



RESEARCH ARTICLE

STUDY ON ORGANOCHLORINE RESIDUE IN FORAGING DUCKS

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ABSTRACT

The study was carried out to detect the Organochlorine (pesticide) residue in foraging ducks in Kerala State of India. Thirty foraging ducks were collected from paddy fields in three foraging regions viz., Kuttanad, Palakkad and Thrissur and the organochlorine (OC) residues in their crop content and body fat were estimated using Gas Chromatography. The results shown that the samples from Kuttanad contained  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$  isomers of Hexachloro cyclohexane (HCH), metabolites of Dichloro Diphenyl Trichloroethane (DDT), Dicofol and  $\alpha$ -Endosulphan. The combined residue of these compounds accounted to 0.0018 ppm in crop content and 0.0117 ppm in fat sample. The samples from Palakkad contained  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$  isomers of HCH, isomers of DDT, Dicofol,  $\alpha$ -Endosulphan and Dieldrin. The combined residues were 0.0152 and 0.0419 ppm in the crop content and fat respectively. The samples from Thrissur showed the presence of isomers of HCH, primary derivatives of DDT and  $\alpha$ -Endosulphan. The combined residues in these samples were also negligible (0.0033 and 0.0077 ppm in crop and fat samples respectively). Even though the residues of many compounds present in the samples from all regions, the detected levels were well below the Maximum residue Limits (MRL) of these compounds in poultry.

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INTRODUCTION

According to a report from Assocham (The Associated Chambers of Commerce and Industry of India), pesticide consumption in India is the lowest at 0.5 kg per hectare as against 17 kg per hectare in Taiwan, 12 in Japan, 6.6 in Korea, 7 in USA and 2.5 in Europe. The report also points out that Indian food and agricultural products contain substantial quantities of pesticide residues as its farmers make indiscriminate use of fertilizers, in the absence of adequate education. It has been observed that many of the environmental problems that have come to light in the past 40 years have been caused by just one class of chemicals: organochlorines. This class includes DDT, HCH, endosulfan, dicofol, aldrin and dieldrin. The chemicals as a class include carcinogens, endocrine disruptors, and substances that harm nervous, reproductive, and immune systems. Many organochlorines are persistent, lasting in the world for years before degrading. Organochlorines have been found everywhere, even in areas where the chemicals have never been used. They are very persistent in the environment because of their resistance to chemical and microbial decomposition especially when protected by deep layers of soil. The body fat, being the main storage area for pesticides, retains the compounds in the body for an extra ordinary length of time. Kerala, the south peninsular region of India, has a

unique system of duck rearing. The vast areas of paddy fields after harvest form a potential and sustainable feed resource for ducks in the foraging system of rearing. Under this system, there is every chance of intake of the pesticides by the ducks due to the indiscriminate use of the compounds in paddy cultivation. The nature of pesticides and their residue levels in animal tissues in these regions have not been investigated so far. Therefore, in this study an attempt was made to detect the residue levels of organochlorine pesticides present in the crop content and body fat of foraging ducks from three distinct foraging regions in Kerala.

MATERIALS AND METHODS

Organochlorine residue analysis of foraging ducks from three regions viz., Kuttanad, Palakkad and Thrissur was carried out using Gas Liquid Chromatography. Thirty ducks were procured immediately after foraging in paddy fields, sacrificed and their crop (feed storage organ) and fat tissue were collected for further studies. The samples collected were stored in deep freezer for further analysis. The crop contents (Fig 1) were weighed separately, dried and processed for estimation of pesticide residue. The fat samples were also subjected to further processing for detection of pesticide residue.

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Fig 1. Crop contents of foraging duck

### Pesticide residue analysis

Specific cleanup procedures were carried out for complete removal of interfacing impurities and extraction of pesticide residues from the collected samples before introduction in to the Gas liquid chromatograph.

### Residue extraction from the crop contents

Samples collected were dried and powdered and two grams of the sample was taken in an extraction thimble. The thimble was introduced in to a soxhlet extraction unit and extracted with 200 ml petroleum ether for six hours. This extract was concentrated to 10 ml in a vacuum flash evaporater and quantitatively transferred to a 100 ml separating funnel. Fifteen milliliter acetonitrile saturated with petroleum ether was added and shaken well and the layers were allowed to separate.

The bottom layer containing pesticide was transferred to a one litre separating funnel having 600 ml water, 100 ml petroleum ether and 40 ml saturated sodium chloride solution. Extraction with acetonitrile was repeated for two more times and the bottom layer was collected in the same one litre separating funnel, shaken well and allowed to separate. The bottom aqueous layer was transferred to another one litre separating funnel containing 100 ml petroleum ether. It was also shaken well and allowed to separate. The aqueous layers were discarded and the petroleum ether layers from the two were pooled, washed with 100 ml of distilled water three times and dried with anhydrous sodium sulphate, then vacuum flash evaporated. Five gram anhydrous sulphate was placed at the bottom of a glass column of size 30 mm x 450 mm and 25g of activated florisil was added to the top of sodium sulphate. Another 10 g of sodium sulphate was added above the florisil. After wetting the column with petroleum ether, transferred the acetonitrile clean up sample using small quantities of petroleum ether. The column was eluted initially with 200 ml of 6 per cent diethyl ether in petroleum ether, followed by 200 ml of 15 per cent diethyl ether in petroleum ether. These elutes were pooled together and evaporated to dryness in vacuum flash evaporator. The dry matter obtained was taken in two millilitre petroleum ether for injection in to GLC.

