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Persistent organochlorine pesticide residues in feed and fodder as a potential source of contamination in food of animal origin

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ABSTRACT

The presence of varying types of toxic substances in feed and fodder is undesirable as from there they enter into the animal body system and redistribute themselves in different tissues and deposited there for a long time depending on nature of the toxicant. Residues of pesticides like organochlorines (OC) are often found in feed and fodder originating from different sources. Ultimately, these residues are transferred through the food chain and bioaccumulated in human body. The objective of the study was to investigate the extent and level of contamination in commonly used feed and fodder collected from different places with organochlorinated pesticide (OCP) residues. The results revealed that 34.6% of 318 samples were positive with residues of targeted OCPs. While hexachlorocyclohexane (HCH) residues were detected in 26.73% samples, endosulfan and dichlorodiphenyltrichloroethane (DDT) residues were found present in 11.95 and 11.64% samples respectively. Dicofol could be found in only 3 samples. In terms of concentration only 3.14 to 2.2% samples exceeded the limiting values of HCH and DDT respectively. Rest of the samples were either free or were contaminated al a level below limiting value. Endosulfan residues were present at a very low level (3–22 ppb). So, in terms of concentration and percentage of positive samples exceeding the limiting value of respective pesticides it can be concluded that the feed and footers can be considered as safe so far as OCP residues were concerned.

Key words: Feed, Fodder, Organochlorine pesticides, Residues

Presence of OCPs in feed and fodder is quite common as evidenced from published literature both from India (Kannan *et al.* 1992, Singh *et al.* 1997, Gupta *et al.* 2000, Prasad and Chhabra 2001, Kang *et al.* 2002, Deka *et al.* 2004, Sharma *et al*. 2005, Nag and Raikwar 2011, Karabasanavar *et al.* 2012) and abroad (Pierson *et al.* 1982, Lovell *et al.* 1996). There is a direct link between the safety of feed and the safety of food of animal origin. Feed production must, therefore, be subjected to the same way as food production, quality assurance including safety systems based on internationally accepted principles as consumers worldwide expect protection from hazards occurring along the entire food chain. Therefore, it is imperative and essential to monitor feed and fodder, which are normally offered to animals for consumption, for pesticide residues and other toxicants as a measure of risk assessment associated with animal and human health. With this objective the present study was undertaken to monitor the extent and level of persistent OCPs present in various types of feed and fodder offered to animals.

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MATERIALS AND METHODS

Sampling: Different types of concentrate feed and straws like oilseed cakes of mustard, linseed, sesamum, cotton, pulse byproducts i.e. chunnies (powdered outer seed coat) of gram, lentil, pea, urd, arhar, cereal straw (wheat, paddy), cereal by products (chunnies of oat, rice, wheat flour) and *pashu ahar* (commercial concentrate mixture) were collected from different places like Jhansi, Gwalior, Kanpur, Lucknow and Agra, which are commonly used for feeding the milch and meat animals. Random sampling procedure was followed and after collection samples were brought to the laboratory and kept in deep freezer at –20°C until analysed.

Similarly green fodders (GF) like sorghum, oat, berseem, cowpea and different grasses were collected from farmers' field at Jhansi during 2008–10. Fresh green fodders after collection were brought to the laboratory and analysed as soon as possible.

Extraction and clean up of samples: Methods given by Luke *et al.* (1975) and Nakamura *et al.* (1994) with modification were followed. The samples were ground in a mill to a fine powder. A representative of 20g sample was taken for extraction. Samples were put in thimbles made up of cellulose filter paper and covered with previously solvent extracted cotton. Thimbles containing the feed

samples were extracted in soxhlet apparatus for 8 h in hexane. The extract was dried, concentrated and cleaned up by employing partitioning and column chromatography. Green fodder (50g) sample was blended with acetonitrile in a mixer grinder and filtered through a filtering funnel using Whatman filter paper (No.1). Clean-up of samples was done same as in the case of feed samples.

Gas chromatographic analysis: The qualitative and quantitative determination was done in gas chromatography on a equipment fitted with Ni^{63} electron capture detector. The column used was fused silica capillary having dimension of 30 m ×0.32 mm id × 0.25µm film thickness (CP-Sil 5CB). The operating conditions of GC were : Injection port temperature, 260°C (split 1:10); detector, 300°C; column, 180°C for 1 min then @ 3°C/min to 250°C for 5 min (Programme); carrier gas: N_2, @ 1ml/min, make up 30ml/min. The OC pesticides were identified and quantified based on the external standard (99.5%) solution injected initially and after every 5 samples.

