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DDT and HCH Residues in Muscle and Organs of Buffaloes

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ABSTRACT

Organochlorines (OC) pesticides viz., DDT (Dichloro Diphenyl trichloroethane) and HCH (Hexachloro Cyclo Hexane) residues in muscle and organs of buffaloes collected from Hyderabad city were estimated by gas chromatograph. Majority of the analysed muscle and organs samples of buffaloes contained residues of metabolites of DDT - p,pDDT (para para Dichloro Diphenyl Trichlore ethane), p,pDDD (para para Dichloro Diphenyl Dichloroethylene) and p,pDDE (para para Dichlorodiphenyl Dichlore Ethane) and isomers of HCH (á , â, ã and ä HCH) pesticides. However, the levels of contamination in all the analysed tissue were below the maximum residue limit. Among the residues, the concentration of ä HCH (0.263 ppm) was more followed by p,pDDT (0.156 ppm), p,pDDE (0.114 ppm), â HCH (0.111 ppm), p,pDDD (0.084 ppm), á HCH (0.061 ppm) and ã HCH (0.057 ppm). The highest concentration p,pDDD and â HCH were found in muscle, whereas the level of p,p 4-DDT, á HCH, ã HCH and ä HCH were highest in liver. The kidneys showed more amount of p,pDDE.

Key words: *Pesticide residues, DDT, HCH, buffalo muscle, organs.*

INTRODUCTION

Buffalo is an important food animal and buffalo meat contributes about 31% of total meat production in India. Being lean and less in cholesterol content, buffalo meat has very good demand in export market. India's export of meat and meat products during the year 2009-2010 fetched Rs 6365.62 crore, of which buffalo meat was of Rs. 5480.60 crores (APEDA, 2010). The presence of chemical residues in meat has been an important issue for many years. Consumer demands food that is free from any chemical residue. Further, the presence of the residues is also a major bottleneck in the acceptance of food commodities by the importing countries. Due to low cost and versatility in action against various pests, DDT (Dichloro Diphenyl trichloroethane) and HCH (Hexachloro Cyclo Hexane) pesticides were extensively used in the country for about last 5 decades (Kannan et al. 1992). Higher stability and persistence of these chemicals in the environment led to the contamination of foodstuffs, especially those having high fat content such as meat and milk products. Transfer and accumulation of these chemicals in human body results in health problems, particularly endocrine dysfunction, birth defects, carcinomas, neurological disorders besides weakening the immune system (Brody and Rudel, 2003). It is therefore necessary to assess the profile of food products for various residues at regular intervals to ensure that public health is not endangered by violative residues. Hence a study was carried out to estimate the organochlorine pesticides viz., DDT and it s metabolites (p,pDDT - para para Dichloro Diphenyl Trichlore ethane ; p,pDDE - para para Dichlorodiphenyl Dichlore Ethane and p,pDDD - para para Dichloro Diphenyl Dichloroethylene) and HCH (a, b, ã and d isomers) residues in the meat, liver and kidneys of buffaloes.

MATERIALS AND METHODS

Sample collection and processing: Muscle, liver and kidney (fourteen samples) of buffaloes were

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collected from Municipal corporation slaughter house of Hyderabad and analysed for the presence of DDT and HCH pesticide residues. Methods of Tonkabony *et al*. (1981) was followed for the extraction of pesticide residues from the tissues. The procedure involves grinding of 15 g portion from each of the minced samples along with twice the quantity of the anhydrous sodium sulphate in a pestle and mortar. Resultant free flowing granular tissue material thus formed was extracted for organochlorine pesticides residues with petroleum ether at 50 to 55°C in a Soxhlet apparatus (Soxplus, Chennai, India) for 6 h.

Extraction of fat and pesticide residues: The method of AOAC (1995) with slight modifications was employed for clean-up of fat extracted from the samples. The extracted fat diluted with 15 ml petroleum ether extracted thrice with 30 ml of acetonitrile saturated with petroleum ether in the 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 clean up of samples to remove the residual fat was performed by colomn chromatography method using activated anhydrous sodium sulphate and Florisil. Elution was carried out with 200 ml of 6% eluting solvent at 40-45 drops per minute and the elute was concentrated in a vacuum evaporator (Lab Tech, Korea).

Estimation of pesticide residues: One micro litre of the reconstituted sample was injected into a gas chromatograph (Shimadzu GC-2010, Japan) equipped with an electron capture detector (ECD). Instrumental settings were as follows: Temperature of injection port, column and detector were 260, 80-220 and 300^0 C, respectively with N_2 as carrier gas at flow rate of 5 ml/ minute. This injection mode was splitless. The retention time along with the areas of the peak 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.

Recovery experiment: Meat samples were fortified with the working standards (0.01 and 0.1 ppm) of investigated compounds to estimate the recovery by following the procedure described above to ascertain the efficiency of extraction. The minimum limit of detectability obtained in this study for various metabolites of DDT and isomers of HCH cyclodiene compounds was 0.001 ppm, while the minimum limit of quantification was 0.01 ppm. 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). Residue levels of pesticides were corrected according to their recoveries and expressed as mg/kg (ppm).