### Residue extraction from fat tissue

Approximately two grams of fat tissue was weighed and homogenized with anhydrous sodium sulphate and this powder was transferred to a column pre- wetted with petroleum ether

and the fat was carefully extracted with 200 ml petroleum ether. The extract was then subjected to further clean up procedures in the same method as done for crop content samples.

### Analysis on gas liquid chromatography

Quantification of pesticide residues in the collected samples were done using gas liquid chromatography as per the method specified by Sharma (1979) and FDA (1977). GLC analysis was performed on a Hewlett-Packard Agilent 6890 series GC with electron capture detector (ECD) having  $^{63}\text{Ni}$  as the radioactive source and equipped with HP enhanced integrator algorithm.

### Detection and estimation

The chromatograph of samples and pesticides standard were obtained under identical conditions of GLC. Residues were detected by the combination of their retention time with the standard and the quantity by comparing the area with the standard using the HP enhanced integrator algorithm. Sum total of pesticides in the samples were quantified by the formula

$$\text{Pesticide residue in ppm} = \frac{X}{V_1} \times \frac{V}{M} \times \frac{1}{10^3}$$

X = Integrator reading in picogram

$V_1$  =  $\mu\text{l}$  of the sample injected

V = Total volume of cleaned up sample in ml

M = Weight (g) of sample taken for extraction

## RESULTS

The samples collected from foraging ducks of Kuttanad revealed that the organochlorine residues present in the crop content and fat of foraging ducks ranged from 0.00014 to 0.00044 ppm and 0.00011 to 0.0089 ppm respectively (Table 1). The samples in this region contained  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$  isomers of Hexachloro cyclohexane (HCH), metabolites of Dichloro Diphenyl Trichloroethane (DDT), Dicofol and  $\alpha$ -Endosulphan. The total residue level of HCH in the crop content and fat samples were 0.001 and 0.01 ppm respectively and that of DDT were 0.00045 and 0.00088 ppm respectively (Fig 2).

The combined residue of all the compounds accounted to 0.0018 and 0.0117 ppm in crop content and fat respectively. The samples collected from foraging ducks of Palakkad (Table 2) contained  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$  isomers of HCH, isomers of DDT, Dicofol,  $\alpha$ -Endosulphan and Dieldrin. The residue level in the crop content ranged from 0.0002 to 0.0075 ppm and that in fat samples ranged from 0.00014 to 0.0135 ppm. The total residue of HCH in the crop content and fat samples were 0.015 and 0.031 ppm respectively and that of DDT were zero and 0.00032 ppm (Fig 3). The combined residues were 0.0152 and 0.0419 ppm in the crop content and fat respectively. The samples estimated in crop contents and fat of foraging ducks from Thrissur (Table 3) showed that only isomers of HCH, primary derivatives of DDT and  $\alpha$ -Endosulphan were present

**Table 1. Mean residue (ppm) of organochlorines detected in the crop content and fat of foraging ducks from Kuttanad**

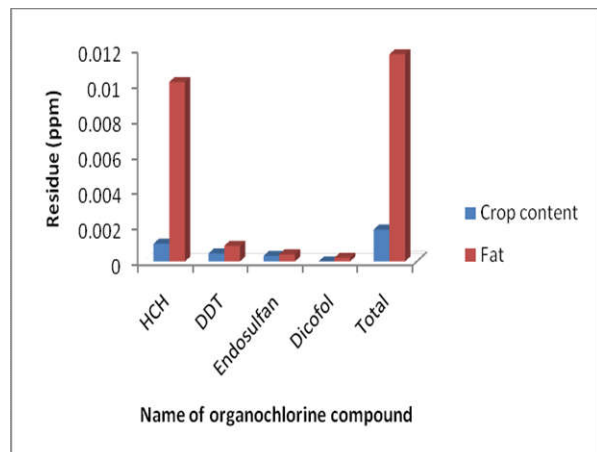
Name of organochlorine compound	Residue in crop content	Residue in fat
$\alpha$ -HCH	0.00016	0.00058
$\beta$ -HCH	0.00044	0.00066
$\gamma$ -HCH	0.00014	0.0089
$\delta$ -HCH	0.00026	0.00011
p, p'-DDE	0.00017	0.00038
o, p'-DDT	0.00028	0.0005
$\acute{\alpha}$ -Endosulphan	0.00031	0.0004
Dicofol	0.0000	0.0002
<b>Total</b>	<b>0.0018</b>	<b>0.0117</b>

