MULTI-RESIDUE ANALYSIS OF SOME ORGANOCHLORINE PESTICIDES IN COMMERCIAL BROILER MEAT, MILK AND CHICKEN EGGS BY GAS CHROMATOGRAPHY-ELECTRON CAPTURE DETECTOR (GC-ECD)

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Thesis submitted to the

KARNATAKA VETERINARY, ANIMAL AND FISHERIES SCIENCES UNIVERSITY, BIDAR

in partial fulfilment of the requirements

for the award of the degree of

DOCTOR OF PHILOSOPHY

in

VETERINARY PHARMACOLOGY AND TOXICOLOGY

By

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CERTIFICATE

This is to certify that the thesis entitled "***MULTI-RESIDUE ANALYSIS OF SOME ORGANOCHLORINE PESTICIDES IN COMMERCIAL BROILER MEAT, MILK AND CHICKEN EGGS BY GAS CHROMATOGRAPHY-ELECTRON CAPTURE DETECTOR (GC-ECD)***" submitted by **Mr. LOKESHA, L.V., I.D. No. DVHK 1414** in partial fulfilment of the requirements for the award of **DOCTOR OF PHILOSOPHY** in **VETERINARY PHARMACOLOGY AND TOXICOLOGY** of the Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar is a record of bonafide research work carried out by him during the period of his study in this University under my guidance and supervision, and the thesis has not previously formed the basis for the award of any degree, diploma, associate ship, fellowship or other similar titles.

Bengaluru
June, 2017

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Affectionately Dedicated to

My beloved Mother

Rathnamma

My beloved Guide

Dr. Jagadeesh S. Sanganal

My beloved friend

Dr. Anitha Kumari, A.M.

and

Family

ACKNOWLEDGEMENT

With great fervency I take this opportunity as my prerogative and honor to express my deep sense of appreciation, warm reverence and wholehearted indebtedness to ***Dr. Jagadeesh S. Sanganal,*** *M.V.Sc., Ph.D., Professor & Head, Department of Veterinary Pharmacology and Toxicology, Veterinary College, Bengaluru and Chairperson of my Advisory Committee for his incredible attention, supervision, timely advice, guidance, encouragement, suggestions and help during my Ph.D., programme.*

I am greatly indebted to ***Dr. N. B. Shridhar,*** *M.V.Sc., Ph.D., Associate Professor, Department of Veterinary Pharmacology and Toxicology, Shivamogga, my advisory committee member, for his encouragement, suggestions and help throughout my Ph.D., programme.*

I am profoundly thankful to ***Dr. H.D. Narayana Swamy,*** *M.V.Sc., Ph.D., Professor, Department of Veterinary Pathology, Veterinary College, Bengaluru, my advisory committee member, for his keen support, timely and valuable suggestions during my research work.*

I express my heartfelt thanks to ***Dr. V. Girish Kumar,*** *M.V.Sc., Ph.D., Professor & Head, Department of Veterinary Biochemistry, Veterinary College, Bengaluru, my advisory committee member, for his support, timely and valuable suggestions during my research work.*

I would be immensely delinquent if I do not express my bonafide gratitude to ***Dr. K.S. Rao****, M.V.Sc., Ph.D., DABT., Advinus Therapeutic Private Limited, Bengaluru, my advisory committee member for his valuable guidance and suggestions during my research .*

I am thankful ***to Dr. N. Prakash,*** *M.V.Sc., Ph.D., Professor & Head, Department of Veterinary Pharmacology and Toxicology, Veterinary College, Shivamogga, for his suggestions and help during my Ph.D., programme.*

I take this opportunity to extend my deep sense of gratitude to who have helped me immensely during this study and for constant support and help in all possible ways. I express my appreciation to ***Mr. Shekar*** *and* ***Dr. Yogesh Gowda, S.,*** *My best friends for their co-operation and encouragement during the course of the study and stay in department, I gratefully acknowledge their valuable help.*

It is my obligation to thank my seniors ***Drs. Ravi Kumar, C & Sunil Chandra, V., and my friends Dr.Prashantkumar Wahge*** *and* ***Dr. Ranjith, D****., for their kind suggestions and constant support throughout my Ph.D programme.*

I am highly indebted and I owe my wholehearted thanks and gratitude to doctoral and post graduate friends, ***Drs. Sahadev, Suresh, L., Manjula, V., Shobha, K.S., Shrikanth, Veena, M.P., Sarvesh, Srikala Devarakonda, Sunil Patel, Vijay Kumar, Rajanna, Shivshankar, Muniraju, H.M., Hanumantha Vadder, Bharath Kumar*** *for their support, encouragement and help in one way or another.*

I thank the supporting staff ***Smt. Akkayamma, Smt. Pushpa and Smt. Nagamma****, Department of Pharmacology and Toxicology, for all the help.*

I am highly indebted and I owe my wholehearted thanks and gratitude to my family friends and my well wisher ***Smt/Sri Anuradha Theerthaprasad*** *and his family for their support, encouragement and help in one way or another during my doctoral degree programme.*

It is my family members without whose support & encouragement, I would not be in this position today. At this explicable moment, I wish to express my gratitude to

my father Venkatappa, mother Smt. Rathnamma, my aunt Smt. Sharada, L.E., my uncle Shreekanteshwara, T and my aunt Smt. Indira, Smt. Shivamma, my brother Neelakanta, my sister/s Roopa, L.V., Deepa, L.V., Gunavathi, S., Chandana, S., Sindu and Aishwarya and my brother in law Shivakumar , my kotta bangaaru lokam Alludu (Krishna Manohar) and my niece *Amrutha* for their moral support which is invaluable & inexpressible. It's their whole hearted love and never ending moral support which helped in completion of my research work successfully

Last but not the least, I would like to place on record my heartfelt thanks to all those who have directly or indirectly helped me in completion of my research work successfully.

June, 2017

Bengaluru *(Lokesha, L.V.)*

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LIST OF ABBREVIATIONS

@	At the rate of
α	Alpha
β	Beta
cm	Centimetre(s)
°C	Degree(s) Celsius
δ	Delta
CV	Coefficient of variance
DDD	Dichloro diphenyl dichloroethane
DDE	Dichloro diphenyl ethylenediene
DDT	Dichloro diphenyl trichloroethane
etc	Et cetera
EU	Europian union
FAO	Food and agriculture organisation
Fig	Figure
γ	Gama
g	Gram(s)
GC-ECD	Gas chromatography electron capture detector
h	Hour(s)
HPLC	High performance liquid chromatography
HCH	Hexachloro cyclohexanre
ICMR	Indian Council of Medical Research
IARC	International Agency for Research on Cancer
kg	Kilogram(s)

Kg/ha	Kilogram per hectre
LD_{50}	Lethal dose 50
LC MS	Liquid chromatography mass spectrometry
LOD	Limit of detection
LOQ	Limit of quantification
<	Less than
mg	Milligram(s)
mg/kg	milligram(s) per kilogram
min	Minute(s)
µ	Micro
µl	micro liter(s)
µg	Microgram(s)
ml	Milliliter(s)
MS	Mass spectrometry
>	More than
MW	Molecular weight
MMT	Million metric tons
MRLs	Maximum residue levels
ng/ml	Nanogram/ milliliter
ng/l	Nanogram/liter
NOAEL	No observed adverse effect level
OCPs	Organochlorine pesticides
Ppb	Parts per billion
rpm	Rounds per minute

RSD	Relative standard deviation
%	Per cent
SF	Separating funnel
SD	Standard deviation
SPME	Solid- phase micro extraction
Std	Standard
viz	Namely
WHO	World Health Organization

Introduction

I. INTRODUCTION

As vast majority of the population in India are engaged in agriculture and agricultural development continues to remain the most important objective of Indian planning and policy and in the process of development of agriculture, pesticides have become an important tool as a plant protection agents for boosting food production. However, exposure to pesticides both occupationally and environmentally causes a range of human health problems. India is the largest producer of pesticides in Asia and ranks 12^{th} in the world for the use of pesticides (Abhilash and Singh, 2008).

Although Indian average consumption of pesticide is far lower than many other developed countries but India is the fourth largest pesticide producer in the world after US, Japan and China. The production of pesticides in India is approximately 85 TMT (Thousand metric tonnes), about 50 TMT of this quantity is used annually and insecticides alone account for 71% (Bhattacharyya *et al.*, 2009) of this consumption. The consumption of pesticides in Indian agriculture is comparatively low (0.5 kg/ha), (only 3.75% of global consumption) as compared to 12.0, 7.0, 6.6, and 3.0 kg/ha in Japan, USA, Korea and Germany, respectively. Different patterns of pesticide production and its use has been analyzed and insecticide use is around 75% in the country compared to 32% in the world. Herbicide is used only 12% compared to worldwide consumption of 47%. Carbamate and synthetic pyrethroids are most globally used (45% together). Organophosphate constitutes 50% of the consumption and bio-pesticides are used only up to 1% compared to 12% worldwide.

Pesticides can be defined as any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating pests. Pests can be insects, rodents, weeds and a host of other unwanted organisms. Thus, pesticides occupy a rather unique position among the many chemicals that we encounter daily, in that they are deliberately added to the environment for the purpose of killing or injuring some form of life.

Pesticides, human health and safety of the environment have been a global concern. Organochlorine compounds have a high toxic effects and persistence in the environment, posing considerable hazards to the biotic system. They pose a major threat to the living organisms in the environment, as they are lipid soluble and non biodegradable. The problem becomes more serious when bioaccumulation of these lipophilic compounds are taken into consideration (Doyle, 2004) and their high toxicities, chemical, biological stabilities, lipophilicities make these compounds more prone to bio accumulate along the food chain (Biziuk *et al*.,1996). Pesticide residues were accumulated in adipose tissue of meat and fat rich dairy products to the significant levels of contamination (Bentabol and Jadral, 1995), this fact has caused concern since meat and dairy products play role in human nutrition.

Ideally, their injurious action would be highly specific for undesirable targets; in fact, however, most pesticides are not highly selective, but are generally toxic to many non target species, including humans. It has been observed that the pesticides exposures are increasingly linked to immune suppression, hormone disruption, diminished intelligence, reproductive abnormalities and cancer (Murphy, 1986).

The main pathway of the human exposure to organochlorine compounds is food, especially the dairy products (Hura and Carmen., 1999). The accumulation of organochlorine compounds in fatty foods is still a matter of major concern although the use of most organochlorine compounds had been banned or restricted in industrialized countries since the 1970s, due to the uncertainty about the adverse effects that those residues may have after a lengthy exposure at low doses (Kodba and Voncina, 2007, Soler *et al*., 2007). These compounds or residues can generate certain harmful effects on humans as well as on animals (Daston *et al.,* 1997).

The residues of these pesticides are accumulated in milk producing animals such as cow and buffaloes, if they feed on contaminated grass/hay and in inhaled air. Being highly lipophilic, organochlorine pesticides are primarily stored in fat rich tissues in the animals and subsequently translocated and excreted through the milk, meat and eggs. They get accumulated in fat rich dairy products such as butter, cheese and as such, consumers of milk and dairy products could be exposed to these residues (Kannan *et al.,*1992; Bentabol and Jordal, 1995; Waliezewski *et al*.,1997). This becomes a great concern with respect to food safety in India, as most Indians consume milk daily as a custom, either in the form of beverages or in its various forms such as butter, butter milk, curd, cheese and sweets made out of milk.

The toxicity of these residues slowly cause ill effects. Residues accumulate in fatty tissues, thus building up in the vital organs such as liver, heart, kidney, thyroid, mammary gland and testes. Several health effects ranging from systemic effects on cardiovascular, respiratory and genotoxic effects have been reported (Kalpana, 1999) and

they are shown to be potential endocrine disrupts in humans even for low level exposures (Calborn *et al*.,1993).

With the advancement of the analytical instruments and analytical methodologies, the sensitivity, precision and accuracy of the methods currently used for detection of organochlorine pesticides and its residues are very high, so that the organochlorine pesticide residues from cow milk, chicken meat and chicken eggs may be detected at microgram per kilogram (µg/kg) fat level.

Chromatography is an analytical technique which has been used for isolation, purification and separation of organic and inorganic compounds including qualitative and quantitative estimation of compounds. The volatile organic compounds (VOCs), poly-aromatic hydrocarbon and pesticides have been analyzed by gas chromatography technique.

A number of different selective detectors can be coupled with GC for analyzing organochlorine pesticides, including electron capture detector (ECD), halogen specific detector (XSD), electrolytic conductivity detector (ELCD) and atomic emission detector (AED). GC-ECD is the most commonly used detection method with low detection limits. The determination of pesticides residues in the environment and in food is necessary for ensuring that human exposure to contaminants, especially by dietary intake does not exceed acceptable level for health. Consequently, robust analytical methods have to be validated for carrying out both research and monitoring programmes, and thus for defining limitations and supporting enforcement of regulations.

Gas chromatography - electron capture detector (GC-ECD) is a commonly used analytic technique in many research and industrial laboratories for quality control as well as identification and quantification of compounds in a mixture. GC-ECD is also a frequently used technique in many environmental and forensic laboratories because it allows for the detection of very small quantities. A broad variety of samples can be analyzed as long as the compounds are sufficiently thermally stable and volatile. In an effort to provide a more concise exploration of existing toxicants bio-monitoring methodology that is relevant today. These methods possess limits of detection (LODs) that span a wide range; some are suitable for only occupational or forensic applications while those with LODs near or lower than the μg/l are useful for detecting incidental environmental exposures. In addition, these methods have been used to measure toxicants and/or their metabolites in a variety of matrices including urine, serum, breast milk, saliva, and post-partum meconium.

Hence, in this context, pesticide safety, regulation of pesticide use, proper application technologies, and integrated pest management are some of the key strategies for minimizing human exposure to pesticides. There is a dearth of studies related to these issues in India. The present study is aimed to detect the residual concentration of some common OCPs in the commercial broiler meat, eggs and milk. Since, they may be present in lesser quantity; it may not be possible to detect them using the existing routine techniques. Hence, a sensitive detection method using Gas Chromatography-Electron Capture Detector (GC-ECD) is selected for the study.

Objectives of the study are as follows

I. Collection of commercial broiler meat, milk and eggs from Bengaluru, Mysuru and Shivamogga areas of Karnataka.

II. Standardisation of the detection methods for the residues of some organochlorine pesticides in commercial broiler meat, milk and eggs.

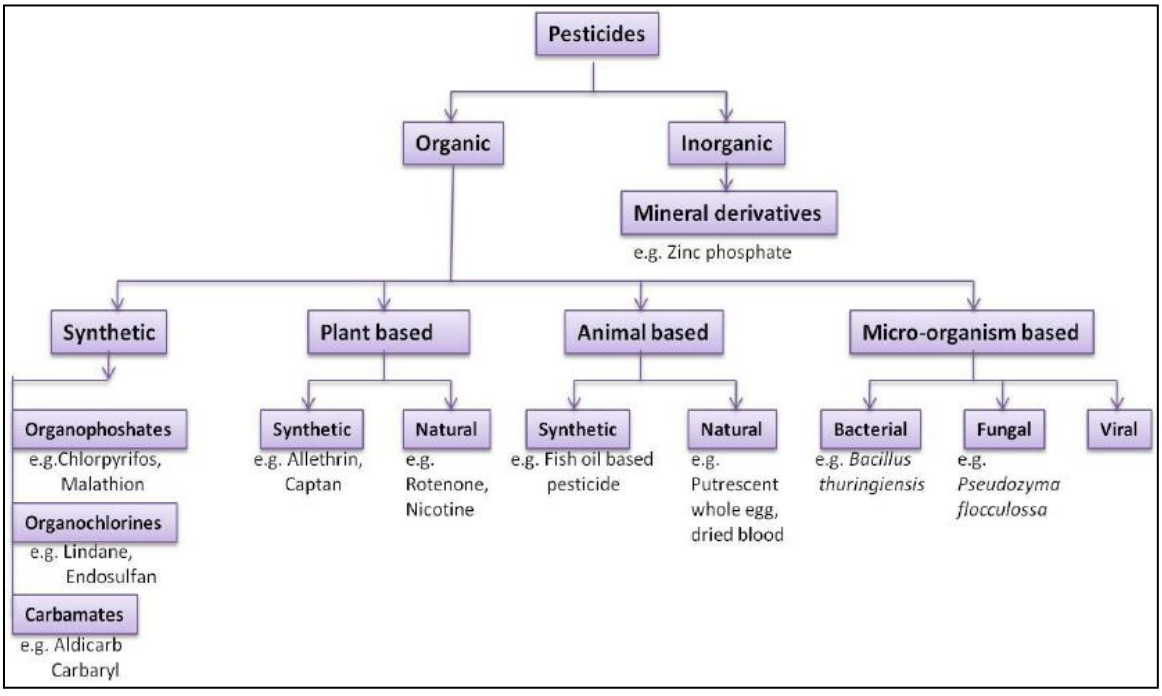
III. Comparing the concentration of residues of organochlorine pesticides with standard Maximum Residue Level (MRLs) values.

Review of Literature

II. REVIEW OF LITERATURE

2.1 Pesticides

Pesticides can be defined as any substance or mixture of substances intended for preventing, destroying, repelling or mitigating pests. Pests can be insects, rodents, weeds and host of other unwanted organisms (Ecobichon, 2001a). Thus pesticide occupy unique position among the many chemicals that we encounter daily, in that they are deliberately added to the environment for the purpose of killing or injuring some form of life. Ideally, their injurious action would be highly specific for undesirable targets. The most common classification of pesticides relies on the target species they act on. The major classes of Pesticides are depicted below.



(Source: https://www.researchgate.net/figure/226507214)

2.2 Organochlorine pesticides

Organochlorine pesticides (OCPs) are ubiquitous contaminants, the occurrence of which in the environment is of special concern due to long persistence to degradation and the toxicity of their constituents (Simonich and Hites, 1995; Tolosa *et al*., 1995).

Organochlorine pesticides, include the chlorinated ethane derivatives, such as dichloro diphenyl trichloroethane (DDT) and its metabolites *viz*; dichloro diphenyl dechloroethane (DDD), dichloro diphenylethylenediene(DDE), dichlorodiphenylacetic acid(DDA), hexachlorocyclohexane(HCH), cyclodienes (aldrin, dieldrin, endrin), chlordanes (heptachlor, heptachlor epoxide, cis-chlordane, trans-chlordane, cis nonachlor, trans-nonachlor, and oxychlordane), HCHs (α,β,γ,δ-isomers), mirex and industrial chemicals like PCBs, are ubiquitous environmental pollutants (Kannan *et al*., 1992; Loganathan *et al*., 1995; Senthil Kumar *et al*., 2005).