Statistical Analysis: The data obtained from the study were subjected to one and two way analysis of variance (ANOVA) to determine the differences in DDT and HCH residues contents among the different tissues analyzed. Significant differences among the means were determined by Tukey honestly significant difference (HSD) test. All statistical computations were performed using the SPSS 10.0 for Windows software (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Among tissues, muscle showed higher levels of fat (2.88 %), followed by liver (2.23 %) and kidney (1.85%) samples. The higher fat content in muscle compared to organs is in accordance with Campbell and Brett Kenney (1994), who reported higher fat in muscle tissues than organs. In the present study, the recovery of various organochlorine pesticides from spiked meat samples was above 80 %. The efficiency of extraction methodologies are evaluated based on

the recoveries of residues and a recovery of 75-102 % is considered as acceptable (Solymos *et al*., 2001). Bedi *et al*. (2005) also reported a similar level of recovery.

DDT Residues: p,pDDT, p,pDDE and p,pDDD are important metabolites of DDT. In the present study, 71.43 % of analyzed buffalo tissue samples showed presence of DDT residues (Table 1). Higher incidence of contamination might be due to intake of contaminated feed, fodder and water. This finding correlates well with reports stating prevalence of high level of DDT residues in grazing field, soil, water bodies, feed and fodders from various parts of India (Ahuja and Awasthi, 1993 and Kumari *et al*., 2008). Further, high stability in the environment (Tonkabony *et al*., 1981) and illegal use of technical grade DDT (mixture of p,pDDT 80% and o,pDDT 20%) in agriculture might be the reasons for higher detection frequency. The overall mean residual concentrations of total DDT was 0.355 ppm. None of the samples showed the residual concentrations of total DDT above MRL of 7 ppm specified by MFPO (1973) and PFA (2004). Tripathi *et al.* (1973) reported that the DDT level in the fat tissues of buffaloes aged below 5 years and 5 to 10 years were 0.278 and 0.640 ppm, respectively.

The most prominent metabolite of DDT in buffalo muscle was p,pDDD, followed p,pDDT and p,pDDE, whereas liver tissues had high concentration of p,pDDT, followed p,pDDE and p,pDDD. However, in kidney p,pDDE was more followed by p,pDDT and p,pDDD. Overall, the residue level and incidence of p,pDDT were more as compared to p,pDDE and p,pDDD. This is in accordance with Madarena *et al.* (1980), who also found that p,pDDE and p,pDDT were the main contaminants among the DDT residues in meat. Among tissues, muscle showed significantly higher accumulation of DDT metabolites than kidney and liver. This might be due to the high fat content in the muscle tissues, which favours accumulation of more amounts of strongly lipophilic organochlorine compounds (Sallam and Morshedy, 2008).

Table 1. Mean residual levels (ppm) of DDT and HCH pesticide residues in tissues of buffaloes

Pesticide / metal residues	Muscle	Liver	Kidney	Overall mean	Percentage of occurrence
p,p DDE	$0.109^{b} \pm 0.046$	$0.062^{a} \pm 0.006$	$0.172^{c} \pm 0.048$	0.114^{xy}	64.29
p,pDDD	$0.196^{b} \pm 0.005$	$0.023^{a} \pm 0.009$	$0.033^{a} \pm 0.010$	0.084^{x}	42.86
p,pDDT	0.168 ± 0.005	0.170 ± 0.009	0.131 ± 0.004	0.156^{y}	71.43
Total DDT	0.473	0.255	0.336	0.355	71.43
á HCH	0.061 ± 0.017	0.064 ± 0.032	0.058 ± 0.007	0.061^{x}	50.0
â HCH	0.138 ± 0.025	0.103 ± 0.028	0.093 ± 0.046	0.111^{y}	50.0
ã HCH	0.051 ± 0.013	0.065 ± 0.017	0.055 ± 0.014	0.057^{x}	71.43
ä HCH	0.241 ± 0.127	0.282 ± 0.059	0.268 ± 0.053	0.263^{z}	57.14
Total HCH	0.491	0.513	0.474	0.493	71.43

Means bearing different superscript between columns (a,b,c) and between rows (x,y,z) differ significantly (P<0.05)for a particular pesticide. Total DDT-sum of p,p DDE, p,p DDD and p,p DDT; Total HCH - sum of á, â, ã and ä HCH

HCH (1, 2, 3, 4, 5, 6-hexachloro cyclo hexane) Residues: Among the various isomers, the occurrence of ã HCH (71.43 %) were more followed by ä (57.14%), á and â (50.0%). The higher incidence of ã HCH (lindane) might be due to the fact that the technical HCH (á HCH- 60 %, â HCH- 5-6 %, ã HCH 13 % and ä HCH <1 5-6 %) has been banned since 1997 and only the lindane (ã HCH) is permitted for agricultural use. Similarly, Kalra and Chawla (1980) reported more level of ã HCH. The presence of other isomers (á HCH, â HCH and ä HCH) indicates the possibility of misuse of technical HCH or commercially available ã -HCH may also contain various other isomers. However, none of the

