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Organochlorine Pesticide Residues in Broiler and Desi Chicken Meat of Hyderabad

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ABSTRACT

An experiment was conducted to estimate certain organochlorine pesticides (DDT-DichlorodiphenylTrichloroethane, HCH – Hexachloro Cyclo Hexane and Cyclodiene compounds (aldrin, dieldrin, endrin, endrin aldehyde, endosulfan, endosulfansulphate, heptachlor and heptachlor epoxide) residues in meat samples of broiler and desi chicken collected from retail markets of Hyderabad. A total of 60 samples (each 15 meat and fat samples of broiler and desi chicken) were analysed for the presence of organochlorine pesticides (OCPs) residues using a gas chromatograph equipped with an electron capture detector. The percentage of contamination was higher in broiler meat samples (70 percent) compared to desi chicken (6.66 percent). Among the OCPs - DDT, HCH, aldrin, endrin, heptachlor and endosulfan residues were detected in broiler meat where as, only DDT and HCH residues were detected in desi chicken meat. The overall concentration of DDT, HCH, aldrin, endrin, heptachlor and endosulfan residues in broiler meat were 0.101, 0.167, 0.085, 0.035, 0.02 and 0.057 ppm, respectively and the overall concentration of DDT and HCH residues in desi chicken meat were 0.05 and 0.03 ppm, respectively. The study revealed that the market samples of desi chicken meat had lower incidence and levels of residues as compared to that of broiler chicken meat samples and the concentration of pesticide residues in both broiler and desi chicken were higher in fat samples compared to meat samples. Further, the levels of pesticide residues recorded in the study were lower than the maximum residue limit prescribed by Food Safety Standards Regulations (Contaminants, toxins and Residues) 2011.

Key words : *Organochlorine pesticide residues, Broiler chicken, Desi chicken, Meat, Fat*

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INTRODUCTION

Chicken meat has become very popular among meat consumers in India because of its taste, nutritional value, free from religious taboos, affordable price and easy availability. Chicken meat production in India is estimated at around 2.33 MMT and thus regarded as the fifth largest in chicken meat in the world.

The presence of chemical residues, especially pesticides in meat has been an important issue, because of possible transfer and accumulation of these chemicals in the human body, which results in health problems, particularly endocrine dysfunction, birth defects, carcinomas, neurological disorders and weakening of the immune system (Brody and Rudel 2003). Pesticides are one of the important chemical contaminants of foodstuffs, especially those having high fat content such as meat and milk products. Among the pesticides, the residues of organochlorine pesticides such as DDT (Dichloro Diphenyltrichloroethane) and HCH (Hexachloro Cyclo Hexane) pesticides were extensively noticed in foodstuffs due to their higher stability and persistence in the environment. Periodical screening foodstuffs for the presence of various organochlorine pesticides residues is therefore necessary to ensure the public health. Studies carried out to investigate the relative presence of pesticide residues on organic as opposed to conventional products show lower presence of pesticide residues in organic food, although organic food may not be defined as pesticide free (Kouba 2003). It is believed that the desi chicken, which are raised in the backyards carry less pesticide burden because they feed on natural vegetation, insects and not commercial feed, which is an important source of residues. However, the paucity of information is available on the levels of pesticide residues in desi chicken raised in free range systems in India. Hence, a study was carried out to estimate the levels of residues of organochlorine pesticides viz., DDT and it's metabolites (p,p'DDT - para para Dichloro Diphenyl Trichlore ethane ; p,p'DDE - para para Dichlorodiphenyl Dichlore Ethane and p,p'DDD - para para Dichloro Diphenyl Dichloroethy lene) and HCH (α, β, $\gamma$and δ isomers) and cyclodiene compounds (aldrin, dieldrin, endrin, endrin aldehyde, endosulfan, endosulfansulphate, heptachlor and heptachlor epoxide) in the meat samples of broiler and desi chicken.

