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Pesticides and heavy metals residues in pig tissues in Hyderabad

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ABSTRACT

A study was conducted to estimate certain organochlorine (OC) pesticides (DDT, HCH and cyclodiene compounds) and heavy metals (lead and cadmium) residues in muscle and organs of pigs collected from Hyderabad city. The residues of organochlorine pesticides and heavy metals were found in majority of the analysed muscle and organs samples of pigs. The contamination pattern of OC pesticides in tissues of pigs were in the descending order of p,p'DDT- para para dichloro diphenyl trichlore ethane (0.213 ppm), p,p'DDE- para para dichlorodiphenyl dichlore ethane (0.132 ppm), p,p'DDD- para para dichloro diphenyl dichloroethylene (0.058 ppm), α endosulfan (0.037 ppm), β HCH - hexachloro cyclo hexane (0.028 ppm), δ HCH (0.022 ppm), endosulfan sulfate (0.018 ppm), α HCH (0.017 ppm), β endosulfan (0.016 ppm), aldrin (0.016 ppm) and γ HCH (0.014 ppm). However, the levels of contamination were quite low and well below the maximum residue level. Among tissues, muscle showed the highest concentration for p,p'DDD; p,p'DDT; β HCH; aldrin; α endosulfan; β endosulfan and endosulfan sulfate, whereas liver showed the highest mean concentration for γ HCH and δ HCH. The concentrations of p,p'DDE and α HCH were highest in kidneys. The heavy metals residues were found in all the analysed meat and organs samples. In most of the cases, the levels of contamination were low and below the maximum residue level except in case of lead, where 40.9% samples exceeded the maximum permitted limit. Among tissues, kidney showed highest concentrations for both lead and cadmium.

Key words: Heavy metals, Organochlorines pesticides, Pig tissues, Residues

Organochlorine (OC) pesticide like dichloro diphenyl trichloroethane (DDT) and hexachloro cyclo hexane (HCH) constituted two-third of the total consumption in the country for about last 5 decades, because of their low cost and versatility in action against various pests (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. As a consequence, humans are exposed to greater dietary levels of organochlorines (Fromberg *et al.* 2011). The accumulation of organochlorine compounds in body fat due to their strong lipophilicity induce changes in biotransformation of endogenous and exogenous compounds resulting in variety of health problems, particularly endocrine dysfunction, birth defects, carcinomas, neurological disorders besides weakening the immune system (Brody and Rudel 2003).

Contamination of food crops through root uptake of metals from soil and water facilitates the entry of heavy metals into the food chain (Bhupalraj *et al*. 2006). Transfer of heavy metals into human body results in their accumulation in vital organs causing disorders such as necrosis of liver, damage of nephrons in kidney, haemotological aberrations and decline in growth rate (Sugunan and Vinci 2002). It is therefore necessary to assess the profile of domestic produce for various residues at regular intervals to ensure that public health is not endangered by violative residues.

The present study was carried out to estimate the organochlorine pesticides, viz. DDT - dichloro diphenyl trichloro ethane and its metabolites (p,p'DDT - para para dichloro diphenyl trichlore ethane; p,p'DDE - para para

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dichlorodiphenyl dichlore ethane and p,p'DDD - para para dichloro diphenyl dichloroethylene), cyclodiene compounds (aldrin, heptachlor and endosulfan) and HCH-hexachloro cyclo hexane (α, β, γ and δ isomers) and heavy metals (lead and cadmium) residues in the meat and organs of pig.

MATERIALS AND METHODS

Sample collection and processing for pesticides residues estimation: Fourteen samples (muscle, liver and kidney) of pigs (reared under semi intensive system) were collected from retail markets of Hyderabad and analysed for the presence of organochlorine pesticides residues. A 15 g portion from each of the minced samples was ground 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 for 6 h as outlined by Tonkabony *et al*. (1981).

Extraction of fat and residues: The fat extracted from the samples was subjected to clean-up employing the procedures of AOAC (1995) with slight modifications. The extracted fat diluted with 15 ml petroleum ether was 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 column chromatography method using activated anhydrous sodium sulphate and Florisil. Elution was carried out with 200 ml of 6% eluting solvent at 40–45 drops/min and the elute was concentrated in a vacuum evaporator.