Organochlorine pesticides are chlorocarbons; they are also called as persistent organic pollutants (POPS), because organochlorines have extremely strong bonds between their chlorine and carbon components and are attracted to fats. They enter the soil by deposition from air, drift, or by washing-off from plant surfaces during rainfall or irrigation. These compounds are mainly found associated with organic matter in soil and lipid tissues of organisms because of their strong hydrophobic (lipophilic) character (Ningombam and Priyankar. 2013).

The problem with this strength due to, once organochlorine pesticides are used, they stay around for a long time, not only in the water supply and in the soil but also

accumulate in human and animal body fats posing problems to human health (Ejobi *et al*., 1996).

Organochlorine pesticides have long history of wide use in agriculture (Loganathan and Kannan., 1994). From the 1940s to the 1970s and 1980s, the organochlorine insecticides widely used in agriculture to increase the yield and improve the quality of the crops (Nawaz, 2003), insect control, malaria control programs and animal productions (Beyer and Biziuk, 2008).

In Indian agriculture, 54% of the total pesticides are consumed on cotton cultivation and nearly 20-25% are used for the control of sucking pests and bollworm. DDT and malathion are still preferred by the small farmers because they are cost effective, easily available, and display a wide spectrum of bioactivity. Out of the total consumption of pesticides, 80% are in the form of insecticides, 15% are herbicides, 1.46% is fungicides and less than 3% are others. In comparison, the world wide consumption of herbicides were 47.5%, insecticides were 29.5% and fungicide was 17.5% and others account for 5.5% only (Puri *et al.*, 1999). In India usage of several of OCPs like aldrin, chlordane, DDT, dieldrin, endrin, heptachlor and HCH has been restricted or banned for use during the last decade (Singh *et al*., 2007).

The organochlorine insecticides important primarily because of ecological considerations: Carlson in 1962 warned that organochlorine compounds could pollute the tissues of virtually every life form on the earth, the air, the lakes and the oceans, the fishes that live in them and the birds that feed on the fishes (Carlson, 1962) because of

their environmental persistence and high lipophilicity, exposure to these compounds continues, most notably through the diet.

The dairy cows were mainly exposed to these contaminating residues through their food (Mallatou *et al*., 1997; Martinez *et al*., 1997, Nag *et al*., 2008). After the residues have been metabolized, they are stored in the fat reserves from which they enter in to circulation and are eliminated through milk (Cerkvenik and Vesna. 2000, Nag *et al*., 2008).

2.2.1 DDT

DDT (1,1,1-trichloro-2,2-bis (p-chlorophenyl)ethane or dichloro-diphenyl-trichloroethane) was extensively and widely used chemical to control insects on agricultural crops, insects that carry diseases like malaria and typhus and sanitary purpose (Pandit *et al*., 2001, Devi *et al*., 2013). It was estimated that about 25000 MT of chlorinated pesticides was used annually in India and DDT accounted for 40% of this group (Mathur, 1993).

DDT was first synthesized in 1874, but it was not until 1939 that Swiss biochemist Paul Hermann Muller discovered its potency as an all-purpose insecticide.

Although DDT has been banned for agricultural use, India has sought exemption under Stockholm Convention for use of 10,000 tons of DDT for restricted use in the public health sector. The National Malarial Program (NAMP) used 3750 tons of DDT in the year 2001, in rural and peri-urban areas for residual spraying (Gupta, 2004).

Technical grade DDT is made from chloral hydrate, chlorobenzene and sulfuric acid. Dichloro diphenyl dichloroethane (DDD) degradation product or metabolite of DDT, is one of the most studied organochlorines because of its ubiquity and potential toxicity (Xianyu *et al*., 2014).

DDT metabolites leave the body mostly in urine, but may also leave by breast milk. These chemicals are carcinogenic, mutagenic and teratogenic and also reported to posses estrogenic activity (Falck *et al*., 1992).

Human breast milk is the preferred sole source of nutrients during early infancy. Breast feeding can help strengthen the infant immune system, and promotes mother-child bonding. However, breast milk can be a source of exposure to organochlorine pesticides in the breast fed infant. Chemicals with estrogenic properties administered chronically at high levels promote mammary gland carcinogenesis in experimental system and growth of estrogen-responsive human breast cancer (Lippman *et al*., 1977).

These pesticides have deleterious effects on the immune system and increased amounts of DDE and DDTs are detected in certain cancerous tissues (Krieger *et al*., 1994) and DDTs has linked with pancreatic cancer and increase in breast cancer (Dich *et al*., 1997).

2.2.2 Endosulfan

Endosulfan is a broad-spectrum insecticide-cum-miticide, which is extensively used on many important crops and use of endosulfan on agricultural crops has been permitted in the country (Ningombam and Priyankar, 2013).

Endosulfan was used to control a number of insects on food crops such as grains, tea, fruits and vegetables and on non-food crops such as tobacco and cotton. Endosulfan was recommended by agriculture scientists to the farmers for controlling most of the economically significant pests such as *Helicoverpa armigera* (American bollworm) and *Spodoptera litura* (Asian armyworm) in India. *H. armigera* alone has caused about Rs 5,000 crore of crop losses in India (Gujar *et al*., 2006)

Endosulfan is one of the most abundant organochlorine pesticides in the global atmosphere and is capable of undergoing long range transport to remote locations such as the Arctic. Degradation of endosulphan results in two isomers, α- and β-endosulfan, does occur in temperate/tropical soil and aquatic systems, both by abiotic and biotic processes, although this is highly dependent on the prevailing environmental conditions. Endosulfan sulfate is the major metabolite and this recalcitrant compound has been detected in air and is present in remote mountain lake sediments (Weber *et al*., 2010).

2.2.3 Lindane

Dutch chemist Teunis van der Linden (1884–1965)- discovered gamma-hexachlorocyclohexane and it was named after him. Lindane consists of five isomers constitutes alpha-HCH : 55-80%, beta-HCH: 5-14%, gamma-HCH: 8-15%, delta-HCH: 6-10% and epsilon-HCH:1-5% (Willet *et al*., 1998).

Lindane –a persistent organic pollutant and preferred pesticide by the small farmers because they were cost effective, easily available and they display a wide spectrum of bioactivity (Ningombam and Priyankar, 2013).

The insecticide lindane was classified as “carcinogenic to humans” (Group 1). Lindane, the γ-isomer of hexachlorocyclohexane, has been used extensively for insect control in agriculture and for treatment of human ectoparasites. Occupational exposures have occurred among agricultural workers and pesticide applicators; however, the use of lindane is now banned or restricted in most countries. Lindane is lipophilic, readily absorbed via all routes of exposure, and distributes widely in the body (Lancet Oncol, 2015).

Exposure to large amounts of lindane can harm the nervous system and caused headache and dizziness to seizures, convulsions and more rarely, death. Prenatal exposure to β-HCH, an isomer of lindane and production byproduct, has been associated with altered thyroid hormone levels and could affect neurological development (Lopez *et al*., 2010).

Epidemiological cohort and case control studies of non-Hodgkin lymphoma in several countries provided sufficient evidence in humans for the carcinogenicity of lindane (Alavanja *et al*., 2014). The United states Agricultural Health Study, a large prospective cohort study with detailed exposure assessment, reported statistically significant increases in non-Hodgkin lymphoma risk with increasing occupational exposure to lindane (Blair *et al*., 1998)

The most environmentally significant isomers were γ-HCH, α-HCH, and β- HCH. All the three isomers have moderately high vapour pressures and high water solubility compared to other organochlorine contaminants therefore, generally detected in the atmosphere or bodies of water (Walker *et al*., 1999).

γ -Hexachlorocyclohexane (lindane) is an organochlorine insecticide that can act through dermal, ingestion, and respiratory routes. It has been used to control insect damage to crops, stored products, in seed application, on animals and for public health pests such as head lice (Blair *et al*., 1998).

Alpha-HCH one of the five stable isomers of technical HCH, a pesticide formerly used in agriculture. In general, HCHs are among the most studied pesticides with respect to their environmental fate and effects (Breivik *et al*., 1999).

The fate of HCH in the environment and the efficiency of its microbial degradation, depends on certain biotic and abiotic factors like availability of HCH degrading microbes, temperature, pH, moisture, texture and organic content of soil, etc. (Lal, 1983).

These factors vary from site to site depending on seasonal changes and properties of the soils and are responsible for variation in concentration among sampling sites and within two depths at the same site (Van *et al*., 1997). Frequent occurrence of -HCH in higher concentration (Nayak *et al*., 1995) in spite of complete restriction on the use of technical HCH may also be due to its highly persistent nature (Dogra *et al*., 2004).

2.2.4 Aldrin

Aldrin was used to control soil insects such as termites, corn rootworm, wireworms, rice water weevil and grasshoppers to protect crops such as corn and potatoes. Aldrin is under complete ban in India, which has been used as anti-termite agent

against potato crops earlier but aldrin residues in ground water were observed (Singh *et al*., 2007).

Aldrin was readily and rapidly converted to dieldrin in the environment and its fate is closely linked to that of dieldrin. Aldrin is readily metabolised to dieldrin in both animals and plants and therefore Aldrin residues are rarely present in animals and then only in very small amounts (http://www.popstoolkit.com/about/chemical/aldrin.aspx).

2.2.5 Dieldrin

Dieldrin has been used in agriculture for the control of soil insects and several insect vectors of disease but this latter use has been banned in a number of countries due to environmental and human health concerns. Principle contemporary uses are restricted to controlling termites and wood borers and against textile pests. Dieldrin binds strongly to soil particles and hence is very resistant to leaching into groundwater. Volatilization was an important mechanism of loss from the soil and because of its persistent nature and hydrophobicity, dieldrin is known to bioconcentrate (http://www.popstoolkit.com/about/chemical/aldrin.aspx).

Dieldrin when used in agriculture, the chemicals can enter the soil, wash into nearby surface water or volatize in air. Once in the environment, they can accumulate in living organisms. Once present in soil or water, dieldrin breaks down very slowly, does not easily evaporate into the air and can bind very tightly to soil particles. Plants take up dieldrin residues directly from the soil. In animals, including humans, dieldrin is stored in the fat and leaves the body very slowly (http://www.popstoolkit.com/about/chemical/aldrin.aspx).

In countries where dieldrin is still used for termite control, household air may be a significant exposure pathway for animals and human beings. Dieldrin was ubiquitous in breast milk and it was found in more than 99 percent of samples tested in most countries. Because dieldrin is attracted to fat, the level of dieldrin in a mother's milk is generally about six times higher than the level in her blood. However, despite dieldrin's widespread presence in breast milk, the World Health Organization (WHO) has concluded that dieldrin residues in breast milk may not pose a significant risk to the infant. WHO found, surprisingly, that the concentration of dieldrin in the blood and bodies of breastfeeding babies did not increase with age during their first six months of life (http://www.popstoolkit.com/about/chemical/aldrin.aspx).

2.2.6 Chlordane

Chlordane was broad spectrum contact insecticide used on agricultural crops including vegetables, small grains, maize, other oilseeds, potatoes, sugarcane, sugar beets, fruits, nuts, cotton, and jute. It has also been used extensively in the control of termites. Chlordane was highly insoluble in water and soluble in organic solvents. Chlordane was semi-volatile and can be expected to partition into the atmosphere as a result. It binds readily to aquatic sediments and bioconcentrates in the fat (http://www.popstoolkit.com/about/chemical/aldrin.aspx).

2.2.7 Chlordecone and Mirex

These two organochlorine insecticides were introduced for use against fire ants and leaf eating insects. Among the two, chlordecone has most studied because of episode involved 148 workers in chlordecone producing factory in Hopewell, Verginia (Taylor *et*

al., 1978). Chlordecone induces hepatic drug metabolizing enzyme system and causes hepatospleenomegaly in rats and humans. It was not mutagenic but can induce liver tumours in rodents (Smith, 1991). Chlordecone also causes reproductive toxicity in animals likely by mimicking the effects of excessive estrogens. Low or absent sperm count was found in chlordecone exposed workers (Guzelian, 1982).

2.3 Method validation and detection of organochlorine pesticide residues in Milk

Losada *et al*. (1996) determined the residue levels in raw bovine milk and the residue levels of the organochlorine pesticides (alpha-HCH, lindane, heptachlor-epoxide, aldrin, endrin, dieldrin, o,p'-DDE, p,p'-DDE and p,p'-DDT) and compared with the maximum levels allowed by the European Union (EU) in these foods. The highest incidence percentage of the 10 insecticides measured was for lindane, followed by alpha-HCH and aldrin. Moreover, the highest mean residue level was for alpha-HCH. None of the samples analyzed exceeded the maximum levels allowed by the EU.

Martinez *et al*. (1997) investigated the presence of organochlorine pesticides (α-HCH, β-HCH, lindane, aldrin, dieldrin, heptachlor, heptachlor epoxide, chlordane and the isomers and metabolites of DDT) in pasteurized milk and associated health risks. The samples (95%) contained one of the isomers of the HCH group and 12.9% of them exceeded the maximum residue limit permitted by the European Union, 6 samples went over that limit for heptachlor epoxide and 74.63% of the samples contained chlordane at higher concentrations than those permitted by the legislation. None of the samples exceeded the limit for the DDT group.

Pandit *et al*. (2001) carried out a study to monitor the milk and milk product (curd, milk powder and butter) samples of various brands from different cities in Maharashtra, India to detect the occurrence of persistent organochlorine pesticide residues. The measurements were made using a gas chromatograph- electron capture detector system. The trace levels of DDT and HCH were detected in the samples. All levels of organochlorine pesticide residues in milk and milk products were below the maximum permissible limits given by the FAO/WHO.

John *et al*. (2001) conducted the survey during 1993–1996 to investigate the magnitude of contamination of bovine milk with organochlorine pesticide (OCP) residues from Jaipur city, Rajasthan, India. Milk samples, i.e., dairy (toned and whole) and buffalo milk, were collected seasonally and pesticide residues were assessed using a gas chromatograph (GC) with an electron capture detector (ECD). The results indicated that all the milk samples were contaminated with dichlorodiphenyltrichloroethane (DDT) and its metabolites (DDE and p,p′-dichlorodiphenyldichloroethane [DDD]), isomers of hexachlorocyclohexane (HCH; α, β, and γ), heptachlor and its epoxide, and aldrin. Seasonal variations of these pesticide residue levels were also observed in all the milk samples. Samples collected during winter season were found to contain higher residue levels as compared to other seasons.

Gonzalez-Rodriguez *et al*. (2012) developed a new analytical method to determine more than 40 multiclass pesticides in different kinds of processed (whole, skimmed and powdered) and unprocessed (goat and human) milk samples using solid-phase microextraction (SPME). A comparative study between headspace (HS) and

direct immersion (DI) was carried out. The effect of milk dilution and the use of acid to reduce the influence of the matrix in DI-SPME mode were also evaluated. DI of the SPME fiber into previously diluted and acidified milk samples achieved the best sensitivity results.

Pesticides were determined using low-pressure gas chromatography-tandem mass spectrometry (LP-GC-MS/MS). Both of the selected techniques have been shown to be effective at reduced fat interference and can determine analytes present at very low concentrations (limits of quantification between 0.02 and 1.00 μg/l). Performance characteristics such as linearity, recovery, precision, and lower limits, together with an estimation of the measurement uncertainty using validation data, are presented for each pesticide.

All of the pesticides presented recovery rates of between 81 and 110% and precision values lower than 12% (expressed as the relative standard deviation). The overall uncertainty of the method was estimated at three different concentrations (10, 25 and 50 μg/l) and was lower than 25.5% in all cases. The proposed analytical methodology was applied to the analysis of target pesticides in 35 samples: 15 commercial, 3 human and 17 goat milk samples. The metabolite p,p′-DDE was the compound most frequently found in both the breast and goat milk samples, at concentration levels <20 μg/l. However, pesticide residues were not found in any of the other 15 commercial milk samples (skimmed, powdered and whole milk) analyzed.

Giampiero *et al*. (2006) carried out an experiment to determine the contamination of raw milk by the organophosphorus pesticides in Italy and to evaluate the opportunity

to start specific procedures of risk management along the milk production chain. Among the 135 samples analyzed, 37 were positive in traces and 10 showed an organophosphorus contamination ranging from 5 to 18 μg/kg. The higher results were recorded in the samples collected during the autumn-winter period. The main pollutants detected were acephate and chloropyriphos. Their concentrations were lower than the maximum residue level fixed by the European Commission.

Maria *et al.* (2008) developed a simple and rapid method based on solid-phase microextraction (SPME) technique followed by gas chromatography with micro electron-capture detection (GC-μECD) was developed for the simultaneous determination of more than 30 pesticides (pyrethroids and organochlorinated among others) in milk. A fractional factorial design was performed to assess the influence of several factors (type of fiber coating, sampling mode, stirring, extraction temperature, and addition of sodium chloride) on the SPME procedure and to determine the optimal extraction conditions. After optimization of all the significant variables and interactions, the recommended procedure was established as follows: SPME (using a polydimethylsiloxane (PDMS)/divinylbenzene (DVB) coating) of 1 mL of milk sample diluted with milli-Q water (1:10 dilution ratio), at 100 °C, under stirring for 30 min. The proposed method showed good linearity and high sensitivity, with limits of detection (LOD).

Subir *et al*. (2008) monitored the bovine milk from different places in Bundelkhand region of India was carried out to evaluate the status of organochlorine pesticide (OCP) residues. Out of a total of 325 samples 206 (63.38%) were contaminated with residues of different OCPs. The average concentration of total HCH was 0.162

mg/kg. Among the different HCH isomers the frequency of occurrence of α-isomer was maximum followed by δ, γ and β. Endosulfan (α, β, sulfate) was detected in 89 samples with mean concentration of 0.0492 mg/kg while total DDT comprising of DDT, DDE and DDD was present in 114 samples having mean concentration of 0.1724 mg/kg. Dicofol was positive in 17 samples.

Ashnagar *et al.* (2009) carried out an experiment to detect the residues of seven important oraganochlorine insecticides in 35 milk samples marketed in the city Ahwaz, Iran. The detection and measurement of the toxins were achieved by using HPLC with UV detector. The results obtained has showed that lindane (0.042 mg/kg) and DDT (0.28 mg/kg) are detected and exceeded the standard limits recommended by FAO/WHO.