MATERIALS AND METHODS

A total of 60 samples, each 15 meat and fat samples of broiler and desi chicken were randomly collected from retail markets of Hyderabad. Approximately 100 gm of meat (leg and breast) and 10 gm of fat from each broiler (6 weeks of age) and desi chicken were collected from local markets at random and

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packed in clean polythene bags. The packaged samples were then labelled and transported in ice to the National Research Centre on Meat, Chengicherla, Hyderabad and processed immediately or stored at –20^{0}C until analysis.

The extraction of fat, partitioning and clean up of the pesticides from the meat and fat samples were done as per AOAC (1995). The extracted fat was diluted with 15 ml petroleum ether and extracted thrice with 30 ml of acetonitrile saturated with petroleum ether in a 125 ml separator. Each time, the acetonitrile portion was drained into a one litre separator containing 650 ml water, 40 ml saturated sodium chloride solution and 100 ml petroleum ether. The one litre separator containing the acetonitrile extracts was shaken thoroughly. The aqueous layer separated was drained into another one litre separator to which 100 ml petroleum ether was added and shaken thoroughly with care (back extraction into petroleum ether). After discarding the aqueous layer, the petroleum ether portion was combined with that in the first one litre separator, washed with two 100 ml portions water and the washings were discarded. The cleanup of samples to remove the residual fat was performed by column chromatography method using activated anhydrous sodium sulphate and Florisil. Elution was carried out with 75 ml of 6 per cent eluting solvent at 40-45 drops per minute followed by 75 ml of 15 per cent eluting solvent at 40-45 drops per minute. The elute was collected in a 500 ml concentrating flask and dried completely in a vacuum evaporator. Reconstitution based on the concentration of the residues with n-hexane of known volume was carried out.

One micro litre of the reconstituted sample was injected into a gas chromatograph (Varian 450 GC, Netherlands) equipped with an electron capture detector (ECD). Instrumental settings were as follows: Temperature of injection port, column oven and detector were 260, 80-260 and 300^{0}C, respectively with N_2 gas flow rate of 1 ml/ minute. This injection mode was split 1:10 ratio. The retention time along with the areas of the peaks were recorded. The organochlorine pesticides were quantified from individually resolved peak areas with corresponding peak areas of standards. For every set of ten samples, a procedural blank consisting of all reagents and glassware used during analysis was run for interference and cross contamination.

Chicken meat samples were fortified with the working standards (0.01 and 0.1 ppm) of investigating compounds to estimate the recovery by following the procedure described above to ascertain the efficiency of extraction. The data obtained from the study were statistically analysed and interpreted. Data were subjected to independent samples T-test to determine the differences in the OCPs among the fat and meat samples of broiler and desi chicken. All statistical computations were performed using the SPSS 10.0 for Windows software (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

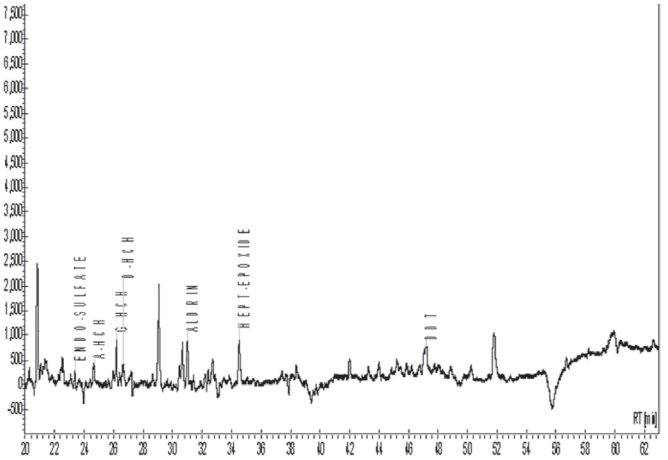
In the present study, the recovery of various organochlorine pesticides from spiked meat samples was above 80% and is in agreement with FDA recommendations (FDA 1994). The efficiency of extraction methodologies were evaluated based on the recoveries of residues and a recovery of 75-102 % is considered as acceptable (Solymos *et al*. 2001). Doong and Lee (1999), Bedi *et al*. (2005) and Muthukumar *et al.* (2010) also reported a similar level of recovery.

