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Research Article

OCCURRENCE OF SOME ORGANOCHLORINE PESTICIDE RESIDUES IN POULTRY FEED AND MEAT

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ABSTRACT

In the present study, gas chromatography-electron capture detector technique was standardized for the simultaneous detection and quantitation of residues of organochlorine pesticides viz., HCH, lindane and endosulfan from poultry feed and meat. Validation parameters showed that the values for the system precision (% RSD for all analytes were <0.1% for area and 0.04% for retention time), linearity (r^2>0.99), specificity and accuracy (83.7- 92.7% and 84.3– 92.0% in feed and meat, respectively) were within accepted range and demonstrated system suitability for analysis of farm samples. A total of 50 samples each of poultry feed and meat collected from poultry farms of Hisar, Haryana were analyzed for the detection of HCH, lindane and endosulfan sulphate. Mean concentration of ΣHCH in poultry feed and meat was 618.281 and 310.368 µg/kg, respectively, and that of Σendosulfan in poultry feed and meat were 135.626 and 195.057 µg/kg, respectively. Comparison of pesticide concentration in each positive sample of meat with national and international MRLs showed that lindane was responsible for maximum violations. It was concluded that the poultry feed may be a significant source of pesticide residues in poultry meat.

Key words: Pesticide residues, HCH, lindane, endosulfan, chicken meat, poultry feed

India is world's 5th largest producer of poultry meat (Thaper, 2010). Despite of nutritional importance of poultry products, its food safety aspect is a matter of great concern. A large number of pesticides being used in the agriculture sector lead to indirect exposure of farm animals including poultry birds through feed and water. It leads to accumulation of pesticide residues in animal/ poultry products and subsequent dietary exposure of human beings. This low dose-long time exposure has been linked epidemiologically with adverse health effects such as immunosuppression, hormone disruption, diminished intelligence, reproductive abnormalities and cancer (Crisp *et al*., 1998; Hurley *et al.,* 1998; Khurana and Chauhan, 2005). The lipophilic nature, hydrophobicity and low chemical and biological degradation rates of organochlorine (OC) pesticides have led to their widespread accumulation in food chain (John *et al.,* 2001; Bedi *et al.,* 2005; Aulakh *et al.,* 2006) and subsequent magnification of concentrations in human, a topmost consumer in the food chain (Surendernath *et al.,* 2000).

Presence of pesticide residues in poultry meat and eggs has considerable international implications because poultry products are traded throughout the world (Lovell *et al*., 1996). Perusal of the pesticide residue data in India indicated their presence in vegetables as wells as foods of animal origin (Bhushan, 2006). Most of the commonly encountered residues of pesticides in food are OCs followed by organophosphates and carbamates (Kulkarni and Mitra, 1990). In India, especially Haryana, there is paucity of reports related to occurrence of pesticide residues in poultry feed/ feed ingredients and meat. Therefore, the present investigation was planned with the objective to standardize the gas chromatography (GC) technique for the detection and quantification of OC residues.

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MATERIALS AND METHODS

Collection of Samples: The present work was carried out in the Department of Veterinary Public Health and Epidemiology, LUVAS, Hisar. Fifty samples each of poultry feed/ feed ingredients and chicken meat were collected in polyethylene bags from various poultry farms in and around district Hisar of Haryana.

Chemicals and Reagents: Pesticide reference standards (α-HCH, β-HCH, γ-HCH (lindane), δ-HCH, α-endosulfan, β-endosulfan, endosulfan sulphate) of purity >98.0% and chemicals viz. aluminium oxide, ethyl acetate and HPLC grade n-hexane were procured from, Sigma Aldrich Corporation, Bengaluru, India. Whereas, petroleum ether (60^0C), anhydrous sodium sulphate were procured from Sisco Research Laboratories Pvt. Ltd., Mumbai, India.