**Table 2. Mean residue (ppm) of organochlorines detected in the crop content and fat of foraging ducks from Palakkad**

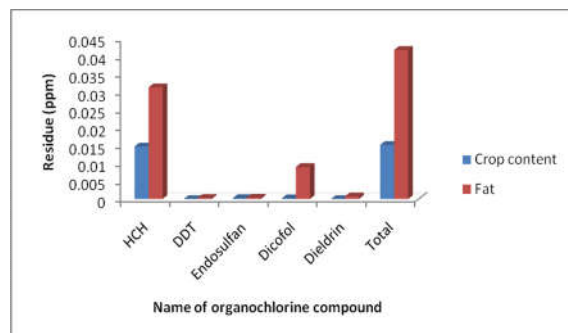
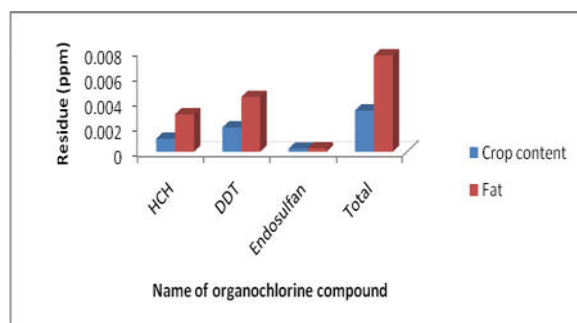
Name of organochlorine compound	Residue in crop content	Residue in fat
$\alpha$ -HCH	0.00086	0.0021
$\beta$ -HCH	0.0045	0.0092
$\gamma$ -HCH	0.0075	0.0135
$\delta$ -HCH	0.0019	0.0066
p, p'-DDE	0.0000	0.00014
o, p'-DDT	0.0000	0.00018
$\acute{\alpha}$ -Endosulphan	0.00024	0.0004
Dicofol	0.0002	0.009
Dieldrin	0.0000	0.00077
<b>Total</b>	<b>0.0152</b>	<b>0.0419</b>

**Table 3. Mean residue (ppm) of organochlorines detected in the crop content and fat of foraging ducks from Thrissur**

Name of organochlorine compound	Residue in Crop content	Residue in Fat
$\alpha$ -HCH	0.00015	0.00025
$\beta$ -HCH	0.0006	0.00135
$\gamma$ -HCH	0.0001	0.0008
$\delta$ -HCH	0.0002	0.0006
p, p'-DDE	0.0018	0.0042
o, p'-DDE	0.00014	0.00018
$\acute{\alpha}$ -Endosulphan	0.00026	0.00028
<b>Total</b>	<b>0.0033</b>	<b>0.0077</b>

**Fig 2. Organochlorine residues in foraging ducks from Kuttanad**

in the samples. The combined residues in these samples were also negligible. The total residue of HCH in the crop content and fat samples were 0.001 and 0.003 ppm respectively and that of DDT were 0.002 and 0.004 ppm (Fig 4)

**Fig 3. Organochlorine residues in foraging ducks from Palakkad****Fig 4. Organochlorine residues in foraging ducks from Thrissur**

## DISCUSSION

Report on the Australian National Residue survey results (2001) showed that the Maximum Residue Level (MRL) for various organochlorine pesticides in poultry fat are 0.2 ppm for endosulphan, aldrin and dieldrin compounds, 0.3 ppm for HCH and 5.0 ppm for DDT and its metabolites. MRL specified by Pesticide Manufacturers and Formulators Association of India (PMFAI) are 7.0 ppm for DDT, 2.0 ppm for HCH and 0.2 ppm for aldrin and dieldrin in poultry meat. In the present study the residue level detected for various organochlorine compounds were well below their Maximum Residue Level (MRL) specified in poultry. The residue levels detected in fat samples of foraging ducks from three regions were higher than those detected in corresponding crop samples which indicates the lipophilic action of these compounds. This finding agrees with Singh *et al.*, (1970) and George and Sundararaj, (1995) who reported highest residue of DDT and its metabolites in adipose tissue than meat, blood and other organs of laying hens. Lipophilic action of DDT was also reported by Furusawa (2002) with higher residue levels in egg yolk than blood and liver and Aulakh *et al.*, (2006) with high concentration of organochlorine residues in chicken egg yolk than muscle. In this study among the various Organochlorine pesticides (OCPs) detected, HCHs and its isomers had higher contribution to the total OCPs in Kuttanad and Palakkad region which confirms the finding of Dhananjayan and Muralidharan 2010. The results of the present study indicated that the residues from a series of organochlorine

compounds are present in the paddy fields of Kerala. Since the organochlorine residues detected in the samples from three foraging regions in the study were below their MRL specified in poultry, the eggs and meat of foraging ducks could be considered safe for human consumption. However, the farmers must be educated about the harmful residual effects of the compounds in human and animal population by their indiscriminate use in agriculture fields.

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