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Ch Bindu Kiranmayi
Assistant Professor, Dept. of Veterinary Public Health and Epidemiology, NTR College of Veterinary Science, Gannavaram, Krishna District, Andhra Pradesh, India

N Krishnaiah
Professor & University Head, Dept. of Veterinary Public Health & Epidemiology, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana, India

M Muthu Kumar
Senior Scientist, National Research Centre on Meat, Chengicherla, Boduppal Hyderabad, Telangana, India

N Subhashini
Assistant Professor, Dept. of Veterinary Public Health and Epidemiology, NTR College of Veterinary Science, Gannavaram, Krishna District, Andhra Pradesh, India

T Srinivasa Rao
Associate Professor & Head, Dept. of Veterinary Public Health and Epidemiology, NTR College of Veterinary Science, Gannavaram, Krishna District, Andhra Pradesh, India

T Madhava Rao
Professor and University Head, Dept. of Veterinary Public Health and Epidemiology, College of Veterinary Science, Tirupati, Chittoor District, Andhra Pradesh, India

Correspondence
Ch Bindu Kiranmayi
Assistant Professor, Dept. of Veterinary Public Health and Epidemiology, NTR College of Veterinary Science, Gannavaram, Krishna District, Andhra Pradesh, India

Detection of organochlorine, organophosphorus and synthetic pyrethroid residues in pork, chicken, fish and fish pond water samples

Ch Bindu Kiranmayi, N Krishnaiah, M Muthu Kumar, N Subhashini, T Srinivasa Rao and T Madhava Rao

Abstract
Pesticides are chemicals used in agriculture to protect crops against insects, fungi, weeds, pests and to protect public health by vector control such as mosquitoes. But pesticides are potentially toxic to humans. They may induce adverse health effects including cancer, effects on reproduction, immune or nervous systems. A study was conducted to detect organochlorine (DDT, HCH and cyclodiene compounds), organophosphorus (Methyl-Parathion, Malathion, Chlorpyrifos and Methyl- Chlorpyrifos) and synthetic pyrethroid (Cypermethrin and Deltamethrin) residues in pork, chicken, fish and fish pond water samples collected from different regions of Andhra Pradesh by Gas Chromatography (GC). All the Organochlorine pesticides (OCP), Organophosphorus pesticide (OPP) residues detected in pork, chicken, fish and fish pond water samples were quite low and well below the maximum residue levels (MRLs) specified by different national and international regulatory bodies. We could not detect the selected synthetic pyrethroids (SYPs) in any of the samples.

Keywords: Organochlorines, organophosphates, synthetic pyrethroids, pesticide residues,

Introduction

The prolong usage of persistent and harmful pesticides have raised a number of concerns, over 98% of sprayed insecticides and 95% of herbicides reach a destination other than their target species, including non-target species, air, water, bottom sediments and food. The pesticide spreads into the environment and has detrimental effect on human health through the contamination of soil, air and water resources and poses significant risks to the environment and non-target organisms, such as beneficial species of insects, soil, micro-organisms, plants and bird and cause development of insect resistance and resurgence of resistance in certain species of pests (Miller, 2004). Pesticides have been used in the public health sector for disease vector control and in agriculture to control and eradicate crop pests for the past several decades (Darko and Acquaah, 2007) [5]. In recent years, the status of chemical pesticides has undergone a sea change from being one of the major instruments of green revolution to an agent polluting air, water and soil and posing serious threat to public health by entering into food chain (Wadhwa, 2000) [32]. Other sources of exposure include drinking water contaminated through crop field run off pollution, misapplication of pesticide to animals or their housing (Heise, 1992) [11]. Higher stability and persistence of these chemicals in the environment led to the contamination of foodstuffs, especially those having high fat content such as milk and meat products (Kannan *et. al.*, 1992) [17]. Fish are the major part of human diet because fish have low risk of coronary heart diseases, hypertension and cancer. The level of residues in fish depends upon the quality of water, feed and sediment. Fish being at the higher level of food chain, accumulate large quantities of residues (Karadede *et. al.*, 2004) [18]. Pesticide residues have been reported to cause cancer, epilepsy, liver and kidney dysfunctions, somatic growth, depression, neuritis, testicular cancer (Sandhu, 1992; Straube *et. al.*, 1999) [26, 30], endocrine dysfunction, birth defects, carcinomas, neurological disorders and weakened immune system (Brody and Rudel, 2003) [4]. Organochloride insecticides like DDT and PCB have been responsible for breast cancer and decreased fertility in human beings (Au *et. al.*, 1999) [2]. DDT and HCH are widely recognized as neurotoxic substances affecting the peripheral and central nervous system, respectively and causing hyper-excitability of nerves and muscles (Hassal, 1983) [10]. DDE is an oestrogen like compound because of its feminizing and emasculating effects, reduction in sperm counts in men (Jeyaratnam, 1985; Kelce *et. al.,* 1995, 1998; Sonnenschein and Soto, 1998) [14, 20, 19, 28].