The percentage of contamination was higher in broiler meat samples (70 percent) compared to desi chicken (6.66 per cent). In general, the order of pesticide contamination in broiler

Table 1 : Retention time and recovery percentages oforganochlorine pesticides in spiked chicken meat samples

Sl. No.	Name of the pesticide	Mean retention time (min)	Mean recovery (%)
1	Endosulfansulphate	23.03	97.07
2	α HCH	24.45	84.46
3	γ HCH	26.52	89.94
4	δ HCH	26.79	94.32
5	β HCH	28.47	92.31
6	Heptachlor	31.25	88.44
7	Aldrin	33.82	94.57
8	Heptachlor epoxide	36.88	95.09
9	Dieldrin	41.09	96.28
10	p,p'DDE	41.45	103.42
11	Endrin	42.45	87.09
12	Endrin aldehyde	44.26	89.56
13	Endosulfan	44.48	97.09
14	p,p'DDD	46.03	101.63
15	p,p' DDT	46.54	101.19

Fig 1: Chromatogram showing organochlorine pesticides residues in broiler Chickenfat



chicken was HCH > DDT > Aldrin > Endosulfan > Endrin > Heptachlor, whereas in desi chicken, only DDT and HCH residues were detected and the order of contamination was DDT > HCH.

The residual concentrations of Σ DDT (sum of p,p'DDE, p,p'DDD and p,p'DDT) was in the range of 0.046 to 0.155 ppm. The overall concentration of Σ DDT was 0.101 and 0.050 ppm, respectively in broiler and desi chicken. A similar level of contamination of broiler chicken with DDT was also reported by Kalra and Chawala (1981) from Ludhiana, Punjab. The overall incidence of Σ DDT was higher in broiler chicken (43.33 percent) compared to desi chicken (3.33 percent). Higher incidence of contamination in broiler chicken compared to desi chicken might be due to the high fat content in the broiler chicken (3.2 % compared to 1.65 %) which favors accumulation of more amounts of strongly lipophilic organochlorine compounds. Gannon *et al.* (1959) also stated that the insecticide residues in various tissues are directly proportional to their fat content. Widespread contamination of the grains, oilseeds and animal feed, which are the major sources of OCPs contamination in broiler chicken might be the reason for higher levels of DDT residues in broiler chicken compared to desi chicken. Further, Aulakh *et al*. (2006) also detected DDT residues in poultry feed at a level of 0.91 ppm. The contamination of soil and water bodies could be the reason for the presence of very low level of pesticide residues in free range desi chickens. This is in accordance with Kannan *et al*. (1992) and Bedi *et al*. (2005), who opined that the extensive use of DDT in agriculture in the past coupled with its unique chemical stability was the reason for widespread contamination. Ghidini *et al.* (2005) also noticed the presence of residues of DDT at very low level (3 to 6.23 ppm) in organic beef from Northern Italy.

The residual concentrations of total DDT in chicken tissues noticed in the present study was very low and in all cases were below the maximum residue limit of 7 ppm specified by Food Safety Standards Regulations (Contaminants, toxins and Residues) 2011. However, a low level of contamination was reported in Great Briton (0.01-0.09 ppm; Findlay and Hamilton 1968) and Italy (0.061 ppm; Madarena *et al*. 1980). The regulated pesticide usage and regular monitoring programme in these countries might be the reason for the presence of low level of pesticide residues in animal tissues.