Hronn *et al*. (2010) designed the study to assess the occurrence of a few organochlorine contaminants and their metabolites in eggs of different marine bird species in Iceland, a country located in the sub-Arctic of the North-Western Atlantic. Previous investigations from Sweden and the Netherlands have shown some obvious differences in contaminant concentrations, including hydroxylated polychlorinated biphenyl metabolites (OH-PCBs) in guillemot (*Uria aalge*) and other bird species.

Eggs from seven marine bird species, Arctic tern (*Sterna paradisaea*), common eider (*Somateria mollissima*), guillemot, fulmar (*Fulmarus glacialis*), great black-backed gull (*Larus marinus*), lesser black-backed gull (*Larus fuscus*), and great skua (*Stercorarius skua*), that all breed in Iceland, were collected and analyzed for several persistent organic compounds and their metabolites. The contaminant levels varied

between the species investigated. The highest concentrations were found in eggs from the Great Skua (18 and 23 μg/g l.w. of CB-153 and 4,4′-DDE, respectively).

The concentration difference was generally 2 orders of magnitude higher in great skua for all organochlorine compounds analyzed with the exception of HCB. HCB did not vary as much between the seven species (ranging from 34 to 710 ng/g). OH-PCB and OH-PCB metabolites congener concentrations and patterns showed differences in metabolic capacity between bird species. *Guillemot* and *Great Skua* seem to distinguish themselves most from other species with the absence of 4-OH-CB187 and low relative levels of 4-OH-CB146 in *Guillemot* and the low abundance of OH-PCBs in *Great skua.*

Sait *et al*. (2011) determined the organochlorine pesticides (OCPs) in three types of milk (cow, buffalo and sheep milk) produced in Afyonkarahisar province of Turkey. The results indicated that these milk specimens were found to be contaminated by 21 different pesticides.

Sixteen OCP residues were detected in sheep's milk and it was followed with 14 pesticides in buffalo's milk and 11 pesticides in cow's milk. Dominant pesticides in all samples examined were beta-HCH in buffalo's, cow's, and sheep's milk in the concentrations of 63.36, 91.32, and 122.98 ng/ml, respectively. Total OCP levels were found to be 243.81 ng/ml in sheep's milk, 151.02 ng/ml in cow's milk, and 133.38 ng/ml in buffalo's milk. Some of the pesticides detected were found to be in the excess amount of the acceptable level regarding the EU regulations.

Julijana *et al*. (2012) established the method to detect the presence of seven important organochlorine pesticides in raw milk produced in farms located in three different regions of the Republic of Macedonia. In Pelagonia region, DDT was most present insecticide and its max value was 0.21% and minimum value of 0.17%. In the region of St. Nikole, most Dominant insecticide was heptachlor in amounts of 0.35%, where in the Skopje region, it was the similar situation, heptachlor was the main organochlorine pesticide present and it was detected at the levels of 0.17% followed by DDT with 0.16%.

Ismet *et al*. (2012) studied and reported the levels and accumulation profiles of OCPs, PCBs and PAHs in 47 breast milk samples obtained from a Mediterranean city, Mersin. High resolution analyses were performed by a gas chromatography coupled with mass spectrometer (GC–MS). Dichloro diphenyldichloroethane (4.4′-DDE) was the dominant pollutant. Betahexachlorocyclohexane (ß-HCH), dichlorodiphenyl trichloroethane (4.4′-DDT), dieldrin, hexachlorobenzene, oxy-chlordane, cis-heptachlor epoxide were the other main OCPs detected.

Mean levels of PCB congeners and WHOPCB-TEQ were 9.94 and 0.001 ng/g lipid, respectively. PCB 153 showed the highest concentration (3.37 ng/g of lipid), followed by PCB 138 and 180. For the dioxin-like PCBs, PCB 118 was the dominant (0.97 ng/g lipid). Naphthalene, phenanthrene, pyrene and fluoranthene were the major PAHs among the 16 PAHs detected.

Xianyu Chen *et al*. (2014) developed an analytical method for quantification of organochlorine, organophosphate, carbamate and pyrethroid insecticide residues in cow

milk, human milk and baby formula. A total of 25 compounds were included in this method. Sample extraction procedures combined liquid-liquid extraction, freezing- lipid filtration, solid phase extraction.

Target compounds were analyzed using gas chromatography with electron impact ionization- tandem mass spectrometry. In this experiment 10 human milk samples collected from anonymous donors, 10 cow milk samples and 10 baby formula samples purchased from the local grocery stores in the United States. Hexachlorobenzene, p,p-dicofol, o,p-DDE, p,p-DDE and chlorpyrifos were found in all the samples analyzed. They found detectable levels of permethrin, cyfluthrin and fenvelarate in some cow milk samples but not in human milk samples or baby formula samples.

Dasheng *et al.* (2015) collected the breast milk of 142 pregnant mothers in 2011–2012 in Shanghai, China and analyzed for 27 compounds of organochlorine pesticides (OCPs). All the samples were collected during lactation and the detection rates were in a range of 65.5 to 100%. In particular, metabolites of 2,2-bis(chlorophenyl)-1,1,1-trichloroethane (DDT) such as 2 chloro-1,1-bis(4-chlorophenyl)ethylene (DDMU), 2,2-bis(4-chlorophenyl)ethanol (DDOH), bis(4-chlorophenyl)ketone (DBP), and 4,4′dichlorodiphenylmethane (DDM) were detected in most milk samples. DDTs, hexachlorobenzene (HCB), and hexachlorocyclohexane (HCH) were dominant OCPs with mean levels of 316, 49.8, and 41.5 ng.g-1 lipid content, respectively, whereas levels of methoxychlor, aldrins, heptachlor, chlordane and endosulfan were fairly low (0.87–5.6 ng/g of lipid). Milk concentrations of OCPs were weakly correlated with maternal age, body weight, and body mass indexes (BMIs).

Ioannis *et al*. (2015) carried out an experiment to detect the occurrence of residues of DDT and its metabolites in 196 cow milk samples of various pasteurized commercial types collected from the Greek market. Residue levels were determined by GCMS analysis. In 97.4% of the samples at least one DDT isomer or one of the DDT metabolites was detected, in levels not exceeding the maximum permitted residue level by the EU.

Meghdad *et al.* (2015) reviewed the 710 National and International articles and texts related to chlorinated pesticides residues and breast milk. The majority of the reviewed articles indicated that presence of two or more organochlorine pesticides in collected samples of breast milk.

Dobrinas *et al*. (2016) reported the analysis of 37 compounds comprising polycyclic aromatic hydrocarbons (PAHs), organochlorine and organophosphate pesticides (OCPS and OPPS) in milk powder (one brand each of commercial infant formulae, follow-on formulae and baby formulae purchased from a local supermarket in Romania). The selected analytes were investigated using gas chromatography–mass spectrometry (GC-MS), gas chromatography with electron capture detector (GC-ECD) and gas chromatography with thermionic sensitive detection (GC-TSD). The estimated limits of detection for most target analytes were in the μg/kg level (range 0.001–0.320 μg/kg).

In most of the samples the organochlorine pesticides values were under the limit of detection. Exceptions were heptachlor epoxide and endosulfan sulphate, the last of which was found in all analyzed samples at low concentrations. It was found detectable

levels of ethoprophos, parathion-methyl, chlorpyrifos, prothiofos, guthion, disulfoton and fenchlorphos in most of the analysed samples.

Maria *et al.* (2016) evaluated the presence of organochlorine pesticides in samples of forage, soil, water, and milk in four units of an organic production system for cow´s milk (samples of forage, milk, soil, and water) in Tecpatan, Chiapas, Mexico. The organochlorine pesticides were extracted from forage, soil and water based on the USEPA (2005) guideline and from milk based on the IDF 1991 guideline.

The pesticides were identied and quantified by gas chromatography with electron capture detector (GC-ECD). In general, the highest average concentration of total pesticides was found in the samples of milk and forage (311 ± 328 and 116.5 ±77 ng/g respectively). Although, the production systems analyzed are organic, organochlorine pesticides were detected in all environmental samples (forage, soil, water, and organic milk). Although no values surpassed the limits of Mexican and International regulation it is advisable that a monitoring program of contaminants in these production systems is continued.

Warangkana *et al*. (2016) developed and validated the analytical method for measuring 11 OP pesticide residues in human plasma and breast milk. Analytes in both plasma and breast milk samples were extracted with acetone and methylene chloride, cleaned-up using aminopropyl solid phase extraction cartridges, and analyzed by gas chromatography with flame photometric detection.

The optimized method exhibited good linearity, with the coefficients of determination of 0.996–0.99 and <7% error about the slope. Extraction recoveries from spiked plasma and breast milk samples at low and medium concentrations (0.8–5.0 and 1.6–10 ng/ml respectively) ranged from 59.4% (ethion) to 94.0% (chlorpyrifos). Intra-batch and inter-batch precisions ranged from 2.3–18.9% and 5.8–19.5%, respectively. Method detection limits of plasma and breast milk ranged from 0.18–1.36 and 0.09–2.66 ng/ml respectively.

Sharma *et al*. (2016) monitored and survey was conducted for the important pesticides (organochlorine, organophosphate and synthetic pyrethroids) in bovine milk samples, water, fodder and animal feed collected from the eight different blocks of Jaipur, Rajasthan. Sixty four samples each of wheat (*Triticum*) straw and bajra (*Pennisetum glaucum*) stover, 32 samples each of lucerne (*Medicago sativa*), water, indigenous cow milk, 64 samples each of exotic cow and buffalo milk were analyzed. Gas chromatography results revealed that pesticides in milk, water and fodder samples were absent. The attributed reason being the low consumption and prevailing environmental conditions, viz. soil type, surface water depth, intensity and length of sunlight exposure, temperature etc. which leads to the faster degradation of these pesticides.

Mojtaba *et al*. (2016) developed the preconcentration method for the extraction and determination of traces of multi-residue pesticides using solid-phase extraction (SPE) coupled with dispersive liquid–liquid microextraction and gas chromatography–mass spectrometry (GC–MS). The proposed method resulted in good linearities (R2 > 0.9915)

over the ranges of 1–10,000 ng/kg, limits of detection (LODs) in the range of 0.5–1.0 ng/kg at S/N = 3, and precision of RSD% of < 11.8. Under optimal conditions, the preconcentration factors were obtained in the range of 2362–10,593 for 100 ml sample solutions.

Comparison of the proposed method with other ones demonstrated that SPE–DLLME method provides higher extraction efficiency and larger preconcentration factor for determination of pesticides residues. Further, it is simple, inexpensive, highly sensitive, and can be successfully applied to separation, preconcentration and determination of the pesticides (and other noxious materials) in different real food samples.

Chris *et al*. (2017) developed and validated a dispersive solid phase extraction–liquid chromatography tandem mass spectrometry method with electrospray ionization to determine the multiclass pesticide residues and their metabolites in food of animal origin. A simple and low-cost sample preparation procedure using freezing as the clean-up step was used to identify and quantify analytes belonging to 39 different chemical classes in meat and milk matrices. Mean recoveries in the range of 70–120% with relative standard deviations <10% were obtained for the majority of the analytes.

The limit of quantification of the method was 10 µg/kg. The matrix effects were statistically evaluated and the quantification of the analytes was conducted using calibration curves constructed with matrix matched calibration standards covering concentrations from 5 to 200 µg/kg. The proposed method was applied in 86 samples of

animal origin taken from the Greek market, two of which were found positive for pesticides.

Muhammad *et al*. (2017) screened the 200 milk samples from 20 randomly selected dairy farms for the incidence of organochlorine pesticide residues to evaluate the safety of milk in Faisalabad region. The results revealed that overall buffalo milk samples in winter (85%) and in summer (78%) were more contaminated as compared to cow milk samples 83 and 75 % in respective seasons. The residues of cyhalothrin were found only in summer season in milk of both species.

Permethrin residues were detected at higher levels than perfinofos while DDT and methamedophos were found undetectable. The mean levels of permethrin were 0.042 and 0.033 mg/kg in buffalo milk samples and 0.045 and 0.043 mg/kg in cow milk in winter and summer season, respectively. Perfinofos residues were found to be the least contaminated pesticides with mean values of 0.0006 and 0.0013 mg kg-1, respectively in winter season, and 0.004 and 0.0025 mg/kg in summer season.

All analyzed pesticide residues in milk samples in both seasons were below the maximum residual limit (MRL) values as described by European Union (EU) but milk samples contaminated with α, β-endosulfan and endosulfan sulphate exceeded their respective Food and Agriculture Organization's (FAO) established MRLs both in winter and summer.

2.4 Method validation and Detection of Organochlorine pesticide residues in meat

Elizabeth and Hamilton (1968) conducted a surveillance study to estimate the organochlorine pesticide residues in samples of eggs, poultry meat, liver or fat and poultry feed. Among the 114 meat, liver or fat samples analyzed, the content of -BHC in 7 samples exceeded 0.1 ppm that of dieldrin in 2 equalled or exceeded 0.1 ppm, and that of pp'-DDE in 7 exceeded 0.1 ppm, but all the samples contained less than 0.1 ppm pp'-DDT.

Among the 163 egg samples analyzed, only one sample exceeded 0.1 ppm organochlorine pesticide residue. Samples of poultry feed were also analyzed to evaluate the importance of the food as a source of pesticides residues.

Harvey *et al*. (2000) analyzed the pesticide residues in the market basket food samples from six Canadian cities collected from 1992 to 1996. 136 composites were prepared for each city, representing 99% of the Canadian diet. Residues were found most frequently in peanut butter and butter. DDE, malathion and captan occurred most frequently, while the fungicides chlorothalonil, dicloran and captan were present in the highest concentrations. Processed commodities contained fewer residues and at lower concentrations than the raw products. No residues were detected in either milk or soy-based infant formula.

Vibha *et al*. (2002) documented the biotic and abiotic environmental contamination by pesticides. Pesticides like DDT and its metabolites DDD and DDE, dieldrin, heptachlor, and HCH and its isomers were higher in blood of breast cancer patients when compared with normal women who did not suffer from major diseases like

blood pressure, tuberculosis, diabetes, thyroid dysfunction, arthritis, cancer, etc., and had not undergone any major surgery. The results indicated that organochlorine pesticides taken for analysis were found significantly high in breast cancer patients irrespective of age, diet, and geographic distribution.

Jae *et al*. (2005) developed a multiresidue method for the simultaneous determination of 22 organochlorine and organophosphorus pesticides. The analysis was performed using gas chromatography coupled with electron capture detector. The identification of compounds based on their retention time and on comparison of primary and secondary ions. The optimized method was validated by determining accuracy, precision and sensitivity from the samples fortified at three levels. The proposed method may be used for routine determination of pesticides in fatty matrices.

Rabinder *et al*. (2006) monitored the organochlorine pesticide residues in poultry feed, chicken muscle and eggs at a selected poultry farm. The samples were Soxhlet extracted for 8 h in 200 ml hexane–acetone (1:1, v/v) mixture. The clean-up of the samples was performed by silica gel column chromatography and analysis was done on a gas chromatograph equipped with an electron capture detector. The mean total hexachlorocyclohexane (HCH) and dichlorodiphenyltrichloroethane (DDT), endosulfan sulfate and heptachlor epoxide residues were 0.65, 0.91, 0.42 and 0.02 mg/kg respectively in feed, while respective values for chicken muscle were 0.11, 0.24, 0.10 and 0.07 mg/kg.

Higher residues were encountered in eggs as compared to muscle. None of the muscle samples exceeded maximum residue limits (MRL) for organochlorine pesticides,

while all egg samples had values above the MRL for HCH and heptachlor epoxide and seven egg samples exceeded MRL for DDT residues. The results indicated that poultry feed could be one of the major sources of contamination for chicken and eggs.

Garrido *et al.* (2006) optimized and validated a method for the simultaneous determination of residues of organochlorine (OCPs) and organophosphorus (OPPs) pesticides in meat samples from chicken, pork and lamb. The method was based in the extraction of homogenized meat mixed with sodium sulphate and ethyl acetate in polytron, although both Soxhlet and accelerated solvent extraction (ASE) were also tested. Then, a clean-up step by gel permeation chromatography (GPC) was applied, before the final determination by gas chromatography (GC) coupled to a triple quadrupole (QqQ) mass spectrometry (MS) detection system.

Confirmation criteria of the pesticides were based in the 2002/657/EC European Commission Decision that established at least three identification points (IPs) for organic residues in live animals and animal products. The analytical process was validated in each matrix by the analysis of spiked blank samples. Performance characteristics, such as linearity, detection limit (LOD), quantitation limit (LOQ), precision and recovery were studied. Recoveries and precision values were 70.0–90.0% and 15%, respectively, for the bulk majority of pesticides. LOD < 2.0 and LOQ < 5.0 μg/kg were obtained for all the target compounds, except for acephate.

The proposed analytical methodology was applied to the analysis of the pesticides in 30 samples, 10 from chicken, 10 from pork and 10 from lamb. Only three OCPs, endosulfan α, endosulfan sulfate and dichloran were detected in three different lamb

samples, while endosulfan α was detected in only one pork sample, at concentration levels lower than the LOQ values. However, pesticide residues were not found in any of the other 10 chicken samples analyzed.

Danilo *et al.* (2007) developed an analytical method for the determination of four selected pyrethroid insecticides residue levels in beef meat. Acetone and petroleum ether at 40–60°C were chosen as extraction solvents. A two step clean-up was performed using an Extrelut NT3-C system followed by a Florisil column, with disposable, ready to use cartridges.

Instrumental analysis was carried out on a gas chromatograph equipped with an electron capture detector (GC–ECD), using matrix matched and internal standard calibration techniques. Confirmatory analysis by GC–MS was performed. Recoveries at the EU Maximum Residue Limit (MRL), 0.5 × MRL and 1.5 × MRL levels and the repeatability's were widely satisfactory. The main advantage of the method was the reduction of analysis time as compared with previously published works. The applicability of the method to different matrices and pesticide classes will be investigated.

Khalid and Alaa (2008) determined the organochlorine pesticide residues in a total of 270 meat samples; Comprising the muscle, liver, kidney collected from 90 carcasses (30 each of camel, cattle and sheep) slaughtered in Sharkia Province, Egypt. All samples were analyzed for their residual contents of DDT compounds (DDTs), hexachlorocyclohexane isomers (HCHs), lindane (γ-HCH), aldrin, dieldrin, endrin, hexachlorobenzene (HCB), toxaphene, and chlordane compounds.