OCP, particularly HCH and DDT have become the universal contaminants found in all segments of the environment and food chain (Kaphalia *et. al.,* 1990). To overcome this, OP have replaced the persistent OCP and now are the most frequently used group of insecticides (Juhler, 1997) [15]. Pyrethroids are used to control flies, mosquitoes and other insects as ectoparasiticides. So, the present study is aimed at detection and estimation of certain organochlorine, organophosphate and synthetic pyrethroid pesticide residues in pork, chicken, fish and fish pond water samples collected from different regions of Andhra Pradesh by Gas Chromatography (GC) equipped with electron capture detector (ECD) and Thermionic Specific Detector (TSD).

Materials and Methods

50g each of pork, chicken, fish (40 each) and 100ml of fish pond water (n=40) samples were collected at random from local markets, slaughter houses and local fish ponds from different regions of Andhra Pradesh. Extraction of fat and elution of the pesticides from the fat and water was done by using petroleum ether, acetonitrile and florosil columns as per the procedures given in Pesticide Residue Analysis Manual, ICAR, New Delhi (2007) [24] with slight modifications and the estimation was carried out by GC (ECD and TSD detectors).

Gas chromatograph: A model Varian-450 GC (Germany) with WCOT fused silica 25mx0.25mm ID coating CP-SIL 8CB column for organochlorines and pyrethroids and WCOT fused silica 15mx0.25mm ID coating CP-SIL 5CB column for organophosphates. Electron Capture Detector (ECD) was used for detection of OC and SYPs and Thermionic Specific Detector (TSD) for OPs. The oven temperature programme for GC-ECD: from 80 ^{0}C (2 min), 5^{0}C/min to 150 ^{0}C (2 min), 3 ^{0}C to 180 ^{0}C (3min), 3 ^{0}C to 230 ^{0}C (5 min) and finally at 6 ^{0}C to 260 ^{0}C (6 min) for elution of different OC and SYP pesticides at different time and temperature combinations (Fig.1). The carrier gas (N_2) flow rate was kept in constant flow mode at 1.2 ml/min. Sample (1µl) was injected with split ratio of 10 at 260 ^{0}C.

The oven temperature programme for GC-TSD was from 100 ^{0}C (1 min), 3 ^{0}C/min to 150^{0}C (1 min), 3 ^{0}C to 180 ^{0}C (3min), 3 ^{0}C to 230 ^{0}C (5 min) and finally at 4 ^{0}C to 180 ^{0}C (2 min) for elution of different OP pesticides (Fig.2). The carrier gas (N_2) flow rate was kept in constant flow mode at 1.4 ml/min. Sample (1µl) was injected with split ratio of 10 at 260 ^{0}C. The analysis of extracts was performed in the Gas Chromatography Laboratory, National Research Centre on Meat (NRCM), Hyderabad.

Recovery Studies: Six samples each of pork, chicken, fish and fish pond water samples which did not contain any of the residues were fortified with the working standards (0.01 and 0.1 ppm) to estimate the mean recovery percentage.

Results and Discussion

Recovery studies revealed the recovery percentage ranging from 84.46% - 103.42% for OCP and SYPs and 88.52% - 98.56% for OP residues. The recovery percent of more than 70% is said to be satisfactory (Garrido-Frenich *et. al.,* 2006) [7]. The elution pattern and mean retention time for each of the selected pesticide residue in spiked samples are shown in Fig.1 & 2. The limits of detection (LOD) and quantification (LOQ) were calculated for each of the selected pesticide and the LOD values were ranging from 0.001 to 0.02 ppm and LOQ from 0.01 to 0.025 ppm. The LOD and LOQ values in the present study were well below their respective MRLs indicating that this method was able to detect the given pesticides at sufficiently low level (Bedi *et. al.,* 2005; Jadhav, 2008 and Stefanelli *et. al.,* 2009) [3, 13, 29].