Determination of organochlorine pesticide residual concentrations: One micro litre of the reconstituted sample was injected into a gas chromatograph 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°C, respectively, with N_2 gas flow rate of 5 ml/min. This injection mode was splitless. The retention time along with the height and 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 10 samples, a procedural blank consisting of all reagents and using the same glassware that were 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. 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).

Sample collection and processing for heavy metals residue estimation: The AOAC method (1988) was followed for the estimation of heavy metal residues in tissues samples of pigs. Ten muscle *(L dorsai*) samples and 6 liver and kidney samples of pigs, weighing approximately 250 g were collected at random from retail markets Hyderabad and packed in clean polythene bags. Tissue (5 g) was taken in a 125 ml glass tubes along with glass beads and 25 ml of deionized water added, 10 ml of a 1:1 mixture of concentrated nitric acid (HNO_3) and perchloric acid ($HClO_4$) was added to the contents of test tube. The sample was boiled until the solution become clear. The solution was quantitatively transferred into a 50 ml volumetric flask and made up to 50 ml with deionised water and mixed well.

The estimation of the metals residues was carried out using atomic absorption spectrophotometer. The concentration of the metal to be determined was provided between 0.1 to 0.5 absorbance units. About 2 ml of solution was utilized for each measurement. The working standards were analyzed at the beginning and end of a run and periodically during longer runs. According to the absorbance, the concentration was calibrated and the concentration was measured directly, when the sample was within the linear working range.

Statistical analysis: The data obtained from the study were subjected to 1– and 2-way analysis of variance (ANOVA) to determine the differences in the organochlorine pesticides and heavy metals 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.

RESULTS AND DISCUSSION

Pesticides residues: The overall mean fat content in muscle tissues of pig was 6.83%. Among tissues, muscle showed higher levels of fat (6.83%), followed by liver (4.90%) and kidney (4.0%) 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. They also noticed more fat in liver, when compared to kidney samples.

In the present study, the recovery of various organochlorine pesticides from spiked meat samples was above 80% (Table 1). The efficiency of extraction methodologies are evaluated based on the recoveries of

Table 1. Retention time and recovery percentage for organochlorine pesticides in spiked meat samples

Name of pesticide	Mean retention time (min)	Mean recovery (%)
α HCH	24.844	96.24
β HCH	25.816	84.29
γ HCH	27.148	98.13
δ HCH	27.531	85.06
Heptachlor	34.088	101.82
Aldrin	37.586	79.73
α endosulfan	44.545	89.66
p,p' DDE	47.092	91.54
β endosulfan	48.359	80.00
p,p'DDD	50.062	80.74
Endosulfan sulphate	52.145	82.40
p,p' DDT	54.200	101.13

residues and a recovery of 75–102% is considered as acceptable (Solymos *et al*. 2001).

In general the order of pesticides contamination was DDT > HCH > endosulfan > aldrin. Ahmad *et al*. (2010) also reported a similar trend of pesticide contamination in meats collected from Jordon. Residues of Heptachlor were not detected in any of the samples (Table 2).

DDT residues: p,p'DDE and p,p'DDD are metabolites of DDT and produced during metabolism of ingested technical grade DDT in the liver. Residues of DDT were detected in 78.57% of pig tissue samples analyzed in the present study and the overall mean residual concentrations of total DDT was 0.395 ppm. This might be due to intake of contaminated feed, fodder and water. Several workers from various parts of India have reported presence of high level of DDT residues in grazing field, soil, water bodies, feed and fodders (Kumari *et al*. 2008). Further, the peculiar feeding behaviour of pigs (burrowing), which involves unearthing growing plants and eating them along with roots invariabily ends up with ingestion of some amount of soil. This could be the another probable reason for higher level of contamination in pig tissues. The role of soil as a source of pesticide in animal tissues is in agreement with Herlin and Anderson (1996), who described soil as harbour for high concentrations of environmental contaminants. The ubiquitous nature, high stability in the environment (Tonkabony *et al*. 1981) and illegal use of technical grade DDT (mixture of p,p'DDT 80% and o,p'DDT 20%) in agriculture might be the reasons for higher detection frequency. However, none of the samples showed the residual concentrations of total DDT above MRL specified by national (7 ppm; MFPO 1973, PFA 2004) and international regulatory authorities (5 ppm; Codex and EU). A low level of contamination was reported in developed countries like USA (0.093 to 0.114 ppm; Cambell *et al*. 1965) and Italy (0.0790 ppm; Madarena *et al*. 1980), probably due to early implementation of ban on DDT and regulated usage of OC compounds.