The pesticides under study α-HCH, β-HCH, γ-HCH, δ-HCH, op^l DDE, pp^l DDE, op^l DDD, pp^l DDD, op^l DDT, pp^l DDT, 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 100µg/ml and diluting to a concentration of 1.0 µg/ml with iso-octane. The standard solution was stored in standard stoppered tubes at 4°C in refrigerator.

Method validation and quality control: To check the sensitivity and linearity of the instruments, a 5-point external standard calibration using individual and standard mixture at levels from 0.001µg/ml to 0.01µg/ml was performed initially to establish the detector (ECD) linear range.

Linear regression equation was used to quantify analytes in samples. Calibration of gas chromatograph was done before sample analysis using the standards of pesticides obtained from authentic sources. Qualitative and quantitative analyses were performed by comparing the retention time and peak area of the samples, respectively, with those of the calibrated reference standards.

The mean recovery was determined spiking feed and fodder, previously analyzed so as to ensure the absence of pesticide residues at different levels, using the same extraction and clean up method as done in actual samples. The levels of spiking were 0.001 to 0.01µg/g and the average recovery values were found from 81 to 97.7% varying in different compounds. The limit of detections was 0.001µg/g for HCH (α, γ, δ), 0.002 µg/g for endosulfan (α, β, sulfate), 0.003 µg/g DDT (op^l and pp^l isomers of DDE, DDD and DDT) and 0.005 for β-HCH and dicofol.

RESULTS AND DISCUSSION

Out of 318 (288 feed and 30 green fodder) samples, 110 (98 feed and 12 fodder) samples i.e. 34.6% of the total samples showed the presence of residues of different OCPs like HCH, DDTs, endosulfan and dicofol. The extent of contamination in case of individual samples varied from 17 to 56%, lowest and highest being SSC and CC respectively (Fig. 1).

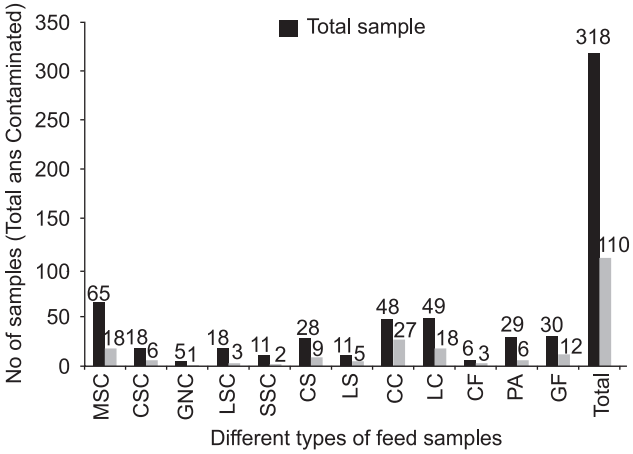

Fig. 1. Level of contamination of different feed samples with OC pesticide residues.MSC, Mustard seed cake; CSC,cotton seed cake; GNC, groundnut cake; LSC, linseed cake; SSC, sesamum seed cake; CS, cereal straw; LS, legume straw; CC, cereal *chunni*; LC, legume *chunni*; CF, cereal flour; PA, *Pashu ahar*; GF, green fodder.

HCH residues: HCH residues were present in 26.73% of the total samples (Table 1). Among the HCH isomers, α and γ-HCH were more frequently encountered than β and δ-HCH. Although β-HCH is considered as the most persistent among all the HCH isomers, it could neither be recorded in samples of oilseed cake like MSC, CSC, LSC, SSC, GNC nor in cereal flour and *pashu ahar*. But, the concentration of β-HCH was highest among all the isomers of HCH detected and the difference from other isomers was statistically significant ($P < 0.05$).

The mean total HCH concentration varied from 0.004 to 0.169 µg/g in different feed and fodder and such concentration of HCH as found in this analysis was also observed in feed samples over different places in earlier studies (Singh *et al.* 1997, Gupta *et al.* 2000, Nag and Raikwar 2011). Out of 5 types of green fodder samples tested, HCH residues were present in 2 types of samples, viz. mustard and paragrass. In straws, *chunnies* and green fodders all 4 isomers were detected.