The mean residual levels of different pesticide residues detected in pork, chicken, fish and fish pond water samples are given in Table.1, 2 & 3. Relatively high concentration of p,p'-DDE among the isomers and metabolites of DDT suggests chronic exposure to DDT (Davies *et. al.,* 1969) [6]. The presence of o,p'-DDT and p,p'-DDT in meat indicates fresh use of DDT and the presence of DDD and DDE indicates the outcome of metabolism of DDT inside the animal body or in the environment. The high incidence and the concentration of DDT metabolites (DDE and DDD) as compared to parent compound DDT indicates previous rather than fresh use (Kannan *et. al.,* 1997) [16]. p,p'-DDE has the highest bio-magnification potential as compared to other isomers/metabolites of DDT (Manirakiza *et. al.*, 2002) [22]. As that of the present study, Rita Kaushal (2008) [25] also reported p,p'-DDE as the predominant, of all the isomers of DDT, followed by p,p'-DDT and p,p'-DDD in in chicken. Most of the OCP are banned or restricted in India almost a decade ago except endosulfan and lindane which are still used for agricultural pest control (Tomar and Parmar, 1998) [31]. Even then their residues are still found in livestock products as OCP are lipophilic and very persistent for more than 20 years (Zia *et. al.*, 2009) [34] and not readily excreted by animals except in milk and tend to accumulate in the fatty tissues which leads to bio-accumulation and bio-magnification when consumed by the organisms of higher tropic level of food chain in an ecosystem (Gill *et al.*, 2010) [9]. Among cyclodiene compounds, endosulfan sulfate was commonly found compared to others. It's usage for agricultural purposes might be the reason (Tomar and Parmar, 1998) [31].

The high incidence of γ-HCH isomer in pork and chicken might be due to the fact that only γ HCH (lindane -contains 99 % of γ HCH) is legally permitted for agriculture use. Herrera *et. al.* (1994) [12], Lazaro *et al.* (1996) [21], Waliszewski *et al*. (1996) [33] and Bedi *et.al*. (2005) [3] also reported high incidence of lindane among various isomers of HCH in different meat samples.

Generally OPP are applied to animals through insecticide impregnated ear tag, spray or dust bags which might be the reason for the presence of OPP residues in animal products. Other common reasons for the presence of OPP residues are contaminated soils, crops, feed, fodder, water and mis-use of pesticides (Zia *et. al.,* 2009) [34]. Though the OPPs are less persistent when compared to others, some of the meat samples were found to be containing OPP residues. It may be due to the regular use of OPP compounds for insect and vector control in livestock houses. OPP residues were not found in any of the fish and fish pond water samples. No SYPs were detected in the samples. The levels of contamination of all the residues detected were quite low and well below the maximum residue levels (MRLs) specified by different national and international regulatory bodies.

Very early age of slaughter (6 weeks) might be a reason for lower level of contamination in broiler chicken tissue samples as compared to pork, as duration of exposure determines the level of pesticide contamination in animal tissues (Singh *et.*

al., 1970; George and Sundararaj, 1995) [27, 8]. Contamination of fish ponds with agricultural run water in many regions of coastal Andhra may be the reason for detection of pesticide residues among fish and fish pond water samples (Amaraneni and Pillala, 2001) [1].

Table 1: Mean residual levels (ppm) of DDT metabolites and HCH isomers in pork and chicken, fish and fish pond water samples

Sample	p,p'-DDT	p,p'-DDD	p,p'-DDE	α-CH	β-CH	γ-HCH	δ-HCH
Pork	0.059 (± 0.002)	0.065 (± 0.003)	0.081 (± 0.002)	0.046 (± 0.001)	0.052 (± 0.009)	0.067 (± 0.007)	0.026 (± 0.009)
Chicken	0.022 (± 0.003)	0.024 (± 0.002)	0.056 (± 0.002)	0.027 (± 0.007)	0.033 (± 0.007)	0.061 (±0.007)	0.018 (± 0.033)
Fish	0.018 (± 0.002)	0.021 (± 0.002)	0.032 (± 0.001)	0.033 (± 0.005)	0.023 (± 0.003)	0.074 (± 0.008)	0.015 (± 0.003)
Fish pond water	0.034 (± 0.002)	0.016 (± 0.001)	0.020 (± 0.001)	0.018 (± 0.003)	0.016 (± 0.002)	0.028 (± 0.004)	0.008 (± 0.001)

Table 2: Mean residual levels (ppm) of different cyclodiene pesticide and SYP residues in pork, chicken, fish and fish pond water samples

Sample	Aldrin	Dieldrin	Endo. Sulfate	Heptachlor	Hepta. Epoxide	Cypermethrin	Deltamethrin
Pork	0.018 (± 0.003)	0.021 (± 0.005)	0.025 (± 0.004)	0.021 (± 0.008)	0.015 (± 0.004)	-	-
Chicken	0.022 (± 0.006)	0.018 (± 0.003)	0.029 (± 0.007)	0.018 (± 0.002)	0.012 (± 0.003)	-	-
Fish	0.014 (± 0.002)	0.014 (± 0.003)	0.047 (± 0.007)	-	-	-	-
Fish pond water	0.016 (± 0.002)	0.012 (± 0.002)	0.082 (± 0.012)	0.014 (± 0.002)	0.011 (± 0.003)	-	-