Preparation of Reagents: Reagents were prepared and calibrated as per the instructions given in Pesticides Residues Manual (AMPRF, 1996). Stock solution of each pesticide (primary standard solution) was prepared in n-hexane. For selection of working range of concentrations, maximum residue limits (MRLs) prescribed by Food

Safety and Standard Authority of India (FSSAI, 2011), European Union (EU, 2006), Codex Alimentarius Commission of WHO (Codex, 2006) were considered. Based on the MRLs, a linearity range (50, 100, 150, 200 and 250 ng/ml) was selected to cover the lowest MRL prescribed. Then appropriate dilutions of secondary standard solution in n -hexane were made to produce a required dilution of working solution.

Sample Extraction and Cleanup: Laboratory method for detection of pesticide residues in feed and meat was standardized as per Pesticide Residue Manual (AMPRF, 1996). Briefly, samples were triturated in a mortar and pestle with sodium sulphate to remove moisture and then Soxhlet extracted with petroleum ether. Fat obtained was subjected to cleanup procedure with aluminum oxide as adsorbent and petroleum ether as eluting solvent by open column chromatography.

Chromatographic Analysis: A Shimadzu gas chromatograph model GC 2010 Plus equipped with auto sampler AOC-20i mounted on a split /splitless injector port connected to ^{63}Ni electron capture detector through Equity 5 capillary column (30 m x 0.25 mm I.D.) was used in the study for analysis of OC pesticides. Initial temperature of column was adjusted to 60°C and held for 0.5 min., then temperature was raised at the rate of 20°C/min to 204°C, 2°C/min to 208°C, 0.5°C/min to 210°C then 20°C/min to 300°C held for 3 min. The total run time was 21.2 min. Split ratio used was 1:47 with column flow at the rate of 4.0 ml/min. Injector and detector temperature was set to 250°C and 320°C, respectively and the injection volume was 1 µl.

Both laboratory and instrumental methods were standardized and validated as per harmonized tripartite guidelines on validation on analytical procedures: methodology (ICH, 1998) and taking into consideration the performance criteria and other requirements for analytical methods laid down by EU (2002).

RESULTS AND DISCUSSION

Standardization and Validation Studies of GC: The system precision was evaluated by studying the reproducibility of the instrumental response with respect to retention time and area of an analyte. Percent Relative Standard Deviation (% RSD) for all analytes was found to be less than 0.1% for area and 0.04% for retention time. Specificity was evaluated by visual observation of chromatograms of blank sample matrix and sample matrix spiked with standard mixture. For poultry feed and meat, chromatographic signals at the retention times of pesticides were absent in blank sample matrix.

The standard calibration curves of the analyzed OC pesticides presented a good regression line (r^2>0.99) in the range of explored concentrations i.e., from 50 to 250 ηg/ml. The graphs showing calibration curve of these pesticides revealed that all concentrations of the OC pesticides under study were collinear and thus calibration curves were further employed for the detection of analytes under study. Limit of detection (LOD) and Limit of quantitation (LOQ) were determined on the basis of standard deviation of the blank sample. Measurement of the magnitude of the analytical background response was performed by the analysis of 10 blank samples and calculating the standard deviations of these responses. Tables 1 and 2 summarizes the LOD and LOQ obtained for each pesticide and for poultry feed and meat. Perusal of tables clearly indicated that the LOD and LOQ for individual analytes were well below their respective MRLs indicating that the method was able to detect the given pesticide at sufficiently low level.

The accuracy in terms of percent recovery of each pesticide in OC pesticide group at five different fortification levels (50, 100, 150, 200 and 250 µg/kg) was evaluated for poultry feed and meat and the results are presented in Tables 1 and 2, respectively. Satisfactory results were found in almost all instances. Recoveries for all analyte-matrix combinations ranged between 83.7- 92.7% in feed and 84.3-92.0% in meat. In general, method showed acceptable recoveries of spiked pesticides within the validation interval as per EU legislation (EU, 2002) i.e. between 70% and 110%. The chromatograph of standard mixture of OC pesticides is shown in Fig 1.