Among the metabolites of DDT, p,p'DDT was the most prominent in broiler chicken followed by p,p'DDE and p,p'DDD. However, in case of desi chicken only p,p'DDT metabolite was detected. Even though the mean residual concentration of p,p'DDE, p,p'DDD and p,p'DDT were higher in fat (0.025, 0.07 and 0.06 ppm, respectively) compared to meat (0.010, 0.02 and 0.016 ppm, respectively), there was a no statistical significant variation due to larger sample variation. Kannan *et al.* (1992) also observed wide variation in the levels of DDT contamination (trace to 0.15 ppm) in meat and animal fat. Higher level of DDT metabolites noticed in fat tissues is in accordance with Singh *et al*. (1970), who demonstrated that fat was the major site of OCPs accumulation in body. Overall, the incidence of p,p'DDT were more as compared to p,p'DDE and p,p'DDD. Higher levels of p,p'DDT and p,p'DDE among the DDT residues in meat was also observed by Madarena *et al*. (1980), Kannan *et al*. (1992) and Wani *et al*. (1998).

The mean residual concentrations of total HCH noticed in the present study were ranged between 0.01 and 0.20 ppm. The level of contamination of chicken tissues with HCH is comparable with data reported by Kalra and Chawala (1981), Aulakh *et al*. (2006) and Muthukumar *et al.* (2010). However, low levels of HCH contamination of broiler chicken samples were reported from Great Briton (Findlay and Hamilton 1968) and Itally (Madarena *et al*. 1980).

The overall incidence and mean residual concentration of Σ HCH (sum of α HCH, β HCH, γ HCH and δ HCH) was higher in broiler (30 per cent and 0.167 ppm) compared to desi chicken (3.33 percent and 0.030 ppm). Kannan *et al*. (1992) reported that the extensive use of technical grade HCH (α HCH 60 %, β HCH 5-6 %, γ HCH 13 % and δ HCH - 5-6 %) in agriculture in the past coupled with their high stability and persistence in the environment appears to contribute to the prevalence of HCH residues in animal tissues. Earlier studies have reported the presence of HCH residues in water (Shukla *et al.* 2006) and feed samples (Beura 2012). However, the concentration of total HCH in the samples analysed in the present study is much lower than the recommended maximum limit of 2 ppm set (for lindane alone) by Food Safety Standards Regulations (Contaminants, toxins and Residues), 2011.

Among the various isomers, the occurrence of α HCH (16.66%) was more followed by δ (10%), β (6.66%)and γ (3.33%) in broiler chicken. In case of desi chicken samples, only α (3.33 %) and γ (3.33 %) isomers were detected. Muntean *et al*. (2003) also reported α HCH to be the most persistent among different HCH isomers in different meats, eggs and milk samples. The higher incidence of α HCH indicates the possibility of misuse of technical grade HCH or commercially available γ HCH may also contain various other isomers. Similarly, Kalra and Chawla (1981) reported more level of γ HCH. However, Aulakh *et al*. (2006) found the highest concentration of α HCH in feed and β HCH in chicken egg and muscles among HCH isomers and opined that possibility of misuse of technical grade HCH

Table 2:Pesticide residue levels (ppm) in tissues of broiler and desi chicken meat