The results indicated that 54.4% (49/90), 51.1% (46/90), 47.8% (43/90), 44.4% (40/90), 33.3% (30/90) and 15.6% (14/90) of the examined carcasses were contaminated with DDTs, HCHs, lindane, aldrin, dieldrin and endrin, respectively. The other contaminants (HCB, toxaphene, and chlordane) were only present in less than 10% of the analyzed carcasses.

Amongst the three meat animal species examined, the incidence of contamination as well as the residual concentrations of all the pesticides detected in camel carcasses were lower than those detected for cattle and sheep. The contamination levels of the studied organochlorines followed the order: DDTs > HCHs > lindane > dieldrin > aldrin > endrin > toxaphene > HCB > chlordane; while the order for the contamination in the analyzed organs was liver > kidney > muscle. Heat treatment of some selected samples (boiling for 1.5 h) produced overall reductions of 40.4%, 55.0%, 32.4%, 33.5%, 29.2%, 42.7% and 38.2% in DDTs, lindane, dieldrin, aldrin, endrin, toxaphene and HCB contents, respectively. The residual contents of the organochlorines detected in all of the contaminated samples analyzed from the three different species were well below the respective maximal permissible limits set by local or international organizations.

Jonathan *et al.* (2008) collected human breast milk samples (n = 30) from mothers within the age range of 19– 40 years from Thohoyandou area, South Africa. DDT and its metabolites were extracted from the milk samples using diethyl ether. The crude extracts were subjected to column chromatography. The eluates were then evaporated on a stream of nitrogen up to 0.5 ml. One µl cleaned extracts were injected into GC-ECD for selected organochlorine compounds. A significant cluster of DDT and its metabolites between the

infants' weight range of 2.5–3.9 kg was observed. Increase in lipid content was followed by a decrease in the sum DDT in the older mothers (27–30). The estimated daily intake varied from 260 to 4,696 ng/g, 10,551 ng/g and 4,237 ng/g for DDE, DDD and DDT respectively.

Gregor *et al*. (2009) developed a method for measurements of organochlorine pesticides (OCPs) in food products. Introduced modifications in method development improved the efficiency of the procedure for samples with high fat content. Recovery values for all analyzed pesticides were over 60% and the reproducibility expressed as relative standard deviation was in the range of 10%. The method is suitable for the determination of OCPs in meat products with high content of fat from low ppb concentration range onward. The limits of detection for examined OCPs were in the range from 0.1 ppb to 2 ppb for lindane and α-endosulfan, respectively.

Patrizia *et al*. (2009) developed and optimized a multiresidue method for the identification/ quantification of organochlorine pesticides (OCPs) and pyrethroids (PYRs) in beef meat samples. The analysis was carried out using gas chromatography coupled with quadrupole mass spectrometry. The performance of the method was investigated in terms of linearity, accuracy, precision, limit of detection and quantification limit. Good linearity was obtained with correlation coefficients higher than 0.98.

Mean recoveries were found in the ranges of 70-110% and 84-99% for the investigated OCPs and PYRs respectively. RSD% turned out to range from 2-15%. LOQ values were in the range of 0.005-0.1mg/kg for either class of compounds. The method developed was successfully tested on 50 commercial beef meat samples from the market

area of Rome proving to be a useful tool in routine multiresidue analysis of OCPs and PYRs for monitoring purposes. None of the compounds of interest were observed above their respective LOQ.

Shinde and Karim (2009) screened the meat and milk samples of sheep maintained in institute and field flocks for the presence of organochlorine pesticide residues viz., DDT, aldrin, endrin, dieldrin, endosulfon, heptachlor and BHC. The levels of organochlorine pesticide residues in meat and milk of sheep were compared with maximum tolerance levels reported. The residues were not detected even at 5 ppb level concentration. The study clearly indicated that meat and milk produced by sheep in the region meets the norms fixed for food safety and are also safe for human consumption. However, regular monitoring and screening of pesticide residues in meat and milk in different regions should be made mandatory for protecting human beings from their hazardous effects.

Muhammad *et al*. (2010) planned the study to determine the contamination of the meat and organs of cattle reared in pesticide spraying areas of Faisalabad, Pakistan. The meat and organs such as liver, lung and kidney were collected from villages situated within the radius of 25-35 km on four different localities (Pensara, Aminpur, Jaranwala, and Sheikhupura roads) in the Northeast and Southwest of city during winter and spring seasons of 2009.

Five pesticides (cyhalothrin, endosulfan, chlorpyrifos, cypermethrin and methyl parathion) were analyzed in the collected meat and organs (n=600) with solid phase microextraction and high performance liquid chromatography techniques. The residue

analysis revealed that about 13, 21, 4, and 2 % muscle samples were contaminated with chlorpyrifos, cyhalothrin, cypermethrin and endosulfan, respectively. The concentration (ppm) of chlorpyrifos (0.373 ± 0.001 vs. 0.297 ± 0.006), cypermethrin (2.962 ± 0.003 vs. 1.789 ± 0.228), endosulfan (12.938 ±0.007 vs. 14.487 ± 4.497) and cyhalothrin residues (4.521 ± 1.143 vs. 4.790 ± 0.933) were non-significantly different ($p>0.05$) in north east and southwest direction, respectively. Similarly, the levels of these pesticides were non significantly different in spring and winter seasons. Parathion-methyl was not detected in muscle samples. The same trend of pesticide contamination was observed in the kidney samples. Three pesticides (chlorpyrifos, cyhalothrin, cypermethrin) were detected in liver and lung samples while endosulfan and methyl parathion were only detected in traces.

Pesticides residues in muscle and organs were found higher than the Maximum Residual Limit (MRL) established by the international health regulatory agencies. Comparative results indicated that chlorpyrifos, lambda-cyhalothrin, cypermethrin and endosulfan residues in muscles were about 34 times, 23 times, 47 times, and 27 times, respectively, higher than the MRL. These findings alarm a threat to the public health and suggested the need to create awareness in dairy farmers regarding the avoidance of pesticide residues in meat.

Ahmed *et al*. (2010) conducted an experiment for detection and determination of some of organochlorine and organophosphorus pesticides in muscles of chicken and bovine in the Egyptian market. The samples were prepared, then extracted, partitioning and clean up processes has been conducted and the aliquots were analyzed by Gas Chromatography using Electron capture detector and Flame Ionisation Detector. The

studies showed that, lot of samples were contaminated with organochlorine pesticides while fewer samples were contaminated with organophosphorus pesticides.

Biswas *et al*. (2010) reviewed the food safety in relation to pesticide and veterinary drug residues and mycotoxins in meat and meat products. Residues in meat and their products are generally classified as naturally present, caused by man and arise secondarily. In the past, most contamination of meat resulted from natural toxicants. However, usage of synthetic chemicals for regular house-hold and agricultural practices while benefiting society has also provided new sources of potential contamination. The levels of pesticide residues were alarming situation in certain countries.

Drug residues in meat are relatively uncommon whereas, aflatoxin or ochratoxin are rarely found. Residues from secondary residues also occur less frequently. This study revealed the causes of residues in meat, types of residues found, their detection methods, incidences and their regulation with emphasis on public health risk and their assessment.

Rafat *et al*. (2010) determined the organochlorine pesticide residues in 519 samples comprising of eggs, chicken and meat (lamb and beef) collected from Jordon. All the samples were analyzed for their residual contents of aldrin, DDT and its metabolites, dieldrin, endosulfan isomers, endrin, HCHs, heptachlor, heptachlor epoxide and HCB using gas chromatography equipped with electron capture detector (GC-ECD). The results indicated that 28%, 20%, and 49% of the examined eggs, chicken and meat samples respectively were contaminated with OCP residues. DDT and HCHs are the most prominently noticed compounds, as they were detected at a high incidence. Whereas on the other hand, heptachlor, heptachlor epoxide, HCB, aldrin and endrin compounds

were only present in less than 7% of the analyzed samples. No residues of op'-DDD, op'-DDT, Dieldrin , endosulfan were detected.

Feride and Emre (2011) conducted the study to develop an easy analytical method for determining α-hexachlorocyclohexane (α-HCH), hexachlorobenzene (HCB), heptachlor (HC), aldrin (ALD), 4,4-dichlorodiphenyldichloroethylene (4,4-DDE) and 4,4dichlorodiphenyltrichloroethane (4,4-DDT) residues by gas chromatography with mass spectrometer (GC–MS) for routine analysis. Chicken muscles were utilized as samples in this study. A florisil packed column was used in sample preparation step and showed good performance. The average precision and accuracy ranged 8.03-24.00% and 4.36-30.67%, respectively. The average recoveries were between 95.74 and 130.67% for various spiking levels (25, 50, 100 ng/g).

The average of inter and intra-day precision was <13 and the limits of detection were ranged 7-19 ng/g, depending on different organochlorinated (OC) pesticides. In conclusion, the extraction method used in the present study was inexpensive, easy and rapid. Additionally, the validation parameters show that the proposed method in this study was sensitive, reproducible and reliable alternative to the normally used methods. Thus, it could efficiently be used in the routine and monitoring studies so that several samples can be run in parallel.

Paramasivam (2011) developed a rapid, simple and efficient multiresidue method and optimized for the identification and quantification of organochlorine pesticides (OCP) and synthetic pyrethroids (SPs) in sheep meat samples. The method consisted of a modified quick, easy, cheap, effective, rugged, and safe sample preparation method.

Samples were extracted with acetonitrile, and the extracts were cleaned up by dispersive solid phase extraction with primary secondary amine (PSA) sorbent and anhydrous magnesium sulphate.

Determination and quantification of OCP residues was carried out using a GC-ECD. Mean recoveries were found in the ranges 70-110% and 84-99% for the investigated OCPs and SPs, respectively, with the RSD was less than 20%. This method was found more efficient and reliable enabling more number of samples to be analyzed in less time. Moreover lipid removal was achieved to a large extent to get desired result.

Bhuvaneshwari and Balu (2012) analyzed the organochlorine pesticides using Gas Chromatography-Mass Spectrometer (Selective Ion Monitoring mode) in the muscle tissues of five fish species such as *O. mossambicus, L. parsia, E. suretensis, C. striata* and *S. wynaadensis* from seven locations of River Cauvery and one location in Veeranam Lake. OCPs viz., DDTs, HCHs, CHLs, cyclodienes, heptachlor, HCB and mirex were detected with varying concentrations among species and locations. Mirex which was not reported in the fish tissues elsewhere reported. The study on the risk associated with the consumption of fish species that had higher concentrations of aldrin, dieldrin and mirex showed significant carcinogenic risk to the human beings.

Violeta *et al*. (2012) conducted a surveillance study to monitor the tissue distribution patterns of organochlorine pesticides in bovine carcasses varied among seasons, geographic locations and tissues. The highest concentrations of DDT during the dry season were detected in lungs from Paso de Ovejas (2,834.90 μg/g lipid) and during the rainy season, Lindane and HCH in muscle and lung samples from Paso de Ovejas

(995.80 and 1,690.10 μg/kg lipid). Estimated daily intakes of γ−HCH and Σ−DDT (3.35 and 1.22 μg/kg) through consumption of muscle tissues from Paso de Ovejas and Puente Nacional during the rainy season showed the highest contribution.

During the rainy season the highest non cancer Hazard Ratios estimated corresponded to γ−HCH (3.97) and DDT (4.39) detected in muscle samples from Puente Nacional. The highest hazard ratios of cancer risk to the 95th centile daily consumption through meat corresponded to p,p'-DDT from Alvarado (7.76E + 06) and from Paso de Ovejas for γ−HCH (1.50E + 05) during rainy season. The results indicated potential non carcinogenic risks to consumer health through meat consumption.

Bayessa and Louis (2013) determined the residue levels of organochlorine pesticides (OCP) in a total of 90 cattle samples comprising meat, liver and kidney collected from carcasses slaughtered in six towns in West Shoa Zone, Ethiopia, (Ambo, Guder, Ginchi, Gedo, Holeta and Tikur Inchini). The pesticides were extracted by solid phase extraction (SPE) and quantification was carried out using GC-MS. A good linearity ($r > 0.998$) was found in the range 0.001–7.00 mg/kg for the samples studied. Most of the pesticides had recoveries in the range 81–99% and values of relative standard deviation (RSD) <7.2% for repeatability and reproducibility, showing good accuracy and precision of the method. The concentration of the studied organochlorines followed the order: p, p' dichloro-diphenyl-trichloroethane (DDT) > endosulfan > o,p′-DDT > lindane > dieldrin > endrin > aldrin > chlorothanolin while the order of contamination in the analyzed organs was liver > kidney > meat.

Heat treatment of the meat, kidney and liver samples (boiling for 90 min.) produced an overall reduction of 62.2%, 44.5%, 37.7%, 29%, 31%, 34.3% and 30.8% in lindane, o, p′-DDT, endosulfan, p, p′-DDT, chlorothanolin, aldrin, dieldrin, and endrin, respectively. Although the residual contents of the organochlorines detected in all the contaminated samples analyzed from the six cities were below the respective maximal permissible levels set by international organizations, samples from Holeta town were more contaminated and may necessitate monitoring as bioaccumulation of these residues may pose health problems in human beings.

Enbaia *et al*. (2014) conducted the study to assess the level of contamination of marine fish with persistent chemicals such as many organochlorine pesticide residues, where most countries suffer from the problems of pollution of marine fish with persistent chemicals which have negative effects on human and animal health as cause of cancer, kidney failure, liver and fetal abnormalities as a result of accumulation in adipose tissue.

The aim of this study was to estimate organochlorine pesticide residues in Libyan fish where they were pulling samples of fish during the months (September, October, and November, 2013) from the local market to market the fish Port of Tripoli Sea, which is the main source of samples fresh. Samples were representative of the types of fatty fish include Round Sardinella (*Sardinella aurita*), European Pilchard (Thunnus thynnus), Yellow Fin Tuna (*Thunnus albacares*) and Bogue (*Boops boops*).

The results showed that there was no concentrations higher than the permissible limit, according to FAO, global in all tissues of the fish, which were estimated by organochlorine pesticides as for estimating vehicle organochlorine fats in fish has been

found that some types of sardines contain high concentrations of endosulfan (5.5058) and heptachlor epoxide (2.4366) and methoxychlor (6.1312) and some types of mackerel contain a high concentration of heptachlor epoxide (8.4513) and some types of tuna contain a high concentration of dieldrin (9.1996) and these concentrations are higher than the permissible limit, according to FAO where concentrations were calculated in mg / kg of fish.

Alexsandro *et al*. (2016) developed an efficient, sensitive, and reliable analytical method for trace analysis of 17 different pyrethroids and chlorpyrifos in the fatty content of animal products, including beef, chicken, eggs, fish, and milk. The method developed was based on an ultrasound extraction using lyophilized samples, a solid phase extraction cleanup with basic alumina and C18 cartridges in tandem and analysis by gas chromatography coupled to tandem mass spectrometry in negative chemical ionization mode.

Recovery values were in the range of 27–128 % with relative standard deviation always below 25 % and chiral analysis of recovery data showed predominance of isomers of cis form over trans. Limits of detection (LODs) ranged from 0.002 to 6.43 ng/g lipid weight and limits of quantification (LOQs) ranged between 0.006 and 21.4 ng/g. The developed methodology was used for the analysis of 25 samples of fatty foods. All samples were positive for at least one of the pesticides, chlorpyrifos, bifenthrin, cyhalothrin, permethrin, cypermethrin, or deltamethrin, with mass fraction levels ranging from 0.03 to 270 ng/g.

2.5 Method validation and Detection of Organochlorine pesticide residues in eggs

John *et al.* (1989) conducted an experiment in 367 domestic fowl (*Gallus domesticus*) eggs collected from 61 farms, residues of 10 pesticides were detected in various combinations and in the following order of frequency: p,p-DDE (in 100% of the eggs), p,p-DDT (98%), dieldrin (95%), lindane (66%), p,p-DDD (46%), o,p-DDT (17%), -HCH (9%), -HCH (5%), endrin (4%) and aldrin (0-5%). No residues of heptachlor, heptachlor epoxide and HCB or PCBs were found.

The mean concentration (0.70 mg/kg eggs; range <0.01-10.25) of total DDT exceeded the residue limit (ERL) of 0-50 mg/kg. The mean dieldrin residue level (0.35 mg kg; range 0.01-14.90) was 3-5 times higher than the estimated residue levels (0-10 mg/kg). Only 3% of the eggs exceeded the estimated residue levels for lindane. The 156 eggs from free-range hens had significantly ($P<0.05$) higher residue concentrations of total DDT, dieldrin and lindane than eggs collected from hens kept in enclosures. The mean ratio [p,p-DDT]/[p,p-DDE] in eggs from enclosed hens (0-97) was significantly higher ($P<0.01$) than in eggs from free-range hens (0-53), indicating that the former had a more direct exposure to p,p-DDT, whereas the latter obtained more of it after environmental conversion to p,p-DDE.

Eggs from a rice growing area had the highest concentrations of all pesticide residues detected. Accumulation ratios indicated that the levels of DDT and lindane in the feed of enclosed hens could account for the levels in the corresponding eggs. The much higher accumulation ratios calculated for the free range hens demonstrated that the feed ingested by these chickens obviously contained ingredients additional to those sampled

and revealed probable extensive environmental contamination by these persistent pesticides. The present results indicated that there is a need to identify sources of dieldrin in the eggs of domestic fowls and, where necessary to investigate local wildlife samples. The amounts of total DDT and dieldrin in eggs in this study seem to be higher than reported from any other country.

Franois Bordet *et al.* (2002) conducted an inter laboratory study to validate a gas chromatographic (GC) method for determination of 21 organochlorine pesticides, 6 pyrethroid pesticides, and 7 polychlorobiphenyl (PCB) congeners in milk, beef fat, fish, and eggs. The method was performed at low contamination levels, which represent relevant contents in food and is an extension of the European standard. It enlarged the applicable scope of the reference method to pyrethroid pesticides and proposes the use of solid-phase extraction (SPE) as a cleanup procedure.