Table 1
Method performance parameters for detection of residues in poultry feed for OC pesticides

Analyte	Limit of detection (mg/kg)	Limit of quantitation (mg/kg)	Accuracy (average recovery %)	Precision (average CV %)
α-HCH	0.0215	0.0531	89.542	10.387
β-HCH	0.0050	0.0111	92.718	5.486
γ-HCH	0.0225	0.0584	91.716	6.188
δ-HCH	0.0086	0.0225	83.782	11.325
α-Endosulfan	0.0148	0.0386	91.819	5.139
β-Endosulfan	0.0207	0.0608	88.822	9.897
Endosulfan sulfate	0.0221	0.0616	86.991	8.308

Table 2
Method performance parameters for detection of residues in broiler chicken meat for OC pesticides

Analyte	Limit of detection (mg/kg)	Limit of quantitation (mg/kg)	Accuracy (average recovery %)	Precision (average CV %)
α-HCH	0.0039	0.0097	92.012	6.086
β-HCH	0.0018	0.0046	90.032	6.809
γ-HCH	0.0039	0.0100	87.783	7.743
δ-HCH	0.0027	0.0074	86.745	8.074
α-Endosulfan	0.0056	0.0132	89.869	7.778
β-Endosulfan	0.0026	0.0073	84.311	10.387
Endosulfan sulfate	0.0029	0.0079	88.019	10.043

The precision of the method was assessed at five concentration levels (50, 100, 150, 200 and 250 µg/kg) on the basis of recovery studies on spiked samples. Repeatability and intermediate precision values, expressed as relative standard deviation (CV percent) were found less than 11.32 for OC pesticides (Tables 1 and 2).

Overall, the multiresidue method followed for simultaneous detection and quantification of OC pesticide residues in poultry feed and meat was subjected to rigorous validation parameters. The system precision values indicated a good consistency in response by the GC instrument used during present study. A good linearity was noted for standards and spiked poultry feed and tissue samples. Absence of interfering peaks in blank samples indicates good specificity of extraction and clean up method. Accuracy and precision of the method were in accepted range as per international guidelines. These results of validation study clearly demonstrated that the present method is suited for routine analysis of OC pesticides from poultry feed and meat samples.

OC Residues in Poultry Feed and Meat: Validated method was applied for analysis of 50 samples each of poultry feed and meat collected from poultry farms in and around of Hisar. The overall samples positive for OC pesticide residues are presented in Table 3. Figs. 2 and 3 show the chromatographs of poultry feed and meat samples found positive for OC pesticides.

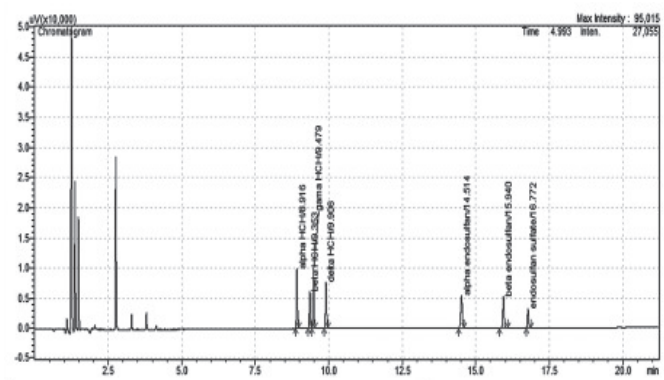

Fig 1. Standard mixture of seven OC pesticides in the concentration of 100 µhg/ml

