrin, heptachlor, α-endosulfan, β-endosulfan, endosulfan sulfate and dicofol. A working standard solution of mixture of all the above pesticides was prepared by combining aliquots of each individual stock solution of 100 μg/mL and diluting to a concentration of 1.0 μg/mL with isooctane. The standard solution was stored in standard stoppered tubes at 4°C in refrigerator.

The mean average recovery varied from 90 to 95% with standard deviation being less than 8 units indicating good repeatability of the method. The limit of detection was 0.001 mg/kg.

Results and Discussion

A total of 325 samples were analysed of which 206 (63.38%) samples were found to be contaminated with different OC pesticide residues. The concentration of OC pesticides in milk, on whole milk basis, is presented in Table 1. Samples were analysed in duplicate and mean results were reported. Among four different isomers of HCH, α-isomer was found to be present in 78 samples out of total 325 samples (24% detection frequency) and its concentration ranged between traces to 0.1983 mg/kg with the mean concentration of 0.0188 mg/kg. Out of 78 contaminated samples concentration in five samples was higher than its prescribed maximum residue limit (MRL) of 0.05 mg/kg (Agnihotri 1999). The predominantly accumulating, most persistent and metabolically stable β-isomer of HCH could be identified in 49 samples (15.07% detection frequency) in the concentration range of traces to 0.9267 mg/kg, the mean concentration being 0.0985 mg/kg. The MRL of 0.02 mg/kg, as designated for β-HCH (Agnihotri 1999), was violated in 27 of 49 contaminated samples. Most active and important isomer of HCH, i.e. γ-isomer (lindane) was detected in 69 samples (21.23% detection frequency) with the concentration range varying between traces to 0.0801 mg/kg and mean concentration being 0.0101 mg/kg. In 20 of the 69 contaminated samples the concentration was higher than its prescribed MRL of 0.01 mg/kg (FAO 2000). The δ-isomer of HCH was positive in 73 samples (22.46% detection frequency) with a mean concentration of 0.0346 mg/kg and its MRL of 0.02 mg/kg (Agnihotri 1999) was exceeded in 18 samples. The mean total HCH concentration was found to be 0.162 mg/kg. In terms of frequency the occurrence of different isomers of HCH residues in milk on whole milk basis was of the order of $\alpha > \delta > \gamma > \beta$. However, with regard to mean concentration in the contaminated samples β-isomer was highest (0.0985 mg/kg) followed by δ-, α- and γ-isomer.

The residues of endosulfan, an insecticide of the cyclodiene group of organochlorines, was also detected in the form of its two isomer α and β and the toxic metabolite endosulfan sulphate. The frequency of α-endosulfan was

Table 1 OC pesticides detected and their concentration (mg/kg whole milk basis)

Pesticide	Total sample	Contaminated sample	No. of sample > MRL	Range of residues	Mean Concentration
α-HCH	325	78 (24%)[a]	5 (6.4%)[b]	Tr–0.1983	0.0188
β-HCH	325	49 (15.07%)	27 (55.1%)	Tr–0.9267	0.0985
γ-HCH	325	69 (21.23%)	20 (28.98%)	Tr–0.0801	0.0101
δ-HCH	325	73 (22.46%)	18 (24.65%)	Tr–0.3489	0.0346
Σ-HCH	325	123 (37.84%)		Tr–0.9283	0.1620
α-Endosulfan	325	77 (23.69%)		Tr–0.0360	0.0065
β-Endosulfan	325	62 (19.07%)		0.001–0.3153	0.0229
Endosulfan Sulfate	325	51 (15.69%)		Tr–0.1316	0.0198
Σ-Endosulfan	325	89 (27.38%)	58 (65.16%)	Tr–0.3748	0.0492
op^I DDD	325	18 (5.53%)		0.0017–0.2126	0.0162
pp^I DDD	325	46 (14.15%)		Tr–0.8179	0.0217
op^I DDE	325	49 (15.07%)		0.0011–0.6095	0.0200
pp^I DDE	325	77 (23.69%)		Tr–0.7304	0.0360
op^I DDT	325	29 (8.92%)		Tr–0.646	0.0235
pp^I DDT	325	26 (8.0%)		0.0012–0.8993	0.0550
Σ-DDT	325	114 (35.07%)	38 (33.33%)	Tr–0.9884	0.1724
Dicofol	325	17 (5.23%)	4 (23.52%)	Tr–0.4537	0.0814

Tr traces

[a] Figures in parenthesis denote percentage of total samples contaminated with respective compounds

[b] Figures in parenthesis denote percentage of contaminated samples with respective compound having concentration higher than its MRL

higher (23.69%) although its mean value (0.0065 mg/kg) was less than that of β-endosulfan (0.0229 mg/kg) which occurred less frequently (19.07%) than the former. Endosulfan sulphate was detected in lesser samples (15.69%) than the two isomers having mean concentration of 0.0198 mg/kg. In terms of total endosulfan 89 samples (27.38% detection frequency) were contaminated with mean total level of 0.0492 mg/kg. As the MRL of endosulfan in milk is only 0.004 mg/kg (FAO 2000) so in 58 contaminated samples the concentration was above the MRL.

Total DDT residues comprising of op^I and pp^I isomers of DDT, DDD and DDE were recorded in 114 samples (35.07% detection frequency) with concentration ranged between traces to 0.9884 mg/kg. The principal contributor to total DDT was pp^IDDE (23.69%) followed by op^IDDE (15.07%) possibly due to metabolism of most DDT in the environment to the more stable DDE. The next major component was pp^IDDD (14.15%) while the frequency of occurrence of DDT isomers (op^I and pp^I) was less (8–9%) than the other metabolites though the mean concentration level was found highest in pp^I DDT (0.055 mg/kg) followed by pp^I DDE (0.0360 mg/kg) and op^I DDT (0.0235 mg/kg). In 58 contaminated samples the residue of total DDT was more than the MRL of 0.05 mg/kg on whole milk basis (Agnihotri 1999).