The most prominent metabolite of DDT in pig muscle and liver tissues was p,p'DDT, followed p,p'DDE and p,p'DDD. However, in kidney p,p'DDE was more followed by p,p'DDT and p,p'DDD. Overall, the residue level and incidence of p,p'DDT were more as compared to p,p'DDE and p,p'DDD. This is in accordance with Madarena *et al*. (1980), who also found that p,p'DDE and p,p'DDT were the main contaminants among the DDT residues in meat.

Table 2. Mean residual levels (ppm) of various pesticides and heavy metal residues in tissues of pigs

Pesticide/metal residues	Muscle	Liver	Kidney	Overall mean	Percentage of occurrence
p,p'DDE	0.155^{b}±0.049	0.063^{a}±0.026	0.178^{b}±0.055	0.136	78.57
p,p'DDD	0.106^{b}±0.044	0.021^{a}±0.008	0.024^{a}±0.008	0.058	50.0
p,p'DDT	0.314^{b}±0.158	0.179^{a}±0.005	0.145^{a}±0.009	0.213	78.57
Total DDT	0.575	0.263	0.348	0.395	78.57
α HCH	0.008^{a}±0.002	0.021^{b}±0.002	0.022^{b}±0.005	0.017	57.14
β HCH	0.048^{b}±0.015	0.018^{a}±0.004	0.019^{a}±0.0002	0.028	71.43
γ HCH	0.016±0.003	0.012±0.002	0.014±0.002	0.014	71.43
δ HCH	0.016±0.004	0.024±0.001	0.027±0.001	0.022	57.14
Total HCH	0.088	0.075	0.082	0.082	71.43
Aldrin	0.015±0.003	0.017±0.002	0.016±0.003	0.016	57.14
α endosulfan	0.050±0.009	0.028±0.003	0.030±0.005	0.037	85.71
β endosulfan	0.020±0.002	0.011±0.004	0.013±0.004	0.016	71.43
Endosulfan sulfate	0.018±0.005	0.018±0.002	0.019±0.008	0.018	42.86
Total endosulfan	0.088	0.057	0.062	0.069	85.71
Heptachlor	BDL	BDL	BDL	BDL	Nil
Lead	$2.129^{a\pm}$0.245	2.453^{b}±0.417	$2.540^{b\pm}$0.424	2.329	100
Cadmium	$0.170^{a\pm}$0.015	0.247^{b}±0.021	$0.250^{b\pm}$0.031	0.212	100

Means in the same row sharing a common superscript letter are not significantly different (P>0.05). Total DDT, sum of p,p' DDE, p,p' DDD and p,p' DDT; Total HCH, sum of α, β, γ and δ HCH; BDL, below detectable level.

Among tissues, muscle showed significantly higher accumulation of p,p'DDD and p,p'DDT metabolites than kidney and liver. Significantly higher level of p,p'DDE was found in muscle and kidney than liver tissues. High fat content in the muscle tissues might have favoured for accumulation of more amounts of strongly lipophilic organochlorine compounds. The influence of fat on the concentration of DDT residues was demonstrated by Sallam and Morshedy (2008).

HCH (1, 2, 3, 4, 5, 6-hexachloro cyclo hexane) residues: Among the various isomers, the occurrence of β and γ HCH (71.43%) were more followed by α and δ (57.14). This may be due to higher chemical stability and lipobilicity of β HCH (Kutz *et al*. 1991). Similarly Kannan *et al.* (1992) also recorded highest level of β HCH isomer. The higher incidence of γ HCH (lindane) might be due to the fact that the technical HCH (α HCH, 60%; β HCH, 5–6%; γ HCH, 13% and δ HCH, 5–6%) has been banned since 1997 and only the lindane (γ HCH) is permitted for agricultural use. Similarly, Kalra and Chawla (1980) reported more level of γ HCH. The presence of other isomers (α HCH, β HCH and δ HCH) indicates the possibility of misuse of technical HCH or commercially available γ HCH may also contain various other isomers. However, none of the samples showed the residual concentration of total HCH above MRL of 2 ppm set (for lindane alone) by India (MFPO 1973, PFA 2004).