Mean concentration along with range for each pesticide in poultry feed and meat is provided in Tables 4 and 5, respectively. The results revealed that mean concentrations of ΣHCH pesticides in poultry feed and meat were 618.281 and 310.368 µg/kg, respectively. In case of Σendosulfan, concentrations in poultry feed and meat were 135.626 and 195.057 µg/kg, respectively. Amongst HCH isomers, the presence of α-HCH and lindane in feed samples may be due to the contamination of poultry feed ingredients by carryover of residues in soil due to past use. The residue levels of HCH found in poultry feed samples analyzed in the present study were lower than those reported by others viz. 0.65 mg/kg and 0.83 mg/kg in poultry feed from Punjab, India (Aulakh *et al.*, 2006, 2007); 32-47 ng/g of β-HCH; 28-41 ng/g for γ-HCH in poultry feed from Serbia (Skrbic, 2007). The contamination levels of different constituents of poultry feed with organochlorine pesticides were studied in detail in India by Rao (1990). The residue levels of ΣHCH found in poultry meat in present study were lower than those reported by others viz. 3.879 mg/kg HCHs in chicken body fat from India (Kaphalia and Seth, 1981) and 100 µg/kg of ΣHCHs in chicken fat from Bangkok (Tanabe *et al.*, 1991) but higher than reported by others viz. 0.11 mg/kg HCHs in chicken muscle from India (Aulakh *et al.*, 2006); 0.03 µg/kg in chicken muscle from Shangai, China (Nakata *et al*., 2002). The residue levels of endosulfan found in poultry feed in present study were lower than those reported by others (Kang *et al.,* 2002; Aulakh *et al*., 2007). However, the residue levels of

Table 3
Number of poultry feed and meat samples positive for OC pesticide residues

Pesticides	Feed (n=50)	Meat (n=50)
α-HCH	39 (78%)	33 (66%)
β-HCH	3 (6%)	5 (10%)
γ-HCH	35 (70%)	34 (68%)
δ-HCH	4 (8%)	15 (30%)
α-Endosulfan	13 (26%)	28 (56%)
β-Endosulfan	1 (2%)	2 (4%)
Endosulfan sulfate	8 (16%)	4 (8%)

Table 4
Mean concentration of OC pesticide residues in poultry feed

Pesticides	Mean conc. on wet wt. basis (µg/kg)	Mean conc. on fat wt. basis (µg/kg)	Range conc. on wet wt. basis (µg/kg)	Range conc. on fat wt. basis (µg/kg)
α- HCH	12.765	187.295	2.996 - 53.697	144.661- 541.439
β- HCH	0.566	15.387	7.983 - 28.293	194.707- 318.588
γ- HCH	20.266	399.311	3.834 - 498.282	157.035 - 12153.22
δ- HCH	0.996	16.287	6.889 - 24.731	103.959 - 256.048
α- Endosulfan	4.409	64.035	6.531 - 52.779	122.417- 482.97
β- Endosulfan	0.581	7.884	29.074	394.223
Endosulfan sulfate	4.666	63.707	7.863-79.928	65.144- 1083.769

Table 5
Mean concentration of OC pesticide residues in poultry meat

Pesticides	Mean conc. on wet wt. basis (µg/kg)	Mean conc. on fat wt. basis (µg/kg)	Range conc. on wet wt. basis (µg/kg)	Range conc. on fat wt. basis (µg/kg)
α- HCH	3.613	128.174	1.134 - 15.825	88.529 - 404.591
β- HCH	0.577	19.135	3.512-9.352	122.797-269.51
γ- HCH	3.814	135.532	0.524-18.324	13.628-478.434
δ- HCH	0.842	27.528	1.273-5.884	56.217-144.57
α- Endosulfan	3.864	150.298	1.621-21.828	123.875-536.314
β- Endosulfan	0.204	11.011	3.901-6.328	235.709-314.826
Endosulfan sulfate	1.024	33.748	7.889-24.896	249.003-753.283