Dicofol, which is itself an acaricide and also a metabolite of DDT, was positive in only 17 samples (5.23% detection frequency). The mean concentration was 0.0814 mg/kg and in four samples it was above the MRL of 0.1 mg/kg (FAO 2000).

The results obtained in other monitoring studies on organochlorine pesticide residues in milk conducted in India and abroad were compared in Table 2 with that of the present study. The mean level of α-HCH (0.0188 mg/kg) in the milk samples of present study was similar or less than those reported in few other studies conducted in India (Mukherjee and Gopal 1993), Spain (Riva and Anadon 1991), Mexico (Waliszewski et al. 1996) and China (Zhong et al. 2003) but still higher than those detected in France (Venant et al. 1991) and Slovakia (Prachar et al. 1995). In case of β-HCH, however, the mean concentration obtained in the present study (0.0985 mg/kg) was comparable only with that of Mexico (Waliszewski et al. 1996) and on the higher side compared to all other reports as mentioned in Table 2. The mean level of most active isomer, i.e. γ-HCH or lindane (0.0101) in the present report was lesser than most of the other studies except for two instances, one from India (Mukherjee and Gopal 1993) and another from Slovakia (Prachar et al. 1995). The γ-HCH level was lower than that of β-HCH which might be due to the fact that the γ-isomer can be transformed in to a more metabolically stable β-isomer which is a predominant isomer accumulating in human and adipose tissues (Kalantzi et al. 2001). Owing to its presence rapid biomagnification of β-HCH takes place in man and its bioconcentration is higher while

Table 2 Comparison of OC levels (mg/kg) in milk from different places

Country	References	α-HCH	β-HCH	γ-HCH	δ-HCH	Σ-HCH	Σ-DDT	Σ-endosulfan
France	Venant et al. (1991)	0.003	0.005	0.024				
Spain	Riva and Anodan (1991)	0.018	0.009	0.024				
China	Zhang (1995)					0.07	0.095	
Mexico	Waliszewski et al. (1996)	0.055	0.099	0.026		0.098	0.057	
India (Delhi)	Mukherjee and Gopal (1993)	0.053	0.014	0.004		0.071	0.150	
Slovakia	Prachar et al. (1995)	0.005	0.006	0.004		0.015	0.413	
India (all)	Agnihotri (1999)					ND-5.12	ND-25.6	
India (Lucknow)	Nigam and Siddiqui (2001)		0.078	0.025			0.015	
India (South)	Surendranath et al. (2002)					0.01–0.71	ND-0.8	
China	Zhang et al. (2003)	0.024	0.011	0.012			0.046	
India (North)	Kathpal et al. (2004)					0.001–0.209	0.001–0.649	0.001–0.154
India (present study)	Nag and Raikwar (2007)	0.0188	0.0985	0.0101	0.0346	0.162	0.1724	0.0492

elimination is slower than for the other HCH isomers (WHO 1992). The levels of δ-HCH could not be compared since no other reports mentioned about occurrence of δ-HCH residues in milk. As regards to the presence of total DDT residues the mean concentration found in the present study was comparable or lesser than most of the other reports from India and Slovakia. However, the value found was still higher than those reported from China, France, Spain and Mexico. Endosulfan is generally presumed to be not passing into the milk. So, almost all the monitoring studies of milk and dairy products in respect of OCP residues have not reported about any occurrence of residues of endosulfan excepting that of Kathpal et al. (2004) who found residues of total endosulfan (comprising of α- and β-isomers and metabolite endosulfan sulfate) to the extent of 0.001–0.154 mg/kg. In the present investigation residues of endosulfan with a mean concentration of 0.0492 mg/kg were recorded. It would be worthwhile to mention here that endosulfan too, like DDT and HCH, excreted through milk in milch animals following ingestion of contaminated feed and fodder (Nag et al. 2007) although the transfer coefficient (the percentage of daily intake of a pesticide excreted in milk each day) in case of endosulfan was very low (0.23–0.33%) as compared to DDT (∼5%) and HCH (3–36% depending on the isomer).

The presence and concentration of HCH, DDT and other OCP residues in milk in the context of present study indicate that though the frequency and level has considerably decreased over the past records of particularly India but still the contamination exists albeit in low scale. The most important reason for this could be that most of the OC compounds have become the persistent environmental contaminants over the decade and have very less degradability. So, they would require much more time to be completely phased out from the system, which on the other hand, is a very distinct possibility since some of the compounds like DDT, lindane, etc. are not totally banned but restricted in their use, as a result of which someway the animals may still be exposed to these compounds. Endosulfan is a very popular and cheap insecticide used for management of a variety of insect pests in a wide range of crops in India. Although the transfer coefficient of endosulfan from feed to milk is very less but still residues of it could be found in milk samples.

Dicofol residues could not be found in many samples because being an acaricide it has a very limited use and that too in specific crops. So feed and fodder offered to animals was normally not contaminated with it.

The result of the study revealed that though contamination of milk with OC residues was observed the number of contaminated samples exceeding the tolerance level of respective pesticides was very less. But, nevertheless, there is need to continue the monitoring study of the organochlorine and other pesticide residues in milk from the view point of human food safety and also to monitor their time tendency after restriction and ban of some of the OCs.

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