Endosulfan residues: Endosulfan residues were present in all kinds of samples excepting GNC and SSC. Both the isomers of endosulfan i.e. a and b and the toxic metabolite endosulfan sulfate were present in most of the contaminated samples, but in CSC only β-endosulfan was detected. Among the different fodders analysed, endosulfan was found positive in sorghum and paragrass. The concentration of endosulfan residues in positive samples was, however, very meager and mean total endosulfan, which is summation of endosulfan α, endosulfan β and endosulfan sulfate present in a single sample, varied from 0.003 to 0.022 µg/g (Table 2). There was no significant difference in the mean concentration of α, β and endosulfan sulfate at 5% level of significance. Presence of endosulfan residues in feed and fodder is nothing unusual, but earlier studies of monitoring

Table 1. Concentration of HCH isomers and total HCH (μg/g) in feed samples

Samples	α-HCH	β-HCH	γ-HCH	δ-HCH	Σ-HCH
Mustard seed cake (n, 65)	0.001–0.019* (0.006±0.007)**	–	0.002–0.0261 (0.009±0.008)	0.0015–0.012 (0.005±0.004)	0.001–0.031 (0.01±0.01)
Cotton seed cake (n, 18)	0.001–0.004 (0.003±0.001)	–	0.002–0.006 (0.0035±0.002)	0.001–0.0011 (0.001±0.00005)	0.001–0.007 (0.004±0.002)
Ground nut cake (n, 5)	0.016	–	–	–	0.016
Linseed cake (n, 18)	0.002–0.016 (0.006±0.007)	–	0.002–0.004 (0.002±0.0009)	0.003	0.003–0.021 (0.01±0.008)
Sesamum seed cake (n, 11)	0.0029	–	0.002–0.005 (0.004±0.002)	–	0.0046–0.0053 (0.005±0.0004)
Cereal straw (n, 28)	0.001–0.003 (0.002±0.0007)	0.052–0.43 (0.241±0.189)	0.001–0.005 (0.002±0.0017)	0.001–0.003 (0.002±0.0009)	0.001–0.433 (0.064±0.15)
Legume straw (n, 11)	0.001–0.026 (0.01±0.01)	0.177–0.456 (0.316±0.139)	0.001–0.01 (0.005±0.0033)	0.001–0.005 (0.003±0.0017)	0.001–0.497 (0.169±0.192)
Cereal *chunni* (n, 48)	0.004–0.092 (0.034±0.029)	0.089–0.639 (0.257±0.223)	0.001–0.039 (0.012±0.012)	0.001–0.068 (0.011±0.019)	0.001–0.663 (0.091±0.136)
Legume *chunni* (n, 49)	0.001–0.011 (0.003±0.0029)	0.089	0.001–0.132 (0.013±0.033)	0.001–0.006 (0.004±0.0021)	0.002–0.138 (0.022±0.039)
Cereal flour (n, 6)	0.002–0.003 (0.003±0.00005)	–	0.002–0.003 (0.003±0.00045)	–	0.005–0.006 (0.006±0.0005)
Pashu ahar (n, 29)	0.001–0.018 (0.008±0.007)	–	0.001–0.009 (0.004±0.0032)	–	0.002–0.027 (0.009±0.009)
Green fodder (n, 30)	0.001–0.002 (0.0015±0.0005)	0.004	0.003–0.004 (0.004±0.0005)	0.002	0.004–0.008 (0.006±0.001)

* Range (Minimum – Maximum); **, figures in parenthesis denote mean±sd.

Table 2. Concentration of endosulfan isomers and sulfate (μg/g) in feed sample

Samples	α-Endosulfan	β- Endosulfan	Endosulfan sulfate	Σ-Endosulfan
Mustard seed cake (n, 65)	0.002–0.016* (0.007±0.0054)**	0.001–0.030 (0.006±0.0094)	0.001–0.004 (0.0024±0.0013)	
Cotton seed cake (n, 18)	–	0.0043	–	0.0043
Ground nut cake (n, 5)	–	–	–	–
Linseed cake (n, 18)	0.0035	0.0017	–	0.0052
Sesamum seed cake (n, 11)	–	–	–	–
Cereal straw (n, 28)	0.001	0.0018	0.0128	0.0128
Legume straw (n, 11)	0.001–0.004 (0.003±0.001)	0.0018–0.01 (0.005±0.004)	0.0022	0.004–0.107 (0.007±0.002)
Cereal *chunni* (n, 48)	0.001–0.016 (0.005±0.006)	0.001–0.004 (0.0017±0.0012)	0.001–0.005 (0.003±0.002)	0.001–0.017 (0.007±0.006)
Legume chunni (n, 49)	0.001–0.007 (0.003±0.002)	0.002	–	0.002–0.03 (0.009±0.01)
Cereal flour (n, 6)	0.001	0.002	–	0.003
Pashu ahar (n, 29)	0.003	0.001–0.002 (0.0015±0.0005)	–	0.0051
Green fodder (n, 30)	0.005–0.014 (0.008±0.004)	0.003–0.016 (0.007±0.005)	0.002–0.02 (0.007±0.007)	0.011–0.05 (0.022±0.016)