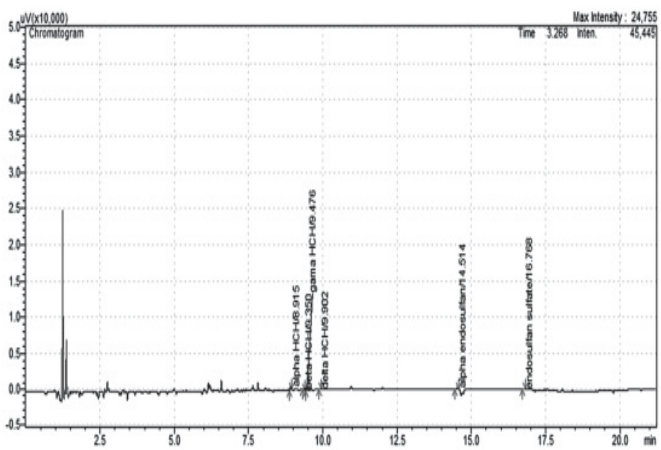

Fig 2. Chromatograph of poultry feed field sample showing presence of OC pesticides

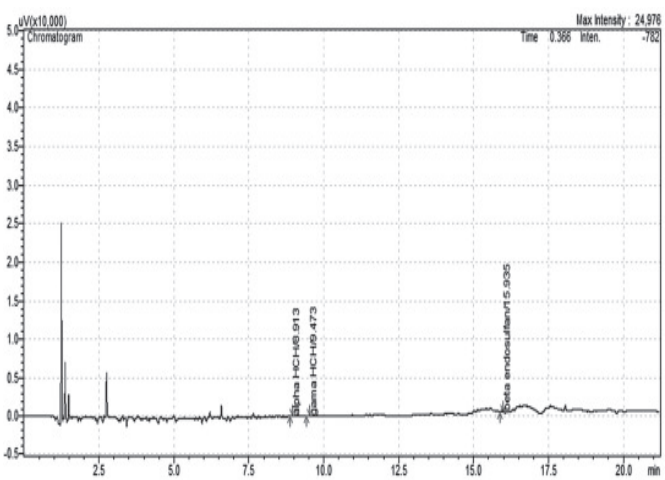

Fig 3. Chromatograph of poultry meat field sample showing presence of OC pesticides

endosulfan found in poultry feed in present study were higher than those reported by others (Deka *et al.,* 2004; Nagra, 2006). The residue levels of endosulfan found in poultry meat in present study were higher than those reported by various workers (Aulakh *et al.*, 2006; Jadhav, 2008).

Comparison of OC Residues with National and International MRLs: The results showing number of samples with violative concentration are presented in Table 6. FSSAI MRLs are available for lindane but not for other isomers of HCH and endosulfan (FSSAI, 2011). As per these MRLs, none of the poultry meat samples exceeded the given tolerance of lindane. According to MRLs given by Codex and EU, lindane was found to be responsible for maximum violations (66%) followed by endosulfan (60%). EU MRLs are separately mentioned for α and β isomers of HCH. According to these MRLs, 24% and 10% samples violated MRLs for α-HCH and β-HCH, respectively.

Overall, based on frequency of detection and concentration of various analytes of OC pesticides, it can

Table 6
Comparison of OC pesticide residue levels in poultry meat field samples with national and international MRLs

Pesticides	Number of samples with violative MRL concentration (mg/kg)		
	FSSAI	Codex	EU
α- HCH	NA	NA	12 (24%)
β- HCH	NA	NA	5 (10%)
γ- HCH	0	33 (66%)	33 (66%)
δ- HCH	NA	NA	NA
Endosulfan	NA	30 (60%)	30 (60%)

be stated that poultry meat samples were laden with all pesticides included in study. The rate of accumulation in poultry meat was correlated well with that of rate of occurrence in poultry feed. From the findings of the present study, it can be concluded that the poultry feed may be a source of pesticide residues in poultry meat. Although as per PFA MRLs, there appeared no hazard by consuming poultry meat and organ tissues for Indian consumers, the export of meat requires constant monitoring to avoid rejection of poultry meat and meat products consignments.

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