After injection of the purified extract onto a GC column, residues were measured by electron capture detection. Food samples (liquid milk, beef fat, mixed fish, and mixed eggs) were prepared, tested for homogeneity and sent to 17 laboratories in France. Test portions were spiked with 27 pesticides and 7 PCBs at levels from 26 to 45, 4 to 27, 31 to 67, and 19 to 127 ng/g into milk, eggs, fish, and fat, respectively. Based on results for spiked samples the relative standard deviation for repeatability ranged from 1.5 to 6.8% in milk, 3 to 39% in eggs, 4.5 to 12.2% in fish and 7 to 13% in fat. The relative standard deviation for reproducibility ranged from 33 to 50% in milk, 29 to 59% in eggs, 31 to 57% in fish, and 30 to 62% in fat. This method showed acceptable intra- and inter laboratory precision data.

Valsamaki *et al.* (2006) developed and validated a multiresidue method for the determination of 20 organochlorine pesticides (aldrin, endrin, dieldrin, α-BHC, β-BHC, γ-BHC, δ-BHC, α-chlordane, γ-chlordane, 4,4′-DDE, 4,4′-DDT, 4,4′-DDD, endosulfan I, endosulfan II, endosulfan sulfate, endrin aldehyde, heptachlor, heptachlor epoxide, endrin ketone and methoxychlor) and eight PCB congeners (PCB 20, 28, 52, 101, 118, 138, 153, 180) in chicken eggs. The samples were extracted by a simple and fast matrix solid phase dispersion (MSPD) method using Florisil as the sorbent material and dichloromethane/ hexane (1:1) as the eluting system. Further purification of the extracts was conducted using a conventional clean-up procedure with concentrated sulphuric acid. Determination and quantitation of PCBs and OCs residues was carried out using a gas chromatograph equipped with an electron capture detector (GC-ECD). A mass spectrometric detector (GC–MS) in the selected ion monitoring (SIM) mode was used for confirmation purposes.

The method detection limits were <0.7 ng/g for all PCBs and OCs and the relative standard deviations for analyses of samples fortified over the range of 10–200 ng/g were <8%. All compounds provided average recoveries (spiked at five concentration levels) ranging from 82 to 110%. The proposed method was used to analyze 30 commercial products taken from local markets in the course of a 3 month sampling campaign.

Aulakh *et al*. (2007) conducted an investigation to monitor organochlorine pesticide residues in poultry feed and eggs at a selected layer farm for 1 year. The samples were Soxhlet extracted for 8 h in 200 ml hexane-acetone (1 : 1, vlv) mixture. The clean up of the samples was performed by silica gel column chromatography. The

residues in cleaned up extracts were estimated using a gas chromatograph equipped with an electron capture detector were 0.83 mg/kg while those of DDT (dichlorodiphenyl trichoroethane) were 0.76 mg/kg, while the total HCH and DDT levels in eggs were 0.28 and 0.42 mg/kg respectively.

The mean levels of endosulphan sulphate in poultry feed were 0.22 mg/kg while the corresponding levels in eggs were 0.08 mg/kg. The study concluded that high levels of organochlorine pesticide residues in poultry feed including total HCH and DDT and their presence in eggs at the farm indicated that poultry feed could be one of the major sources of contamination for eggs. These residues were present despite a complete ban on the use of technical HCH and DDT for agricultural purposes in India.

Muralidharan *et al*. (2008) conducted surveillance study to determine the organochlorine pesticide residues in tissues of five Indian whitebacked vultures and two of their eggs collected from different locations in India. All the samples had varying levels of residues. p,p′-DDE ranged between 0.002 µg/g in muscle of vulture from Mudumali and 7.30 µg/g in liver of vulture from Delhi. Relatively higher levels of p,p'-DDT and its metabolites were documented in the bird from Delhi than other places.

Dieldrin was 0.003 and 0.015 µg/g while p,p′-DDE was 2.46 and 3.26 µg/g in egg one and two respectively. Dieldrin appeared to be lower than the threshold level of 0.5 µg/g. p,p′-DDE exceeded the levels reported to have created toxic effects in eggs of other wild birds. Although varying levels of DDT, HCH, dieldrin, heptachlor epoxide and endosulfan residues were detected in the vulture tissues, they do not appear to be responsible for the present status of population in India.

Tao *et al*. (2009) conducted a surveillance study to determine the concentrations of hexachlorocyclohexane isomers (HCHs) and dichlorodiphenyltrichloroethane and metabolites (DDTs) in Chicken organs, animal feed, droppings, and ambient air at a farm in Beijing. Mean fresh weight concentrations of HCHs and DDTs were 0.122 ± 0.061 ng/g and 0.051 ± 0.038 ng/g in the muscles respectively. These values were 1–2 orders of magnitude lower than those reported in China in 1980. Contaminated feed was the main source of HCHs and DDTs. Only 12.8% of HCH and 3.3% of DDT of the amount consumed were excreted. Accumulated quantities of HCHs and DDTs increased during growth.

Dhananjayan, *et al*. (2011) conducted the study to provide information on the current status of contamination by organochlorine pesticides (OCPs) in eggs and tissues of House Sparrow (*Passer domesticus*), in Tamil Nadu, India. The mean concentration of total hexachlorocyclohexane (HCH) and total dichloro-diphenyl-trichloroethane (DDT) in eggs ranged from 0.01 to 1.81 μg/g and 0.02 to 1.29 μg/g respectively. Concentration of 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (p,p′-DDE) ranged from below detectable limit (BDL) to 0.64 μg/g, representing more than 60% of the DDTs. About 28% of samples had p,p′-DDE levels above the critical concentration associated with reproductive impairment. However, the mean concentrations of cyclodiene insecticides were less than 0.5 μg/g. Although OCPs levels detected in tissues are not indicative of toxicity, continuous monitoring is recommended.

Stephen *et al.* (2015) developed and validated a new multiresidue method for the determination of 33 organochlorine pesticides (OCPs) in various fatty and high water

content food matrices. The OCP residues in foods were extracted with matrix solid-phase dispersion and cleaned up with gel permeation chromatography and Florisil solid phase extraction. The instrumental determination was carried out by a gas chromatograph coupled to a single quadrupole mass spectrometer (MS) with runtime of 11 min.

Besides, negative chemical ionization mode was also studied and evaluated for OCPs' sensitivities. The optimized MS was operated in electron ionization mode and acquiring three selected ions per target compound. The method was validated with various food samples, including edible oil, meat, seafood, eggs, coffee, tree nuts, fruits and vegetables etc. An adequate linear relationship was obtained in the studied concentration range (0.5–10.0 μg/ kg) in sample; the average spiked recovery values were in the range 70–120 % for the two levels of concentration studied in samples: 0.5 and 2.0 μg/kg .

Precision values, expressed as relative standard deviation, were lower than 18% at afore mentioned spiking levels; detection limits and quantification limits were below or equal to 0.1 and 0.5 μg/kg , respectively. Moreover, certified reference materials were used to assess the accuracy of the developed method. Finally, the developed method was successfully applied for the OCPs' determination in real samples.

Materials and Methods

III. MATERIALS AND METHODS

Multi-residue analysis of certain organochlorine pesticide residues in broiler meat, milk and chicken eggs using gas chromatography – electron capture detector was carried out.

3.1 Method development

The method described by International Conference on Harmonization (ICH) and AOAC guidelines was applied for method validation for determination of organochlorine pesticide residues in broiler meat, milk and eggs collected from the commercial outlets of Bengaluru, Shivamogga and Mysuru. The organochlorine pesticides and some of their isomers and degradation products *viz*: alpha-BHC, beta-BHC, lindane delta-BHC, alachlor, aldrin, *op*-DDE, endosulphan-I, *pp*-DDE, dieldrin, *op*-DDD, endosulphan-II, *pp*-DDD, *op*-DDT, endosulphan sulphate and *pp*-DDT were considered for validation.

3.2 Gas chromatography analysis

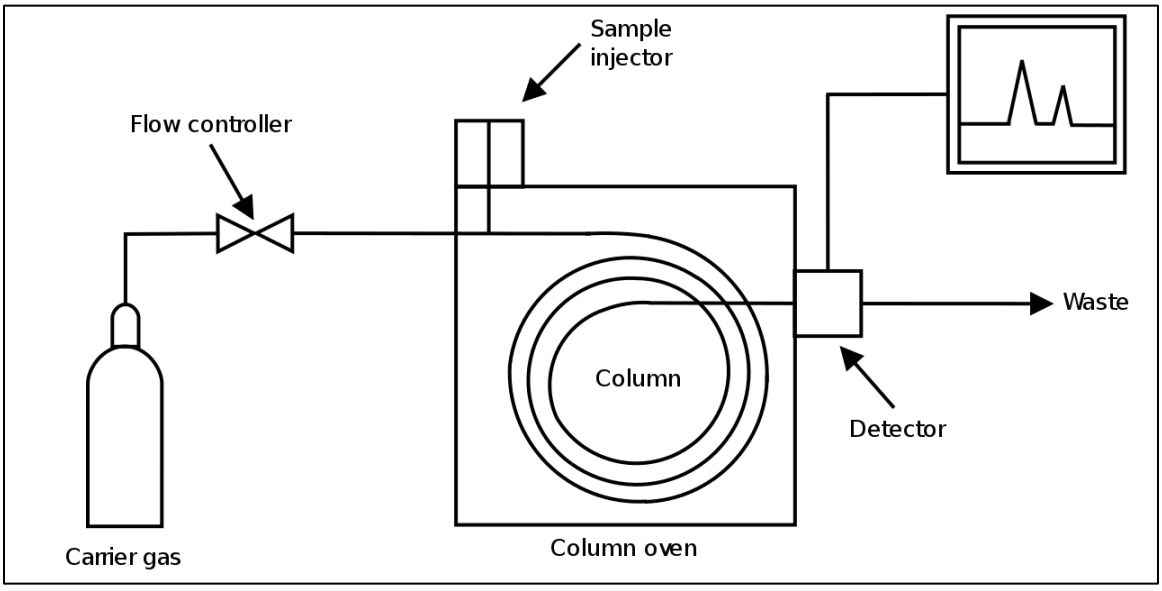
3.2.1 Principle

Chromatography is an analytical technique which has been used for isolation, purification and separation of organic and inorganic compounds including qualitative and quantitative estimation of compounds.

The principle behind the working of gas chromatography is separation technique in which the components of a sample partition between two phases: the stationary phase and the mobile gas phase. According to the state of the stationary phase, gas chromatography can be classified in to gas-solid chromatography (GSC), where the

stationary phase is a solid, and gas-liquid chromatography (GLC) that uses a liquid as stationary phase. GLC is to a great extent more widely used than GSC. During a GC separation, the sample is vaporized and carried by the mobile gas phase (i.e., the carrier gas) through the column.

Separation of the different components is achieved based on their relative vapor pressure and affinities for the stationary phase. The affinity of a substance towards the stationary phase can be described in chemical terms as an equilibrium constant called the distribution constant. The distribution constant (Kc) controls the movement of the different compounds through the column, therefore differences in the distribution constant allow for the chromatographic separation shows a schematic representation of the chromatographic process. Kc is temperature dependent and also depends on the chemical nature of the stationary phase. Thus, temperature can be used as a way to improve the separation of different compounds through the column or a different stationary phase.



(Source: https://www.google.co.in/search/gas+chromatography-gas&gs_l)

Generally, in Gas Chromatography (GC) the mobile phases are gases such as helium (He) or Nitrogen (N_2). GC depends upon temperature programming and boiling point of the compounds. The volatile organic compounds (VOCs), poly-aromatic hydrocarbon and pesticides have been analyzed by gas chromatography equipped with various detectors. Generally, five types of detectors are coupled with GC (GC-ECD, GC-NPD, GC-FID, GCPID and GC-MS). The analysis of compounds on GC depends upon nature of compound, physical and chemical properties of compound. Gas chromatography coupled with electron capture detector (GC-ECD) is used for qualitative as well as quantitative estimation of organochlorine compounds especially organochlorine pesticides.

3.2.2 Gas chromatographic conditions

The chromatography was carried out with gas chromatograph equipped with electron capture detector [GC-ECD] Varian CD-3800 (plate.1). The GC-ECD operating conditions were; capillary column, carrier gas- nitrogen, carrier gas pressure at 20psi, injector temperature was 260^0C, detector temperature was 300° C. The oven temperature program was characterised by, initial temperature of 80°C hold for 2 min, the temperature raised from 80°C to 280°C at 10°C/ min hold for 5 min. Column used for separation was Rtx-5, diameter of the column was 30 meter in leangth and size of the column was 0.25 mm x 0.25µm fused silica capillary column, carrier gas used was nitrogen with purity of 99.99%. Flow rate of the nitrogen gas was at the rate of 1 ml/min and 1µl injections of the final extract manually injected into the GC-ECD system. The total run time of the sample was 28 minutes. The operating conditions were optimized to obtain

the best chromatogram of studied pesticides by direct injection of standard pesticides at different concentrations.

Source/gas parameters for analysis of organochlorine pesticide residues in broiler meat, milk and chicken eggs

Carrier gas : Nitrogen

Carrier gas pressure : 20 psi

Injector temperature : 260^{o}C

Detector temperature : 300^{o}C

Initial temperature : 80^{o}C, held for 2 min

Temperature programme : 80^{o}C to 280^{o}C at 10^{o}C/ min held for 5 min

3.3 Pesticide standard solutions

The pesticide standard solutions were prepared by method prescribed in Official Methods of Analysis and Indian Standard 14628:1999. The main stock solutions of certain organochlorine pesticides were prepared by dissolving the 10 mg of each (the organochlorine pesticide standards viz: alachlor, aldrin, dieldrin, *pp*'-DDT, *op*'- DDT, *pp*'-DDE, *op*'-DDE, *pp*'-DDD, *op*'-DDD, lindane, α-HCH, β-HCH, γ-HCH, α-endosulfan, β-endosulfan and endosulfan sulphate) in 10 ml of methylene dichloride in 20 ml volumetric flask and accordingly dissolved and made up the volume to 10 ml with methylene dichloride.

Intermediate solution of 10 µg/ ml was prepared by pipeting out 1ml of each stock solution in to individual 100 ml volumetric flask and diluted with methylene dichloride.

Working solution 0.1 µg/ml was prepared by pipeting 1 mL solution from the intermediate solution to 100 ml volumetric flask and made up the volume with methylene dichloride.

The spiking stock solutions of organochlorine pesticides and working stock solution of methylene dichloride were prepared and all the solutions were stored at 2-8 ^{o}C.

3.4 Method validation

The selective, sensitive and validated analytical methods for the quantitative evaluation of the organochlorine pesticides and their metabolites are very critical. The validation of the bioanalytical method was done using the method described in International Conference on Harmonization (ICH) and AOAC guidelines for laboratory validation of analytical methods. Analysis of pesticides and their residues in biological samples were performed using calibration standards and quality control samples spiked with reference standards. It was anticipated that the purity of the reference standard used to prepare spiked samples can affect study data. For this reason authenticated analytical reference standards of known identity and purity should be used to prepare solutions of known concentrations. The reference standard should be identical to the analyte.

The fundamental parameters to be considered for validating the system for detecting the organochlorine pesticide residues includes,

1. System Suitability
2. Linearity

3. Limit of detection (LOD) and limit of quantification(LOQ)
4. Method precision
5. Intermediate pretcition
6. Accuracy

3.4.1 System suitability

System suitability can be determined by injecting the 16 standard solutions of analyte concentrations each six times. For each concentration after injecting six times, estimated the average standard deviation and relative standard deviation of the analyte peak area.

3.4.2 Linearity

Linearity of a method validation was validated by preparing a set of minimum of 5 concentration of analyte standards viz; 10, 20, 30, 40 and 50 ppb concentration, with minimum range as appropriate for method under study and generated the standard curve, then plot the values and obtain linear graph for the analyte of organochlorine pesticides considered for residue analysis.

3.4.3 Limit of detection and limit of quantification

Limit of detection and limit of quantification was performed for the individual pesticides in the mixture, limit of detection and quantification were studied in the range of known concentrations by injecting the standard solution each by three times.

Detection limit expressed as DL, and it was calculated using the formula

$$DL = 3.3*\sigma/S$$

and quantification limit was expressed as QL and it was calculated using the formula

$$QL = 10^* \sigma/S;$$

σ - average standard deviation of the response and S - slope of the calibration curve.

3.4.4 Method precision

The method precision was validated by spiking the sample matrix with the mixture of standards concentration two times in same concentration.

3.4.5 Intermediate precision

The intermediate precision was established by spiking the solution with different concentrations by two different analyts.

3.4.6 Accuracy

Accuracy was determined using a minimum of 3 injections over a minimum of 3 concentration levels covering the specified range. Accuracy was taken into consideration by the assay of known added amount of analyte in the sample. Validation of analytical method included the determination of accuracy and recovery at or below the proposed residue detection limit.

3.5 Method development for estimation of OCPs residues in milk

3.5.1 Stock standard solutions

Standard stock solutions were prepared at a concentration of 1000 mg/l by dissolving 1g of assayed reference. Compound purity was assayed to be 96 % or greater, the weight was used without correction to calculate the concentration of the stock

standard. Standard stock solutions were transferred into vials, stored at 4^{o}C and protect from light. Stock standards were checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them. The calibration standards at a minimum of five concentrations for each parameter of interest were prepared through dilution of the stock standards.

3.5.2 Extraction and clean up milk sample for method validation

Milk sample of about 50 ml was taken in measuring cylinder and was transferred into 100 ml volumetric flask and subsequently transferred it carefully into 1 liter separating funnel. To this, 50 ml of methanol and 0.5 g of potassium oxalate was added and mixed well. To this mixture 25 ml of ethyl ether was added and shaked well for one min and then added 25 ml petroleum ether and shake vigorously for 1min. This mixture was transferred to centrifuge tubes and centrifuged at about 1500 rpm for about 5mins.

Then transferred the solvent layer into 1 liter separating funnel (SF) containing 300 ml distilled water and 15 ml saturated sodium chloride (NaCl) solution, re-extracted the residue in separating funnel with 25 ml of ethyl ether and petroleum ether, centrifuge and transferred the solvent layer into separating funnel after each extraction.

Discarded the aqueous layer in SF-I after two extractions. Poured ether solution through the sodium sulphate. Washed with separator with small portions of ether. Collected it in rotary vacuum evaporator flask and evaporated the ether to dry in rotary evaporator at 40^{o}C. Made the final volume to 5 ml by using n-hexane and this solution then injected into GC-ECD.