Table 3: Mean residual levels (ppm) of different organophosphate pesticide residues in pork and chicken samples

Sample	Methyl parathion	Malathion	Chlorpyrifos	Methyl-chlorpyrifos
Pork	0.036 ± 0.007	0.044 ± 0.002	0.039 ± 0.004	0.038 ± 0.005
Chicken	0.038 ± 0.005	0.036 ± 0.005	0.042 ± 0.002	0.033 ± 0.006

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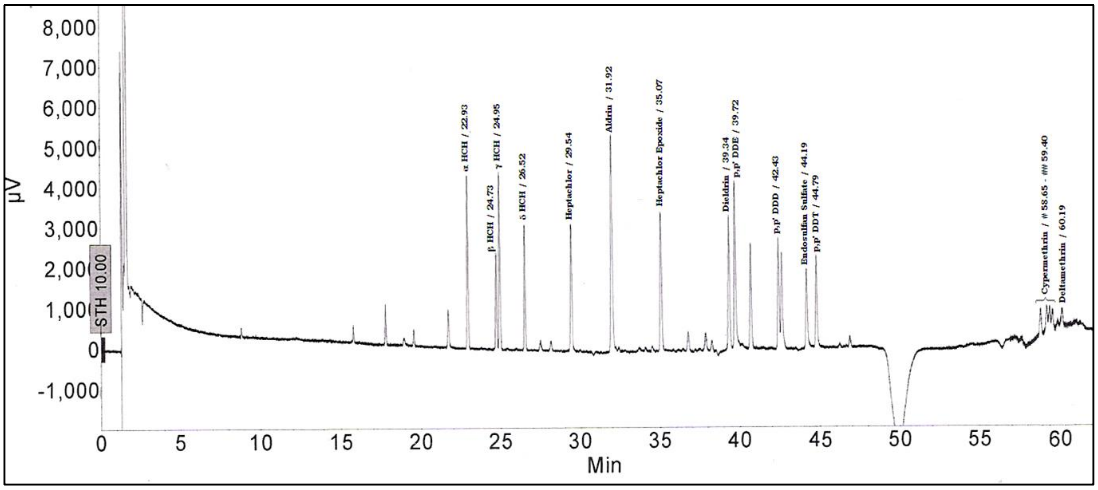

Fig 1: Elution pattern and mean retention time of organochlorine and synthetic pyrethroid pesticides from standard pesticide mixture using ECD detector (0.01 ppm)

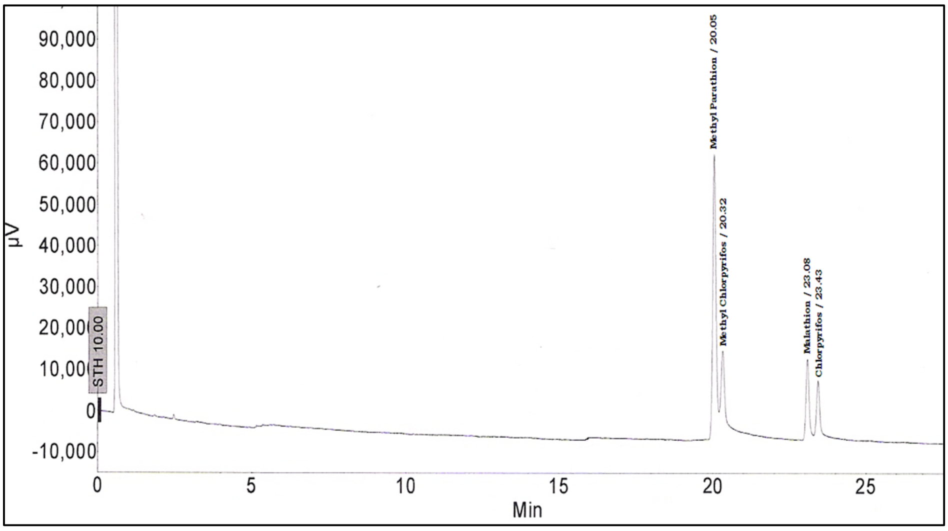

Fig. 2: Elution pattern and mean retention time of organophosphorus pesticides from standard pesticide mixture using TSD detector (0.01 ppm)

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