Fig.1 : Elution pattern of organochlorine pesticides standard mixture (0.01ppm)

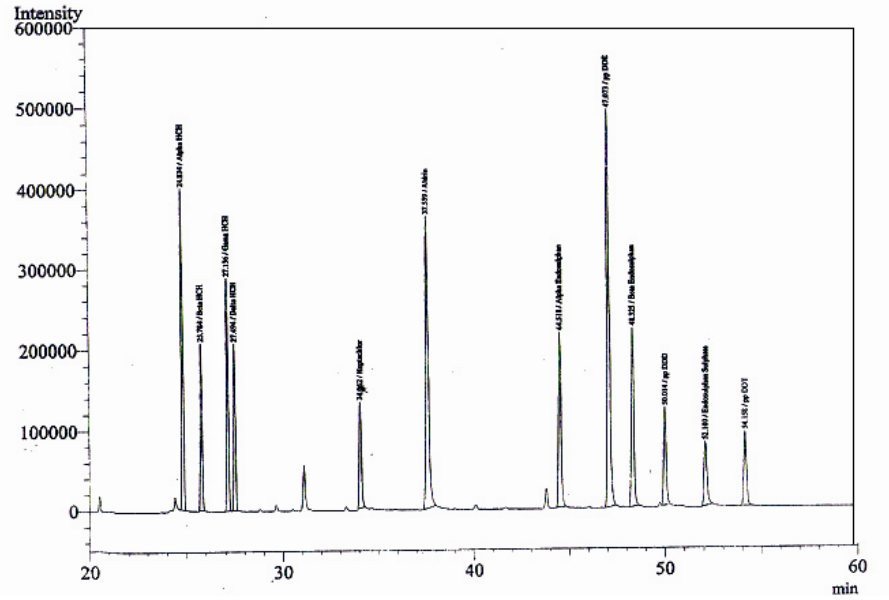
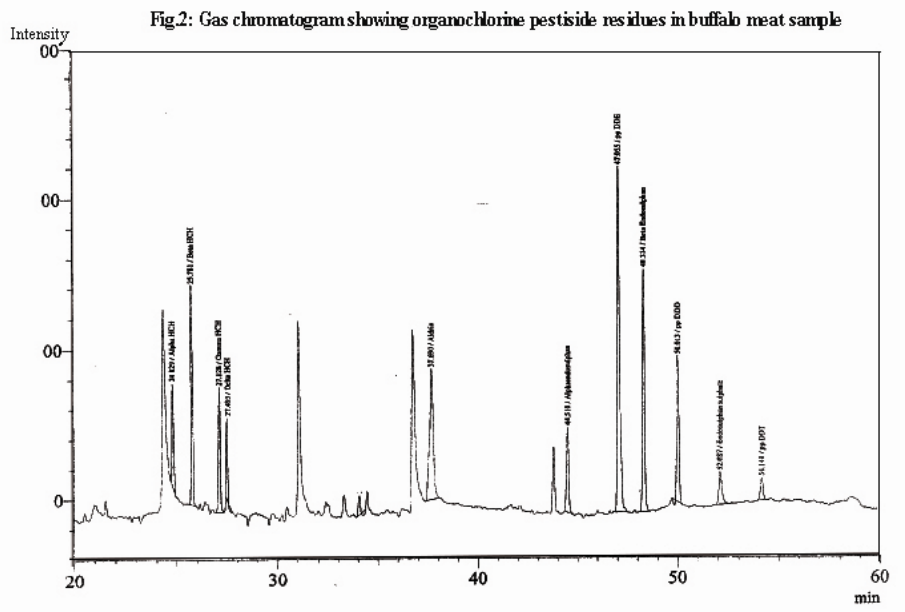


Fig.2: Gas chromatogram showing organochlorine pestiside residues in buffalo meat sample



samples showed the residual concentration of total HCH above MRL of 2 ppm set (for lindane alone) by India (MFPO, 1973 and PFA, 2004).

It can be concluded from the present study that the contamination pattern of OC pesticides in tissues of buffaloes were in the descending order of ä HCH, p,p <1 DDT, p,p <1 DDE, â HCH, p,p <1 DDD, á HCH and ã HCH. Among tissues, muscle showed the highest concentration for p,p <1 DDD, â HCH, whereas liver showed the highest mean concentration for p,p <1 DDT, ã HCH and ä HCH. The concentrations of p,p <1 DDE were highest in kidneys. However, the levels of contamination were quite low and well below the maximum residue level. Therefore consumption of buffalo meat does not pose any health risk for the consumers. Further investigations with a wide range of animal feed and fodder, environment and various animal foods samples are needed to accurately understand the details of source, pathway and magnitude of contamination.

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