3.5.3 Extraction and clean up broiler meat sample for method validation

Broiler meat sample weighing 50 grams was placed in a blender with 100 g of sodium sulphate anhydrous, ground well for few minutes then added 150 ml of petroleum ether, mixed well for two to five minutes. Then decanted the extract through 500 ml Buchnur funnel with What man filter paper. Poured the extract through 40 X 25 mm column of anhydrous sodium sulphate for drying the moisture. The eluant was collected in 500 ml flask and placed in the rotary evaporator to concentrate the extract. The pesticide residues were extracted from fat by using florisil adsorbent. Extraction with 6% diethyl ether in petroleum ether. The elute was concentrated in rotary evaporator, after which it was dried in a test tube at 50 ^{0}C. The dried extract was reconstituted with 0.5 ml of n-hexane for injection into gas chromatogram with electron capture detector.

3.5.4 Extraction and clean up egg samples for method validation

Egg weighing ten to fifteen grams or three grams of yolk was placed in the container containing 30 g anhydrous sodium sulphate and mixed thoroughly to remove water. Then the mixture was soxhlet extracted with 250 ml of petroleum ether at 50^{o}C for 8h. The extracts were processed in a rotary evaporator at 40-60^{o}C to remove petroleum ether and passed over N_2 to ensure dryness. The fat contents were determined by weighing the samples before and after extraction. The fat was dissolved in petroleum ether and extracted in the petroleum ether. This mixture was dried over anhydrous sodium sulphate and concentrated at 30^{o}C on a rotary vacuum evaporator to a volume less than 5 ml. Then transferred this to the florisil column with anhydrous sodium sulphate. An aliquot of each extract was transferred to 2 ml injection vials to be ready for

analysis with gas chromatography electron capture detector. The dried extract reconstituted with 0.5 ml n-hexane for injection.

3.6 Collection of samples

The study included the collection of total of 270 samples, comprising of broiler meat, milk and chicken eggs collected from the commercial outlets of different regions of Karnataka such as Bengaluru, Mysuru and Shivamogga.

30 samples each of broiler meat, milk and chicken eggs total of 90 samples from commercial outlets of Bengaluru were collected and samples were kept in cold ice during transportation and they were stored at -20^{O}C /refrigeration condition until further analysis.

30 samples each of broiler meat, milk and chicken eggs total of 90 samples from commercial outlets of Shivamogga were collected and samples were kept in cold ice during transportation and they were stored at -20^{O} C /refrigeration condition until further analysis.

30 samples each of broiler meat, milk and chicken eggs total of 90 samples from commercial outlets of Mysuru were collected and they were transported to the laboratory in cold chain and they were stored at -20^{O} C /refrigeration condition until further analysis.

3.7 Chemicals and reagents

All the individual pesticide reference standards were obtained from Sigma-Aldrich, India. The organochlorine pesticide standards *viz*: alachlor, aldrin, dieldrin, *pp*'-

DDT, *op*'- DDT, *pp*'-DDE, *op*'-DDE, *pp*'-DDD, *op*'-DDD, lindane, α-HCH, β-HCH, γ-HCH, endosulfan-I, endosulfan-II and endosulfan sulphate were obtained from Merck Chemical, Germany. The purities of organochlorine pesticide standards were more than 99%.

Chemical reagents and solvents for sample extraction and cleanup of broiler meat, eggs and milk samples used were HPLC grade and they were obtained from the Merck Chemicals Germany, including Methanol, sodium or potassium oxalates, standard sodium chloride solution, petroleum ether, methylene dichloride, phosphate buffer, anhydrous sodium sulphate extra pure, hexane, acetone, petroleum ether, activated florisil column and diethyl ether.

3.8 Extraction of milk samples for residue analysis

The sample extraction procedure of milk for the detection of residue of Organochlorine pesticides by Gas Chromatography-Electron Capture Detector (GC-ECD) was done as per the method described in Manual of Methods of Analysis of Foods by Food Safety and Standards Authority of India (2015). Pesticide residues were extracted from milk using following steps.

About 50 ml of milk sample was measured and transferred into 100 ml volumetric flask and transferred it carefully into 1 liter separating funnel. To this, added about 50 ml of methanol and 0.5 g of potassium oxalate and mixed well. To this, 25 ml of ethyl ether was added and shaked well for one min and then added 25 ml petroleum ether and vigorously shaked for 1min. This was transferred to centrifuge tubes and centrifuged this at about 1500 rpm for about 5min. Transferred the solvent layer in to 1 liter separating

funnel (SF-II) containing 300ml distilled water and 15 ml saturated sodium chloride (NaCl) solution, re-extracted the residues in SF-I twice with 25ml of ethyl ether and petroleum ether, centrifuged and transferred the solvent layer into SF-II after each extraction. Discarded the aqueous layer in SF-I after two extractions. Poured ether solution through sodium sulphate. Separator was washed with small portions of ether. Collected it in rotary vacuum evaporator flask and evaporated the ether to dryness in rotary evaporator. Made the final volume to 5 ml by using n-hexane and this solution was injected into gas chromatogram with electron capture detector.

3.9 Preparation of broiler meat samples for residue analysis

Meat samples for organochlorine pesticide residue analysis will be extracted as per the method described by "Association Official Analytical Chemists" (1980) And Pesticide Analytical Manual (1978).

Broiler meat sample of 50 grams were placed in a blender with 100 g of sodium sulphate anhydrous, ground well for few minutes then added 150 ml of petroleum ether, mixed well for 2-5 min and then decanted the extract through 500 ml Buchnur funnel with What man filter paper. The extract was pored through 40 x 25mm column of anhydrous sodium sulphate for drying the moisture. The eluant was collected in 500 ml flask and placed in the rotary evaporator to concentrate the extract. The pesticide residues were extracted from fat by using florisil adsorbent. Extraction was done with 6% diethyl ether in petroleum ether. The elute was concentrated in rotary evaporator, after which it was dried in a test tube at 500^0C. The dried extract was reconstituted with 0.5 ml of n-hexane for injection into gas chromatogram with electron capture detector.

3.10 Preparation of egg samples for residue analysis

The extraction procedure for egg, for detection of residue of OCPs by Gas Chromatography-Electron Capture Detector (GC-ECD) was done as per the method described by "Association Official Analytical Chemists" (1995) and Kodba and Voncina, 2007.

Egg of about 10 to 15 grams or three grams of yolk were placed in the container containing 30 g anhydrous sodium sulphate and mixed thoroughly to remove water. Then the mixture was soxhlet extracted with 250 ml of petroleum ether at 50^0C for 8 h. The extracts were processed in a rotary evaporator at 40-60^0C to remove petroleum ether and passed over N_2 to ensure dryness. The fat contents were determined by weighing the samples before and after extraction. The fat was dissolved in petroleum ether and extracted in the petroleum ether. This mixture was dried over anhydrous sodium sulphate and concentrated at 30^0C on a rotary vacuum evaporator to a volume less than 5 ml. Then transferred this to the florisil column with anhydrous sodium sulphate. The filtrate was dried and the dried extract was reconstituted in 0.5 ml n-hexane for injection.

3.11 Statistical analysis

The detected residue concentrations were calculated using the formula (AOAC, 1980).

$$\text{Residue level (µg/kg)} = \frac{\text{Area of sample x Standard concentration}}{\text{Area of the standard}}$$

The data were analyzed by taking the mean of the samples detected and they are compared with maximum residue limit values.

Results

IV. RESULTS

4.1 Validation of Analytical Procedures

4.1.1 Validation of analytical method for the detection of OCP residues in broiler meat

Method validation was done to support the validity of data used for checking compliance with detected residues of OCPs in broiler meat are presented here

4.1.1.1 System suitability

The observed average relative standard deviation (RSD) for system suitability was 14.2 (Table.1). All the above pesticides were within the limits and system suitability was satisfactory.

4.1.1.2 Linearity

Acceptance criteria for linearity was the correlation co-efficient must be greater than or equal to 0.95 %. The linearity results are depicted in the Table 2. The average linearity (R^2) observed was 0.96%. The calibrated pesticides were within the limits and the results were satisfactory (Fig. 1 -16).

4.1.1.3 Limit of detection and limit of quantification

The limit of detection was 10 ppb and limit of quantification not more than maximum residue limit for each of the pesticide considered.

4.1.1.4 Method precision

The relative standard deviation observed was not more than 7.57 % (Table-3)

4.1.1.5 Intermediate method precision

The relative standard deviation for intermediate method precision observed was not more than 7.57 % (Table-4) for pesticides, all the values were within the limits and it was satisfactory.

4.1.1.6 Accuracy

The observed percentage of recovery was between 86-113 % (Table.5).

All the parameters evaluated for validation were within their respective limits and method validation was satisfactory (Table 6). The standard chromatogram was obtained after method validation using a mixture of some organochlorine pesticides viz: alpha-BHC, beta-BHC. delta- BHC, alachlor, aldrin, *op'*-DDE, endosulphan-I, *pp'*-DDE, dieldrin, *op'*-DDD, endosulphan-II, *pp'*-DDD, *op'*-DDT, endosulphan sulphate, *pp'*-DDT (Fig.49) and chromatogram of extracted blank broiler meat sample depicted in Fig.52. The gas chromatographic retention time and area obtained for 16 organochlorine pesticides using Rtx fused silica column after validation presented in the Table.19.

4.1.2 Validation of analytical method for the detection of OCP residues in milk

Method validation was done to support the validity of data used for checking compliance with detected residues of OCPs in milk are presented.

4.1.2.1 System suitability

The observed average relative standard deviation (RSD) for system suitability was 14.2 (Table.7). The All the above pesticides were within the limits and system suitability was satisfactory.

4.1.2.2 Linearity

Acceptance criteria for linearity was the correlation co-efficient must be greater than or equal to 0.95 %. The linearity results are depicted in the table 8. The average linearity (R^2) observed was 0.96%. The calibrated pesticides were within the limits and the results were satisfactory (Fig. 17 -32).

4.1.2.3 Limit of detection and limit of quantification

The limit of detection was 10 ppb and limit of quantification not more than maximum residue limit for each of the pesticide considered.

4.1.2.4 Method precision

The relative standard deviation observed was not more than 7.57 % (Table-9)

4.1.2.5 Intermediate method precision

The relative standard deviation observed was not more than 7.57 % (Table-10) for pesticides, all the values were within the limits and it was satisfactory.

4.1.2.6 Accuracy

The observed percentage of recovery was between 86-113 % (Table.11).

All the validation parameters evaluated for validation were within their respective limits and method validation was satisfactory (Table 12). The standard chromatogram was obtained after method validation using a mixture some organochlorine pesticides viz: alpha-BHC, beta-BHC, lindane, delta-BHC, alachlor, aldrin, *op'*-DDE, endosulphan-I, *pp'*-DDE, dieldrin, *op'*-DDD, endosulphan-II, *pp'*-DDD, *op'*-DDT, endosulphan sulphate, *pp'*-DDT (Fig.50) and chromatogram of extracted blank milk sample depicted in Fig.53. The gas chromatographic retention time and area obtained for 16 organochlorine pesticides using Rtx fused silica column after validation presented in the table.19.

Plate 1: Gas Chromatography- Electron Capture Detector analytical instrument

4.1.3 Validation of analytical method for the detection of OCP residues in chicken eggs

Method validation was done to support the validity of data used for checking compliance with detected residues of OCPs in chicken eggs are presented.

4.1.3.1 System suitability

The observed average relative standard deviation (RSD) for system suitability was 14.2 (Table.13). The All the above pesticides were within the limits and system suitability was satisfactory.

4.1.3.2 Linearity

The linearity results are depicted in the table 14. The average linearity (R^2) observed was 0.96%. The calibrated pesticides were within the limits and the results were satisfactory (Fig. 33 -48).

4.1.3.3 Limit of detection and limit of quantification

The limit of detection was 10ppb and limit of quantification not more than maximum residue limit.

4.1.3.4 Method precision

The relative standard deviation for method precision observed was not more than 7.57 % (Table-15)

4.1.3.5 Intermediate method precision

The relative standard deviation observed was not more than 7.57 % (Table-10) for pesticides, all the values were within the limits and it was satisfactory.

4.1.3.6 Accuracy

The observed percentage of recovery was between 86-113 % (Table.17).

All the validation parameters evaluated for validation were within their respective limits and method validation was satisfactory (Table 18). The standard chromatogram was obtained after method validation using a mixture of some organochlorine viz: alpha-BHC, beta-BHC. delta-BHC, alachlor, aldrin, *op'*-DDE, endosulphan-I, *pp'*-DDE, dieldrin, *op'*-DDD, endosulphan-II, *pp'*-DDD, *op'*-DDT, endosulphan sulphate, *pp'*-DDT (Fig.51) and chromatogram of extracted blank egg sample was depicted in Fig.54. The gas chromatographic retention time and area obtained for 16 organochlorine pesticides using Rtx fused silica column after validation presented in the table.19.

4.2 Residue analysis of broiler meat, milk and chicken egg samples collected in Bengaluru

Results of estimated residues of organochlorine pesticides in broiler meat, milk, meat and chicken eggs are presented here.

The residue of some organochlorine pesticide *viz*: alachlor, aldrin, dieldrin, *pp*'-DDT, *op'*- DDT, *pp'*-DDE, *op*,-DDE, *pp'* DDD, *op'*-DDD, Lindane, α-HCH, β-HCH, γ-HCH, α-endosulfan, β-endosulfan and endosulfan sulphate are detected in broiler meat, milk and chicken egg samples collected from commercial outlets of Bengaluru.

4.2.1 Organochlorine pesticide residue analysis in broiler meat samples

The mean residue concentration of some organochlorine pesticides (mean concentrations) in broiler meat samples was measured and the values have been summarized in Table 20 and Fig. 55.

Among the thirty broiler meat samples subjected for residue analysis, the results indicated that, 43.29% (13/30), 10% (3/30), 3.33 % (1/30), 3.33 % (1/30) and 3.33 % (1/30) contaminated with DDT and its metabolites, endosulphan and its isomers, dieldrin, alachlor and aldrin respectively. DDT and its metabolites are most detected residues, among them its the *pp'*-DDE is the most frequently detected one, the lowest and highest concentrations detected for *pp*'-DDE was less than limit of detection and 107.64 ppb respectively and the mean concentration of *pp*'-DDE was 32.28 ppb.

The residues of other metabolites of DDTs such as *op'*-DDE, *pp*'-DDD and *op*'-DDT were detected in the concentrations less than the limit of detection (less than 10 ppb levels).

Other residues of OCPs detected were dieldrin and its residue level detected was 22.84 ppb. Aldrin and alachlor were other two OCP residues detected but their concentrations were less than the limit of detection.

4.2.2 Organochlorine pesticide residue analysis in milk samples

The mean residue concentration of some organochlorine pesticides (mean ± SE) in milk samples was measured and the values have been summarized in Table 20 and Fig.56.

Among the thirty milk samples subjected for residue analysis, the results indicated that, 60 % (18/30), 16.67% (5/30), 10 % (3/30), 6.67 % (2/30), 6.67% (2/30) and 3.33 %, (1/30) contaminated with DDT and its metabolites, endosulphan -I, dieldrin, aldrin, alachlor and lindane respectively.

DDT and its metabolites are the most detected residues, among them its the *pp*'-DDE is the most frequently detected one, the lowest and highest concentrations detected for *pp*'-DDE was less than the limit of detection and 240.45 ppb respectively and the mean concentration of *pp*'-DDE was 37.79 ppb.

The residues of other metabolites of DDTs such as op'-DDD residues detected were 116.70 ppb, pp'DDD residue detected was 17 ppb where as *op'*-DDE and *pp*'-DDT residues were detected at the concentration levels less than their limit of detection.

Endosulphan I was another most detected residues of OC pesticide, the mean residue concentration detected was 13.79 ppb and their lowest and highest concentrations were less than limit of detection and 24.61 ppb respectively.

Other OCP residue detected was dieldrin and its mean concentration was 13.64 ppb. Where in aldrin detected in two samples with mean concentration 10.17 ppb and alachlor was detected but their levels less than their limit of detection. Lindane was detected in one sample with residue concentration 86.60 ppb.

4.2.3 Organochlorine pesticide residue analysis in egg samples

The mean residue concentration of some organochlorine pesticides in chicken egg samples was measured and the values have been summarized in Table 20 and Fig. 57.

Among the thirty chicken egg samples subjected for residue analysis, the results indicated that, 36.67% (11/30), 26.67% (8/30), 16.67 % (5/30), 16.67% (5/30), 13.33% (4/30) and 6.67% (2/30) contaminated with DDT and its metabolites, delta-BHC, dieldrin, aldrin, alachlor and endosulphan sulphate respectively.

DDT and its metabolites are most detected residues, among them its the *pp*'-DDE is the most frequently detected one, the lowest and highest concentrations detected for *pp*'-DDE was less than the limit of detection and 25.53 ppb respectively and the mean concentration detected was 10.22 ppb.

Other metabolites of DDTs such as *op*'-DDE residue detected with mean concentration of 94.26 ppb, *pp*'-DDD residue detected with mean concentration of 24.05 ppb, *op*'-DDT residue detected at 26.02 ppb and *op*'-DDE residue was detected 17.33 ppb.

Delta-BHC was another most detected residues of OC pesticide and its lowest and highest concentration detected were 40.43 to 49.12 ppb and its mean residue concentration was 39.38 ppb.

Dieldrin residue detected in samples and its mean concentration 17.25 ppb and its lowest and highest concentrations detected were less than the limit of detection and 47.67 ppb respectively.

Aldrin residue was detected in 5 samples and its mean concentration 13.02 ppb and its lowest and highest concentrations detected were less than the limit of detection and 18.27 ppb respectively. Endosulphan sulphate residue detected in two samples with

mean concentration of 21.66 ppb. Alachlor residue detected in three samples but their levels were below the limit of detection.

4.3 Residue analysis of broiler meat, milk and chicken egg samples collected in Shivamogga

4.3.1 Organochlorine pesticide residue analysis in broiler meat samples

The mean residue concentration of some organochlorine pesticides (mean concentrations) in broiler meat samples was measured and the values have been summarized in Table 21 and Fig. 58.

Among the thirty broiler meat samples subjected for residue analysis, the results indicated that, 30% (9/30), 16.67% (5/30), 13.33% (4/30), 10% (3/30) and 6.67% (2/30) contaminated with DDTs, delta-BHC, dieldrin, aldrin and alachlor respectively.

DDT metabolite *pp'*-DDE was most detected residues, the lowest and highest concentrations detected of *pp'*-DDE was less than the limit of detection and 239.06 ppb respectively and the mean concentration was 84.22 ppb.