* Range (Minimum – Maximum).**, figures in parenthesis denote mean±sd.

mostly ignored it and only concentrated on DDT and HCH. Prasad and Chhabra (2001) found that endosulfan constituted 8% of total organochlorine pesticide residues in overall feed and fodder samples collected from Karnal, India. In the present study, 11.95% samples were contained endosulfan residues. Endosulfan was also detected in feed concentrate samples at Jorhat in Asom (Deka *et al.* 2004) and at Ludhiana in Punjab, India (Kang *et al.* 2002).

DDT and dicofol residues: Residues of DDT expressed as total DDT, represented by op' and pp' isomers of DDT and its isomers/metabolite DDD and DDE, as they were detected in a sample either singly or in different combinations, were found present in 11.64% of total samples tested, except CSC, GNC and CF in which they

were totally absent. In samples of cereal straw and cereal *chunni* all the components of total DDT were present. In general, it was observed that the pp' isomers of DDE, DDD and DDT were more frequently encountered than their op' counterparts. The mean total DDT concentration in different feed samples varied from 0.0015–0.448 µg/g (Table 3) which may be rated as less compared to those reported by Battu *et al.* (1996). Sharma *et al.* (2005), however, could only detect pp' DDT in the range of 0.007±0.005 µg/g in concentrated feed sample collected from Karnal, Haryana.

Among the different green fodders analysed, DDT residues could be found only in mustard fodder and Setaria grass in the form of op' and pp' isomers of DDT in concentrations varying from 0.001 µg/g to 0.023 µg/g with mean total DDT concentration being 0.024 µg/g. A survey of literature revealed only few reports about the occurrence of pesticide residues in green fodders; Gupta *et al.* (2000) could find HCH residues above the MRL in certain green fodders like pearl millet, guinea grass, mustard, fenugreek, oat, china cabbage and lucerne collected from Jaipur, India but they could not detect DDT in any sample. Prasad and Chhabra (2001) reported HCH as high as 15–44 µg/g and DDT (1.3–2.8 µg/g) and endosulfan (1.9–5.9 µg/g) in certain green fodders from Karnal, India. Kang *et al.* (2002) found HCH (0.03–0.82 µg/g) in all the 14 green fodder samples from Ludhiana, Punjab while DDT (BDL–0.18 µg/

Table 3. Concentration of DDT isomers and total DDT (µg/g) in feed samples

Samples	op DDD	pp DDD	op DDE	pp DDE	op DDT	pp DDT	Total DDT	Dicofol
Mustard seed cake (n, 65)	–	0.026–0.034* (0.03±0.004)**	–	0.002–0.058 (0.021±0.022)	0.019	0.012–0.053 (0.032±0.02)	0.002–0.119 (0.038±0.041)	0.05
Cotton seed cake (n, 18)	–	–	–	–	–	–	–	–
Ground nut cake (n, 5)	–	–	–	–	–	–	–	–
Linseed cake (n, 18)	–	–	–	0.0015	–	–	0.0015	–
Sesamum seed cake (n, 11)	–	0.0094	–	–	–	0.0512	0.0606	–
Cereal straw (n, 28)	0.017	0.0188	0.039	0.237	0.026	0.129	0.448	0.002
Legume straw (n, 11)	–	–	–	0.003–0.007 (0.005±0.002)	–	0.022	0.003–0.022 (0.01±0.008)	–
Cereal *chunni* (n, 48)	0.01	0.002–0.059 (0.031±0.028)	0.003	0.001–0.123 (0.025±0.038)	0.003–0.037 (0.015±0.013)	0.009–0.273 (0.071±0.08)	0.012–0.352 (0.088±0.094)	–
Legume *chunni* (n, 49)	–	0.001–0.015 (0.008±0.007)	–	0.009–0.133 (0.054±0.047)	0.007	0.031	0.024–0.133 (0.054±0.041)	–
Cereal flour (n, 6)	–	–	–	–	–	–	–	–
Pashu ahar (n, 29)	–	0.0024	–	–	–	0.0074	0.0098	0.003
Green fodder (n, 30)	–	–	–	–	0.001–0.013 (0.008±0.005)	0.01–0.023 (0.018±0.006)	0.019–0.033 (0.024±0.025)	–

* Range (minimum – maximum).**, figures in parenthesis denote mean±sd.