Cyclodienes compounds: The overall mean concentration of aldrin in pig tissues ranged from 0.015 to 0.017 ppm. Contamination of feed with aldrin residues (Dikshith *et al*. 1989) was already reported in India. However, the reported data on the aldrin residues in animal tissues are sparse. Aldrin levels in the samples analyzed in the present study are much lower than the recommended maximum limit of 0.2 ppm (MFPO 1973).

The residues of endosulfan were detected in 85.71% samples with an overall mean concentrations ranged from 0.057 to 0.088 ppm. Various workers from different parts of country already reported the presence of endosulfan residues in feed (Dikshith *et al*. 1989) and buffalo meat (Pradeepkumar 2006). The present findings are in conformity with earlier reports from various parts of the country.

Heavy metal residues

Lead: The lead residues were detected in the all samples examined and the overall mean concentration of lead residue was 2.329 ppm. More number of pig samples (40.9%) exceeding the stipulated MRL values of 2.5 ppm (MFPO 1973) was observed in this study. As pointed out by Herlin and Anderson (1996), ingestion of soil, which contains high concentration of environmental contaminants in the upper layers, during burrowing, might be a reason for detection of higher lead contamination. This is in accordance with various workers in India, who have documented excess amount of heavy metal residues in animal tissues and products due to contaminated environment (Bhupalraj *et al*. 2006). In the contrary, lower concentration of lead was reported in the muscle and organs of pigs in Swedan (0.019 ppm; Jorhem *et al*. 1991). The study of Doganoc (1996) also revealed that the concentration of lead in muscle, liver and kidney of pig were below detectable limit in Slovenia. Regular monitoring and strict enforcement of laws might be the reasons for the low occurrence of environmental pollutants in food products in more developed countries.

Cadmium: The cadmium residues were observed in all the tissue samples of meat animals collected from Hyderabad city. The overall mean concentration of cadmium residues recorded in the present study was 0.212 ppm. The carry over of heavy metals present in the contaminated environment might be the reason for detection of higher concentrations of heavy metals residues in the present study. This is in accordance with Bhupalraj *et al*. (2006). Based on the MRL (1.5 ppm) specified (PFA 2004) for foods other than infant foods and turmeric powder, none of the sample exceeded the prescribed limit.

The concentration of lead and cadmium residues was significantly higher in organs compared to muscle. As kidney and liver are the filtering organs within the body, metals get accumulated in these organs. Higher concentration of lead and cadmium in liver and kidneys than muscle was also observed by Jevsnik and Doganoc (2003).

The contamination pattern of OC pesticides in tissues of pigs were in the descending order of p,p'DDT, p,p'DDE, p,p'DDD, α endosulfan, β HCH, δ HCH, endosulfan sulfate, α HCH, β endosulfan, aldrin and γ HCH. However, contaminations were quite low and well below the maximum residue level and present no threat to public health on the basis of current toxicological knowledge. Among tissues, muscle showed the highest concentration for p,p'DDD, p,p'DDT, β HCH, aldrin, α endosulfan, β endosulfan and endosulfan sulfate, whereas liver showed the highest mean concentration for γ HCH and δ HCH. The concentrations of p,p'DDE and α HCH were highest in kidneys.

The heavy metal residues were found in all the analysed meat and organ samples. In most of the cases, the levels of contaminations were low and below the maximum residue level except in lead, where 40.9% samples exceeded the maximum permitted limit. Among tissues, kidney showed highest concentrations for both lead and cadmium. Hence, consumption of organs, especially kidney poses more risk of exposure to heavy metals. This study calls for a continuous monitoring programme for animal feed and fodder, environment and various animal foods at different agro-climatic locations in the country involving more number of samples to accurately assess the level of contamination, to understand the national scenario with particular reference to persistent organochlorine pesticides and heavy metal residues.

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