Delta-BHC was another detected residues of OC pesticide and with mean concentration was 15.57 ppb, the lowest and highest range was 11 ppb to 15.32 ppb. The alachlor was another residue detected with 18.18 ppb concentration.

Dieldrin and aldrin were the other residues detected but their levels less than the limit of detection.

4.3.2 Organochlorine pesticide residue analysis in milk samples

The mean residue concentration of some organochlorine pesticides in milk samples was measured and the values have been summarized in Table 21 and Fig. 59.

Among the thirty milk samples subjected for residue analysis, the results indicated that, 53.33 % (16/30), 13.33% (4/30), 10.00% (3/30), 10.00% (3/30), 10.00% (3/30), 6.67% (2/30), 6.67% (2/30), 3.33% (1/30) were contaminated with DDT and its metabolites, endosulphan-I, delta–BHC, aldrin, lindane, beta-BHC, alachlor and dieldrin respectively.

DDT and its metabolites are most frequently detected residues, among them its the *pp'*-DDE is the most frequently detected one, the lowest and highest concentrations detected for *pp'*-DDE was less than the limit of detection and 129.04 ppb respectively and the mean concentration detected was 46.66 ppb. The residues of other metabolites of DDTs such as *op*'-DDE detected but their concentration was less than the limit of detection and op-DDD was another residue detected in the concentration of 15.11 ppb.

Endosulphan-I was another most detected residues with mean concentration of residue was 11.98 ppb.

Delta-BHC residue detected at 16.78 ppb, with lowest concentration and highest concentration detected was 10.83 to 19.14 ppb respectively.

Aldrin residue detected in three samples with mean concentration 15.91 ppb. Lindane residue detected was 98.04 ppb. Beta-BHC concentration detected at 63.98 ppb. Alachlor and dieldrin concentrations detected were 13.16 and 11.33 ppb respectively.

4.3.3 Organochlorine pesticide residue analysis in egg samples

The mean residue concentration of some organochlorine pesticides in chicken egg samples was measured and the values have been summarized in Table 21 and Fig. 60.

Among the thirty chicken egg samples subjected for residue analysis, the results indicated that, 53.33% (16/30), 23.33% (7/30), 13.33 % (4/30), 10.00 % (3/30), 6.67% (2/30), 3.33% (1/30) and 3.33% (1/30) contaminated with DDT and its metabolites, endosulphan-I, dieldrin, endosulphan II, alachlor, aldrin and endosulphan sulphate respectively.

DDT and its metabolites are most detected residues, among them its the *pp'*-DDE is the most detected one, the lowest and highest concentrations detected for *pp'*-DDE was 33.81 to 205.68 ppb respectively and the mean concentration was 94.31 ppb. The residues of other metabolites of DDT such as *op'*-DDE was detected but their residue concentration was less than the limit of detection. *op'*-DDT was detected in the concentration of 228.9 ppb and *pp*'-DDD detected with mean concentration of 98.53 ppb.

Endosulphan-I and endosulphan-II was another frequently detected residue of OC pesticides with 21.55 and 45.17 ppb respectively. Endosulphan sulphate residue concentration was 36.06 ppb.

Lindane residue was detected in four samples with mean residue concentration of 55 ppb. Dieldrin also detected in four samples with mean residue concentration of 27.30 ppb. Alachlor and aldrin were detected in two samples but their levels were less than the limit of detection.

4.4 Residue analysis of broiler meat, milk and chicken egg samples collected in Mysuru

4.4.1 Organochlorine pesticide residue analysis in broiler meat samples

The mean residue concentration of some organochlorine pesticides in broiler meat samples was measured and the values are summarized in Table 22 and Fig. 61.

Among the thirty broiler meat samples subjected for residue analysis, the results indicated that, 16.67% (5/30), 6.37% (2/30), 3.33% (1/30) , 3.33% (1/30) and 3.33% (1/30) contaminated with DDTs, endosulphane I, aldrin, alachlor, alpha-BHC and endosulphan sulphate respectively.

DDT metabolites *op'*-DDE and *op'*-DDD was most frequently detected residues, but their detected residue levels below the level of limit of detection.

Endosulphan- I residue detected was 24.61 ppb and aldrin residue detected was 11.33 ppb. Alpha-BHC and endosulphan sulphate were other two residues detected but their residue levels were below the levels of limit of detection.

4.4.2 Organochlorine pesticide residue analysis in milk samples

The mean residue concentration of some organochlorine pesticides (mean concentration) in milk samples was measured and the values have been summarized in Table 22 and Fig. 62.

Among the thirty milk samples subjected for residue analysis, the results indicated that, 20.00% (6/30), 10.00% (3/30), 6.67% (2/30), 3.33% (1/30) and 3.33% (1/30) were

contaminated with DDT metabolites, beta-BHC, endosulphan-I, dieldrin and lindane respectively.

The *pp'*-DDE and *op'*-DDE are the frequently detected ones but their residue levels were below the limit of detection.

Beta-BHC was another residue detected with concentration of 33.94 ppb and lindane was detected at 45.37 ppb.

Endosulphan-I and dieldrin were the other two residues detected but their levels weer below the limit of detection.

4.4.3 Organochlorine pesticide residue analysis in chicken egg samples

The mean residue concentration of some organochlorine pesticides in chicken egg samples was measured and the values have been summarized in Table 22 and Fig. 63.

Among the thirty egg samples subjected for residue analysis, the results indicated that, 13.33% (4/30), 6.67% (2/30), 3.33% (1/30), 3.33% (1/30) and 3.33% (1/30) contaminated with *op'*-DDE, endosulphan-I, alpha-BHC, lindane and delta-BHC respectively.

The DDT metabolite, *op*'-DDE was detected but their residue levels were below the limit of detection. Endosulphan-I was another residue detected with their concentration below their limit of detection. Other OC pesticides residues detected were alpha-BHC, lindane and delta-BHC and their mean concentrations detected were 24.44, 10.95 and 15.23 ppb respectively.

4.5 Comparison of concentration of residues of organochlorine pesticides detected in broiler meat, milk and chicken eggs collected from commercial outlets of Bengaluru, Shivamogga and Mysuru with Maximum Residue Level (MRLs) values (FAO/WHO and EU-MRLs) and the same has depicted in table 23, 24 and 25 for Bengaluru, Shivamogga and Mysuru respectively

The detected organochlorine pesticide residues in broiler meat, milk and chicken egg samples collected from commercial outlets of Bengaluru, Shivamogga and Mysuru were well within the maximum residue limit. None of the detected residues were more than maximum residue limit values.

Table 1: System suitability of method validation of GC-ECD for detection and quantification of OCP residues in broiler meat

Sl. No.	OC Pesticides	Std-1 Area	Std-2 Area	Std-3 Area	Std-4 Area	Std-5 Area	Std-6 Area	Avg Area	SD	RSD %
1	Alpha-BHC	863	855	617	853	729	692	768	104	13.52
2	Lindane	345	333	352	409	306	351	349	34	9.69
3	Beta-BHC	297	345	255	269	355	273	299	42	14.01
4	Delta-BHC	300	297	230	247	309	260	274	33	11.88
5	Alachlor	4024	4417	3946	3380	3045	3972	3797	496	13.05
6	Aldrin	1110	1155	1028	1001	1022	1225	1090	89	8.13
7	op-DDE	2544	2439	1896	2398	2038	2748	2344	319	13.62
8	Endosulphan-I	1834	1972	1417	1682	1555	1938	1733	221	12.73
9	pp-DDE	1697	1698	1203	1632	1412	1595	1540	195	12.70
10	Dieldrin	2670	2551	2169	2577	2122	2871	2493	292	11.72
11	op-DDD	4557	4330	4740	5242	4640	5491	4833	442	9.14
12	Endosulphan-II	2247	2239	1854	2289	1863	2640	2189	296	13.52
13	pp-DDD	1366	1382	1578	1545	1615	1655	1524	122	7.98
14	op-DDT	859	892	619	856	711	936	812	121	14.92
15	Endosulphan Sulphate	949	781	790	755	669	952	816	113	13.80
16	pp-DDT	1700	1743	1428	1650	1335	1397	1542	175	11.38

Table 2: Linearity of method validation of GC-ECD for detection and quantification of OCP residues in broiler meat

Sl. No.	OC Pesticides	10ppb Area	20ppb Area	30ppb Area	40ppb Area	50ppb Area	Linearity
1	Alpha-BHC	481.2	1069.3	1338.8	2011.1	2541.3	0.987
2	Lindane	648	1107	1459.6	1846.7	2361.1	0.967
3	Beta-BHC	452.9	931.5	1082.9	1602.9	2206.3	0.967
4	Delta-BHC	321.2	844.5	1275.1	1596.4	1935.9	0.988
5	Alachlor	1423.5	3379.5	6126.2	8254.1	9756.0	0.960
6	Aldrin	450.1	986.4	2042.8	2967.4	3919.8	0.950
7	op-DDE	1021.8	2122.1	4435.4	6203.8	7919.1	0.962
8	Endosulphan-I	653.3	1525.1	3401.5	4160.2	4926.0	0.958
9	pp-DDE	853.7	1645.7	3265.4	4333.3	5682.4	0.974
10	Dieldrin	1153.2	2297.8	4112	5935.6	6778.3	0.979
11	op-DDD	2329.4	4217.7	7764.4	11261.0	9989.1	0.979
12	Endosulphan-II	1129.4	2080	3724.5	5766.7	7390.1	0.962
13	pp-DDD	728.6	1401.7	2567.3	3951.9	4486.7	0.968
14	op-DDT	321.5	749.5	1302.1	1762.6	984.1	0.987
15	EndosulphanSulphate	357.9	820.4	1537.2	2451.8	2815.3	0.955
16	pp-DDT	858.2	1675.4	3062.6	4392.3	5566.7	0.976

Table 3: Precision of method validation of GC-ECD for detection and quantification of OCP residues in broiler meat

Sl. No.	OC Pesticides	Std-1 Area	Std-2 Area	Std-3 Area	Avg Area	SD	RSD %	Spl-1 Area	Spl-1 %	Recovery %	Spl-2 Area	Spl-2 %	Recovery %
1	Alpha-BHC	997	1276	1280	1184	163	13.73	2027	171.2	114.12	1745	86.0	86.07
2	Lindane	990	1151	1250	1130	131	11.62	1982	175.4	116.93	1932	97.4	97.44
3	Beta-BHC	979	1190	1279	1149	154	13.43	1928	167.7	111.82	2212	114.7	114.77
4	Delta-BHC	655	688	704	682	25	3.62	1162	170.3	113.55	1087	93.5	93.50
5	Alachlor	14306	14475	12049	13610	1354	9.95	15936	117.1	78.06	18473	115.9	115.92
6	Aldrin	4907	4853	4386	4715	287	6.08	6910	146.5	97.70	7100	102.7	102.75
7	op-DDE	10485	10815	8519	9940	1241	12.49	11901	119.7	79.82	13276	111.5	111.55
8	Endosulphan-I	7866	7607	7675	7716	135	1.74	10588	137.2	91.48	11251	106.2	106.26
9	pp-DDE	7508	8636	6681	7608	981	12.90	8594	113.0	75.30	10192	118.5	118.59
10	Dieldrin	9298	9247	8979	9175	171	1.87	11883	129.5	86.34	13645	114.8	114.83
11	op-DDD	17877	18008	15252	17046	1555	9.12	20291	119.0	79.36	21747	107.1	107.18
12	Endosulphan-II	9165	9888	8233	9095	829	9.12	11020	121.2	80.77	11712	106.2	106.29
13	pp-DDD	6247	6436	5860	6181	294	4.75	8226	133.1	88.73	8296	100.8	100.85
14	op-DDT	3364	3644	3514	3507	140	4.00	4881	139.2	92.78	5024	102.9	102.92
15	Endosulphan Sulphate	4057	4218	4108	4128	82	1.99	6085	147.4	98.29	6278	103.1	103.17
16	pp-DDT	7560	8320	7965	7948	380	4.78	9746	122.6	81.75	10329	105.9	105.98

Table 4: Intermediate precision of method validation of GC-ECD for detection and quantification of OCP residues in broiler meat

Sl. No.	OC Pesticides	Std-1 Area	Std-2 Area	Std-3 Area	Avg Area	SD	RSD %	Analyte 1	Spl-1 %	Recovery %	Analyte 2	Spl-2 %	Recovery %
1	Alpha-BHC	996.6	1276	1280	1184	162	13.72	625	52.78	105.6	896	75.66	100.88
2	Lindane	989.7	1151	1250	1130	131	11.62	997	88.21	176.4	998	88.30	117.73
3	Beta-BHC	979	1190	1279	1149	154	13.41	655	56.99	114.0	882	76.74	102.32
4	Delta-BHC	655.3	688	704	682	25	3.64	324	47.48	95.0	425	62.28	83.04
5	Alachlor	14306	14475	12049	13610	1355	9.95	6859	50.40	100.8	8956	65.80	87.74
6	Aldrin	4907	4853	4386	4715	286	6.08	2158	45.77	91.5	3105	65.85	87.80
7	op-DDE	10485	10815	8519	9940	1241	12.49	4587	46.15	92.3	6089	61.26	81.68
8	Endosulphan-I	7866	7607	7675	7716	134	1.74	3985	51.65	103.3	4698	60.89	81.18
9	pp-DDE	7508	8636	6681	7608	981	12.90	3627	47.67	95.3	5214	68.53	91.37
10	Dieldrin	9298	9247	8979	9175	171	1.87	4398	47.94	95.9	5986	65.24	86.99
11	op-DDD	17877	18008	15252	17046	1555	9.12	7589	44.52	89.0	20154	118.24	157.65
12	Endosulphan-II	9165	9888	8233	9095	830	9.12	4256	46.79	93.6	10548	115.97	154.63
13	pp-DDD	6247	6436	5860	6181	294	4.75	3158	51.09	102.2	4965	80.33	107.10
14	op-DDT	3364	3644	3514	3507	140	4.00	1584	45.16	90.3	3658	104.29	139.06
15	Endosulphan Sulphate	4057	4218	4108	4128	82	1.99	2351	56.96	113.9	3698	89.59	119.46
16	pp-DDT	7560	8320	7965	7948	380	4.78	4058	51.06	102.1	6658	83.77	111.69

Table 5: Accuracy (recovery) of method validation of GC-ECD for detection and quantification of OCP residues in broiler meat

Sl. No.	OC Pesticides	5 ppb Area	10ppb Area	20ppb Area	Linearity	Spike (10ppb)	Spl -1 %	Recovery-1 (%)	Spike (20ppb)	Spl-2 %	Recovery-2 %
1	Alpha-BHC	259.2	501.3	1008.5	0.999	465.2	7.90	92.92	1056.2	10.47	104.73
2	Lindane	345.7	688.2	1256.7	0.991	592.1	8.60	86.04	1152.3	9.17	91.69
3	Beta-BHC	253.4	409.9	805.7	0.984	432.7	10.56	105.56	762.3	9.46	94.61
4	Delta-BHC	186.2	349.1	739.4	0.997	395.8	11.34	113.38	687.5	9.30	92.98
5	Alachlor	724.3	1388.2	3834.1	0.952	1283.2	9.24	92.44	3573.1	9.32	93.19
6	Aldrin	219.5	438.2	829.4	0.997	389.5	8.89	88.89	753.2	9.08	90.81
7	op-DDE	500.8	1059.3	2182.2	0.998	1234.2	11.65	116.51	1985.2	9.10	90.97
8	Endosulphan-I	300.8	685	1350.4	0.997	607.3	8.87	88.66	1156.2	8.56	85.62
9	pp-DDE	400.7	862.3	1756.2	0.998	825.3	9.57	95.71	1531.5	8.72	87.21
10	Dieldrin	509.5	1093.8	2065.1	0.997	989.2	9.04	90.44	1964.2	9.51	95.11
11	op-DDD	1056	2698	5295.1	0.992	2587.6	9.59	95.91	4862.1	91.88	91.82
12	Endosulphan-II	598.4	1062.3	2698.3	0.972	1055.2	9.93	99.33	2485.6	9.21	92.12
13	pp-DDD	305.8	705.2	1398.3	0.996	685.3	9.72	97.18	1245.8	8.91	89.09
14	op-DDT	150.9	299.8	623.5	0.998	318.6	10.63	106.27	589.2	9.45	94.50
15	Endosulphan Sulphate	150.6	293.1	623.7	0.997	286.2	9.76	97.65	597.2	9.58	95.75
16	pp-DDT	485.8	861.7	1724.8	0.996	824.6	9.57	95.69	1538.4	8.92	89.19

Table 6: Parameters of method validation of GC-ECD for detection and quantification of OCP residues in broiler meat

Parameter	Observation	Validation Limits
System Suitability	Avg. RSD 14.2	<RSD 15.0%
Linearity	Avg. R^2 = 0.960	> R^2 = 0.950
LOD&LOQ	LOD-10ppb LOQ less than maximum residue limit	LOQ should be less than MRL (maximum residue limit)
Method Precision	Avg RSD 7.57 %	< RSD 15.0%
Intermediate Precision	RSD 7.57 %	< RSD 15.0%
Accuracy	Avg R^2 = 0.991 Avg 86-113%	> R^2 = 0.950 Recovery between 70%-130%

Table 7: System suitability of method validation of GC-ECD for detection and quantification of OCP residues in milk

Sl. No.	OC Pesticides	Std-1 Area	Std-2 Area	Std-3 Area	Std-4 Area	Std-5 Area	Std-6 Area	Avg Area	SD	RSD %
1	Alpha-BHC	863	855	617	853	729	692	768	104	13.52
2	Lindane	345	333	352	409	306	351	349	34	9.69
3	Beta-BHC	297	345	255	269	355	273	299	42	14.01
4	Delta-BHC	300	297	230	247	309	260	274	33	11.88
5	Alachlor	4024	4417	3946	3380	3045	3972	3797	496	13.05
6	Aldrin	1110	1155	1028	1001	1022	1225	1090	89	8.13
7	op-DDE	2544	2439	1896	2398	2038	2748	2344	319	13.62
8	Endosulphan-I	1834	1972	1417	1682	1555	1938	1733	221	12.73
9	pp-DDE	1697	1698	1203	1632	1412	1595	1540	195	12.70
10	Dieldrin	2670	2551	2169	2577	2122	2871	2493	292	11.72
11	op-DDD	4557	4330	4740	5242	4640	5491	4833	442	9.14
12	Endosulphan-II	2247	2239	1854	2289	1863	2640	2189	296	13.52
13	pp-DDD	1366	1382	1578	1545	1615	1655	1524	122	7.98
14	op-DDT	859	892	619	856	711	936	812	121	14.92
15	Endosulphan Sulphate	949	781	790	755	669	952	816	113	13.80
16	pp-DDT	1700	1743	1428	1650	1335	1397	1542	175	11.38