Table 4. Number and percentage of samples exceeding the limiting value of HCH isomers and DDT

Sample	Pesticide with their limiting value and no. of samples exceeding it				
	α-HCH (0.12)	β-HCH (0.02)	γ-HCH (0.12)	δ-HCH (0.07)	Σ DDT (0.10)
Mustard seed cake	Nil	–	Nil	Nil	1 (1.54%)
Cotton seed cake	Nil	–	Nil	Nil	–
Groundnut cake	Nil	–	–	–	–
Linseed cake	Nil	–	Nil	Nil	Nil
Sesamum seed cake	Nil	–	Nil	–	Nil
Cereal straw	Nil	2 (7.14%)	Nil	Nil	1 (3.57%)
Legume straw	Nil	2 (18.18%)	Nil	Nil	Nil
Cereal *chunni*	Nil	4 (8.33%)	Nil	Nil	4 (8.33%)
Legume *chunni*	Nil	1 (2.04%)	1 (2.04%)	Nil	1 (2.04%)
Cereal flour	Nil	–	Nil	–	Nil
Pashu ahar	Nil	–	Nil	–	Nil
Green fodder	Nil	Nil	Nil	Nil	Nil

Figure in parenthesis denote percentage of total sample of that particular feed analysed. (–) indicates that no sample of that particular feed contained residue of that particular compound.

g) and endosulfan (BDL-1.58 µg/g) were recorded in 2 and 3 samples only. As compared to those reports wherein very high concentrations were recorded, in the present investigation the concentrations in the positive samples were very meager.

Dicofol, a DDT analogue and an acaricide by itself, was found only 3 samples out of 318, one each in MSC, CS and CF at concentration from 0.002 to 0.05 µg/g.

The maximum residue limit or tolerance limit of pesticide in feed materials has not been set in India. However, the limiting value (which may be defined as the concentration of a pesticide in feed and fodder if fed to lactating animals daily, the likely residues in milk will be less than their MRL values and safe for human consumption) of a pesticide in feed can be derived on the basis of its legal permissible limit in milk and its rate of transference from feed to milk. Based on short term feeding experiments on buffaloes, the transfer coefficient of HCH isomer and DDT complex were recommended (Kalra *et al.* 1986, Kapoor and Kalra 1988, 1993). Using these values, the limiting values of different pesticides in feed were calculated. Thus, the limiting values of α-HCH, β-HCH, γ-HCH, δ-HCH and total DDT were found to be 0.12, 0.02.0.12, 0.07 and 0.10 µg/g, respectively (Kang *et al.* 2002). The residue data of feed samples from 4 different places were compared with respect to the above limiting values (Table 4). In α and δ-HCH, concentration levels in contaminated feed samples were below the threshold limit. In respect of β-HCH, 2 samples each of cereal straw (7.14%) and legume straw (18.18%), 1 (2.04%) legume *chunni* and 4 cereal *chunni* (8.33%) samples contained residues higher than its limiting value of 0.02 µg/g. Only one sample (2.57%) of legume *chunni* had γ-HCH concentration above its limiting value. The limiting value of total DDT (0.10 µg/g) exceeded in 1 sample each of MSC (1.54%), cereal straw (3.57%) and legume *chunni* (2.04%), and 4 samples (8.33%) of cereal *chunni*. So, out of a total of 318 samples analysed, only 10 (3.14%) and 7 (2.20%) samples of feed had concentration of HCH and DDT above their limiting values, respectively, and the rest of the samples were either free from those residues or their concentrations were below the respective limiting values. For endosulfan, the data could not be compared as the limiting value was not available.

Pesticide residues can be present in animal feed and fodder resulting from the use of pesticides in the field during crop production and/or during storage and transportation of the materials from one place to another. From the present study it was revealed that about 34.6% samples were containing residues of few OC compounds. Among different OCs occurrence of HCH was more frequent (26.73%) while DDT and endosulfan encountered almost at par, each around 12%. But the levels of residues in the contaminated samples were well below the established tolerance levels. So they would be safe for animal consumption from the view point of cross contamination to milk or meat to be used for human consumption. Regular monitoring of feed samples is necessary for checking the status of residues since contaminated feed and fodder act as the main source of entry of pesticide in animal body.

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