Pesticide residue	Broiler chicken				Desi chicken				Maximum residue limit		
	Meat	Fat	Overall mean	Overall percent of occurrence	Meat	Fat	Overall mean	Overall percent of occurrence	FSSA	FSIS	-USDA
p,p'DDE	0.010										
	(BDL to 0.01)	0.025±0.011 (0.01 to 0.06)	0.018	16.66	BDL	BDL	BDL	NIL	-	-	-
p,p'DDD	0.02	0.07±0.04									
	(BDL to 0.02)	(0.030 to 0.11)	0.045	10	BDL	BDL	BDL	NIL	-	-	-
	p,p' DDT	0.016±0.003	0.06±0.019	0.038	23.33	BDL	0.05				
	(0.01- 0.12)	(0.01 to 0.02)				(0.05)	0.05	3.33	-	-	-
Total DDT	0.046	0.155	0.101	43.33	BDL	0.05	0.05	3.33			
α HCH	BDL	0.040±0.021 (0.010 to 0.120)	0.040±0.021	16.66	BDL	0.02 (BDL to 0.02)	0.020	3.33	-	-	-
ß HCH	0.010 (BDL to 0.010)	0.058 (BDL to 0.058)	0.034	6.66	BDL	BDL	BDL	NIL	-	-	-
γ HCH	BDL	0.050 (BDL to 0.050)	0.050	3.33	BDL	0.01 (BDL to 0.01)	- 0.010	- 3.33	-	-	-
δ HCH	BDL	0.043±0.033 (0.01 to 0.11)	0.043±0.033	10	BDL	BDL	BDL	NIL	-	-	-
Total HCH	0.010	0.200	0.167	30	BDL	0.03	0.030	3.33	-	-	-
Aldrin	BDL	0.040±0.015 (0.01 to 0.08)	0.040	13.33	BDL	-	-	-	-	-	-
Dieldrin	0.010 (BDL to 0.01)	0.077±0.041 (0.01 to 0.20)	0.043	20	-	-	-	-	-	-	-
ΣAldrin	0.010	0.120	0.085	33.33	-	-	-	-	-	-	-
Endosulfan	0.010 (BDL to 0.01)	0.015±0.005 (0.01 to 0.02)	0.013	10	-	-	-	-	-	-	-
Endosulfansulphate	BDL	0.044±0.017 (0.017 to 0.07)	0.044	10	-	-	-	-	-	-	-
ΣEndosulfan	0.010	0.059	0.057	10	-	-	-	-	-	-	-
Endrin	0.010 (BDL to 0.01)	0.040 (BDL to 0.04)	0.025	10	-	-	-	-	-	-	-
Endrin aldehyde	BDL	0.010 (BDL to 0.01)	0.010	3.33	-	-	-	-	-	-	-
ΣEndrin	0.010	0.050	0.035	13.33	-	-	-	-	-	-	-
Heptachlor epoxide	0.01 (BDL to 0.01)	0.03 (BDL to 0.03)	- 0.02	- 6.66	-	-	-	-	-	-	-

BDL= Belowdetection level

for agriculture purpose. There was no significant difference in the level and prevalence among various isomers of HCH in meat and fat samples of broiler and desi chicken due to larger sample variation.

Residues of cyclodiene pesticides could not be detected in any of the analyzed desi chicken samples. However, the residues of aldrin were detected in 33.33 % of broiler chicken samples with an overall mean concentration ranged from 0.01 to 0.12 ppm. However, the residue levels of aldrin in the analyzed samples are well below the maximum residue limit of 0.2 ppm. The presence of residues of aldrin in feed (Dikshith *et al*. 1989), buffalo milk (Saxena and Siddiqui 1982), meat and fat (Kannan *et al*. 1992), broiler chicken (Muthukumar *et al*. 2010) were already reported in India.

The residues of endosulfan were detected in 10 % of broiler chicken samples with an overall mean concentration ranged from 0.01 to 0.059 ppm. However, the residue levels of endosulfan in the analyzed samples are well below the maximum residue limit of 0.2 ppm. Earlier findings of Dikshith . (1989), Amaraneni *et al*. (2000) and Aulakh *et al*. (2006) clearly depicted the widespread contamination of animal feed, milk, meat with endosulfan residues from different parts of the country.

CONCLUSION

It can be inferred from the present study that the market samples of desi chicken meat had lower incidence and levels of residues as compared to that of broiler chicken meat samples and the concentration of pesticide residues in both desi and broiler chicken were higher in fat samples compared to meat samples. Further, the levels of pesticide residues recorded in the study were lower than the maximum residue limit prescribed by Food Safety Standards Regulations (Contaminants, toxins and Residues) 2011.

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