Table 8: Linearity of method validation of GC-ECD for detection and quantification of OCP residues in milk

Sl. No.	OC Pesticides	10ppb Area	20ppb Area	30ppb Area	40ppb Area	50ppb Area	Linearity
1	Alpha-BHC	481.2	1069.3	1338.8	2011.1	2541.3	0.987
2	Lindane	648	1107	1459.6	1846.7	2361.1	0.967
3	Beta-BHC	452.9	931.5	1082.9	1602.9	2206.3	0.967
4	Delta-BHC	321.2	844.5	1275.1	1596.4	1935.9	0.988
5	Alachlor	1423.5	3379.5	6126.2	8254.1	9756.0	0.960
6	Aldrin	450.1	986.4	2042.8	2967.4	3919.8	0.950
7	op-DDE	1021.8	2122.1	4435.4	6203.8	7919.1	0.962
8	Endosulphan-I	653.3	1525.1	3401.5	4160.2	4926.0	0.958
9	pp-DDE	853.7	1645.7	3265.4	4333.3	5682.4	0.974
10	Dieldrin	1153.2	2297.8	4112	5935.6	6778.3	0.979
11	op-DDD	2329.4	4217.7	7764.4	11261.0	9989.1	0.979
12	Endosulphan-II	1129.4	2080	3724.5	5766.7	7390.1	0.962
13	pp-DDD	728.6	1401.7	2567.3	3951.9	4486.7	0.968
14	op-DDT	321.5	749.5	1302.1	1762.6	984.1	0.987
15	Endosulphan Sulphate	357.9	820.4	1537.2	2451.8	2815.3	0.955
16	pp-DDT	858.2	1675.4	3062.6	4392.3	5566.7	0.976

Table 9: Precision of method validation of GC-ECD for detection and quantification of OCP residues in milk

S. No	OC Pesticides	Std-1 Area	Std-2 Area	Std-3 Area	Avg Area	SD	RSD %	Spl-1 Area	Spl-1 %	Recovery %	Spl-2 Area	Spl-2 %	Recovery %
1	Alpha-BHC	997	1276	1280	1184	163	13.73	2027	171.2	114.12	1745	86.0	86.07
2	Lindane	990	1151	1250	1130	131	11.62	1982	175.4	116.93	1932	97.4	97.44
3	Beta-BHC	979	1190	1279	1149	154	13.43	1928	167.7	111.82	2212	114.7	114.77
4	Delta-BHC	655	688	704	682	25	3.62	1162	170.3	113.55	1087	93.5	93.50
5	Alachlor	14306	14475	12049	13610	1354	9.95	15936	117.1	78.06	18473	115.9	115.92
6	Aldrin	4907	4853	4386	4715	287	6.08	6910	146.5	97.70	7100	102.7	102.75
7	op-DDE	10485	10815	8519	9940	1241	12.49	11901	119.7	79.82	13276	111.5	111.55
8	Endosulphan-I	7866	7607	7675	7716	135	1.74	10588	137.2	91.48	11251	106.2	106.26
9	pp-DDE	7508	8636	6681	7608	981	12.90	8594	113.0	75.30	10192	118.5	118.59
10	Dieldrin	9298	9247	8979	9175	171	1.87	11883	129.5	86.34	13645	114.8	114.83
11	op-DDD	17877	18008	15252	17046	1555	9.12	20291	119.0	79.36	21747	107.1	107.18
12	Endosulphan-II	9165	9888	8233	9095	829	9.12	11020	121.2	80.77	11712	106.2	106.29
13	pp-DDD	6247	6436	5860	6181	294	4.75	8226	133.1	88.73	8296	100.8	100.85
14	op-DDT	3364	3644	3514	3507	140	4.00	4881	139.2	92.78	5024	102.9	102.92
15	Endosulphan Sulphate	4057	4218	4108	4128	82	1.99	6085	147.4	98.29	6278	103.1	103.17
16	pp-DDT	7560	8320	7965	7948	380	4.78	9746	122.6	81.75	10329	105.9	105.98

Table 10: Intermediate precision of method validation of GC-ECD for detection and quantification of OCP residues in milk

Sl. No.	OC Pesticides	Std-1 Area	Std-2 Area	Std-3 Area	Avg Area	SD	RSD %	Analyte 1	Spl-1 %	Recovery %	Analyte 2	Spl-2 %	Recovery %
1	Alpha-BHC	996.6	1276	1280	1184	162	13.72	625	52.78	105.6	896	75.66	100.88
2	Lindane	989.7	1151	1250	1130	131	11.62	997	88.21	176.4	998	88.30	117.73
3	Beta-BHC	979	1190	1279	1149	154	13.41	655	56.99	114.0	882	76.74	102.32
4	Delta-BHC	655.3	688	704	682	25	3.64	324	47.48	95.0	425	62.28	83.04
5	Alachlor	14306	14475	12049	13610	1355	9.95	6859	50.40	100.8	8956	65.80	87.74
6	Aldrin	4907	4853	4386	4715	286	6.08	2158	45.77	91.5	3105	65.85	87.80
7	op-DDE	10485	10815	8519	9940	1241	12.49	4587	46.15	92.3	6089	61.26	81.68
8	Endosulphan-I	7866	7607	7675	7716	134	1.74	3985	51.65	103.3	4698	60.89	81.18
9	pp-DDE	7508	8636	6681	7608	981	12.90	3627	47.67	95.3	5214	68.53	91.37
10	Dieldrin	9298	9247	8979	9175	171	1.87	4398	47.94	95.9	5986	65.24	86.99
11	op-DDD	17877	18008	15252	17046	1555	9.12	7589	44.52	89.0	20154	118.24	157.65
12	Endosulphan-II	9165	9888	8233	9095	830	9.12	4256	46.79	93.6	10548	115.97	154.63
13	pp-DDD	6247	6436	5860	6181	294	4.75	3158	51.09	102.2	4965	80.33	107.10
14	op-DDT	3364	3644	3514	3507	140	4.00	1584	45.16	90.3	3658	104.29	139.06
15	Endosulphan Sulphate	4057	4218	4108	4128	82	1.99	2351	56.96	113.9	3698	89.59	119.46
16	pp-DDT	7560	8320	7965	7948	380	4.78	4058	51.06	102.1	6658	83.77	111.69

Table 11: Accuracy (recovery) of method validation of GC-ECD for detection and quantification of OCP residues in milk

Sl. No.	OC Pesticides	5 ppb Area	10ppb Area	20ppb Area	Linearity	Spike (10ppb)	Spl -1 %	Recovery-1 (%)	Spike (20ppb)	Spl-2 %	Recovery-2 %
1	Alpha-BHC	259.2	501.3	1008.5	0.999	465.2	7.90	92.92	1056.2	10.47	104.73
2	Lindane	345.7	688.2	1256.7	0.991	592.1	8.60	86.04	1152.3	9.17	91.69
3	Beta-BHC	253.4	409.9	805.7	0.984	432.7	10.56	105.56	762.3	9.46	94.61
4	Delta-BHC	186.2	349.1	739.4	0.997	395.8	11.34	113.38	687.5	9.30	92.98
5	Alachlor	724.3	1388.2	3834.1	0.952	1283.2	9.24	92.44	3573.1	9.32	93.19
6	Aldrin	219.5	438.2	829.4	0.997	389.5	8.89	88.89	753.2	9.08	90.81
7	op-DDE	500.8	1059.3	2182.2	0.998	1234.2	11.65	116.51	1985.2	9.10	90.97
8	Endosulphan-I	300.8	685	1350.4	0.997	607.3	8.87	88.66	1156.2	8.56	85.62
9	pp-DDE	400.7	862.3	1756.2	0.998	825.3	9.57	95.71	1531.5	8.72	87.21
10	Dieldrin	509.5	1093.8	2065.1	0.997	989.2	9.04	90.44	1964.2	9.51	95.11
11	op-DDD	1056	2698	5295.1	0.992	2587.6	9.59	95.91	4862.1	91.88	91.82
12	Endosulphan-II	598.4	1062.3	2698.3	0.972	1055.2	9.93	99.33	2485.6	9.21	92.12
13	pp-DDD	305.8	705.2	1398.3	0.996	685.3	9.72	97.18	1245.8	8.91	89.09
14	op-DDT	150.9	299.8	623.5	0.998	318.6	10.63	106.27	589.2	9.45	94.50
15	Endosulphan Sulphate	150.6	293.1	623.7	0.997	286.2	9.76	97.65	597.2	9.58	95.75
16	pp-DDT	485.8	861.7	1724.8	0.996	824.6	9.57	95.69	1538.4	8.92	89.19

Table 12: Parameters method validation of GC-ECD for detection and quantification of OCP residues in milk

Parameter	Observation	Validation Limits
System Suitability	Avg. RSD 14.2	<RSD 15.0%
Linearity	Avg. $R^2 = 0.960$	> $R^2 = 0.950$
LOD&LOQ	LOD-10ppb LOQ less than maximum residue limit	LOQ should be less than MRL (maximum residue limit)
Method Precision	Avg RSD 7.57 %	< RSD 15.0%
Intermediate Precision	RSD 7.57 %	< RSD 15.0%
Accuracy	Avg $R^2 = 0.991$ Avg 86-113%	> $R^2 = 0.950$ Recovery between 70%-130%

Table 13: System suitability of method validation of GC-ECD for detection and quantification of OCP residues in chicken eggs

Sl. No.	OC Pesticides	Std-1 Area	Std-2 Area	Std-3 Area	Std-4 Area	Std-5 Area	Std-6 Area	Avg Area	SD	RSD %
1	Alpha-BHC	863	855	617	853	729	692	768	104	13.52
2	Lindane	345	333	352	409	306	351	349	34	9.69
3	Beta-BHC	297	345	255	269	355	273	299	42	14.01
4	Delta-BHC	300	297	230	247	309	260	274	33	11.88
5	Alachlor	4024	4417	3946	3380	3045	3972	3797	496	13.05
6	Aldrin	1110	1155	1028	1001	1022	1225	1090	89	8.13
7	op-DDE	2544	2439	1896	2398	2038	2748	2344	319	13.62
8	Endosulphan-I	1834	1972	1417	1682	1555	1938	1733	221	12.73
9	pp-DDE	1697	1698	1203	1632	1412	1595	1540	195	12.70
10	Dieldrin	2670	2551	2169	2577	2122	2871	2493	292	11.72
11	op-DDD	4557	4330	4740	5242	4640	5491	4833	442	9.14
12	Endosulphan-II	2247	2239	1854	2289	1863	2640	2189	296	13.52
13	pp-DDD	1366	1382	1578	1545	1615	1655	1524	122	7.98
14	op-DDT	859	892	619	856	711	936	812	121	14.92
15	Endosulphan Sulphate	949	781	790	755	669	952	816	113	13.80
16	pp-DDT	1700	1743	1428	1650	1335	1397	1542	175	11.38

Table 14: Linearity of method validation of GC-ECD for detection and quantification of OCP residues in chicken eggs

Sl. No.	OC Pesticides	10ppb Area	20ppb Area	30ppb Area	40ppb Area	50ppb Area	Linearity
1	Alpha-BHC	481.2	1069.3	1338.8	2011.1	2541.3	0.987
2	Lindane	648	1107	1459.6	1846.7	2361.1	0.967
3	Beta-BHC	452.9	931.5	1082.9	1602.9	2206.3	0.967
4	Delta-BHC	321.2	844.5	1275.1	1596.4	1935.9	0.988
5	Alachlor	1423.5	3379.5	6126.2	8254.1	9756.0	0.960
6	Aldrin	450.1	986.4	2042.8	2967.4	3919.8	0.950
7	op-DDE	1021.8	2122.1	4435.4	6203.8	7919.1	0.962
8	Endosulphan-I	653.3	1525.1	3401.5	4160.2	4926.0	0.958
9	pp-DDE	853.7	1645.7	3265.4	4333.3	5682.4	0.974
10	Dieldrin	1153.2	2297.8	4112	5935.6	6778.3	0.979
11	op-DDD	2329.4	4217.7	7764.4	11261.0	9989.1	0.979
12	Endosulphan-II	1129.4	2080	3724.5	5766.7	7390.1	0.962
13	pp-DDD	728.6	1401.7	2567.3	3951.9	4486.7	0.968
14	op-DDT	321.5	749.5	1302.1	1762.6	984.1	0.987
15	Endosulphan Sulphate	357.9	820.4	1537.2	2451.8	2815.3	0.955
16	pp-DDT	858.2	1675.4	3062.6	4392.3	5566.7	0.976

Table 15: Precision of method validation of GC-ECD for detection and quantification of OCP residues in chicken eggs

Sl. No.	OC Pesticides	Std-1 Area	Std-2 Area	Std-3 Area	Avg Area	SD	RSD %	Spl-1 Area	Spl-1 %	Recovery %	Spl-2 Area	Spl-2 %	Recovery %
1	Alpha-BHC	997	1276	1280	1184	163	13.73	2027	171.2	114.12	1745	86.0	86.07
2	Lindane	990	1151	1250	1130	131	11.62	1982	175.4	116.93	1932	97.4	97.44
3	Beta-BHC	979	1190	1279	1149	154	13.43	1928	167.7	111.82	2212	114.7	114.77
4	Delta-BHC	655	688	704	682	25	3.62	1162	170.3	113.55	1087	93.5	93.50
5	Alachlor	14306	14475	12049	13610	1354	9.95	15936	117.1	78.06	18473	115.9	115.92
6	Aldrin	4907	4853	4386	4715	287	6.08	6910	146.5	97.70	7100	102.7	102.75
7	op-DDE	10485	10815	8519	9940	1241	12.49	11901	119.7	79.82	13276	111.5	111.55
8	Endosulphan-I	7866	7607	7675	7716	135	1.74	10588	137.2	91.48	11251	106.2	106.26
9	pp-DDE	7508	8636	6681	7608	981	12.90	8594	113.0	75.30	10192	118.5	118.59
10	Dieldrin	9298	9247	8979	9175	171	1.87	11883	129.5	86.34	13645	114.8	114.83
11	op-DDD	17877	18008	15252	17046	1555	9.12	20291	119.0	79.36	21747	107.1	107.18
12	Endosulphan-II	9165	9888	8233	9095	829	9.12	11020	121.2	80.77	11712	106.2	106.29
13	pp-DDD	6247	6436	5860	6181	294	4.75	8226	133.1	88.73	8296	100.8	100.85
14	op-DDT	3364	3644	3514	3507	140	4.00	4881	139.2	92.78	5024	102.9	102.92
15	Endosulphan Sulphate	4057	4218	4108	4128	82	1.99	6085	147.4	98.29	6278	103.1	103.17
16	pp-DDT	7560	8320	7965	7948	380	4.78	9746	122.6	81.75	10329	105.9	105.98

Table 16: Intermediate precision of method validation of GC-ECD for detection and quantification of OCP residues in chicken eggs

Sl. No.	OC Pesticides	Std-1 Area	Std-2 Area	Std-3 Area	Avg Area	SD	RSD %	Analyte 1	Spl-1 %	Recovery %	Analyte 2	Spl-2 %	Recovery %
1	Alpha-BHC	996.6	1276	1280	1184	162	13.72	625	52.78	105.6	896	75.66	100.88
2	Lindane	989.7	1151	1250	1130	131	11.62	997	88.21	176.4	998	88.30	117.73
3	Beta-BHC	979	1190	1279	1149	154	13.41	655	56.99	114.0	882	76.74	102.32
4	Delta-BHC	655.3	688	704	682	25	3.64	324	47.48	95.0	425	62.28	83.04
5	Alachlor	14306	14475	12049	13610	1355	9.95	6859	50.40	100.8	8956	65.80	87.74
6	Aldrin	4907	4853	4386	4715	286	6.08	2158	45.77	91.5	3105	65.85	87.80
7	op-DDE	10485	10815	8519	9940	1241	12.49	4587	46.15	92.3	6089	61.26	81.68
8	Endosulphan-I	7866	7607	7675	7716	134	1.74	3985	51.65	103.3	4698	60.89	81.18
9	pp-DDE	7508	8636	6681	7608	981	12.90	3627	47.67	95.3	5214	68.53	91.37
10	Dieldrin	9298	9247	8979	9175	171	1.87	4398	47.94	95.9	5986	65.24	86.99
11	op-DDD	17877	18008	15252	17046	1555	9.12	7589	44.52	89.0	20154	118.24	157.65
12	Endosulphan-II	9165	9888	8233	9095	830	9.12	4256	46.79	93.6	10548	115.97	154.63
13	pp-DDD	6247	6436	5860	6181	294	4.75	3158	51.09	102.2	4965	80.33	107.10
14	op-DDT	3364	3644	3514	3507	140	4.00	1584	45.16	90.3	3658	104.29	139.06
15	Endosulphan Sulphate	4057	4218	4108	4128	82	1.99	2351	56.96	113.9	3698	89.59	119.46
16	pp-DDT	7560	8320	7965	7948	380	4.78	4058	51.06	102.1	6658	83.77	111.69

Table 17: Accuracy (recovery) of method validation of GC-ECD for detection and quantification of OCP residues in chicken eggs

Sl. No.	OC Pesticides	5 ppb Area	10ppb Area	20ppb Area	Linearity	Spike (10ppb)	Spl -1 %	Recovery-1 (%)	Spike (20ppb)	Spl-2 %	Recovery-2(%)
1	Alpha-BHC	259.2	501.3	1008.5	0.999	465.2	7.90	92.92	1056.2	10.47	104.73
2	Lindane	345.7	688.2	1256.7	0.991	592.1	8.60	86.04	1152.3	9.17	91.69
3	Beta-BHC	253.4	409.9	805.7	0.984	432.7	10.56	105.56	762.3	9.46	94.61
4	Delta-BHC	186.2	349.1	739.4	0.997	395.8	11.34	113.38	687.5	9.30	92.98
5	Alachlor	724.3	1388.2	3834.1	0.952	1283.2	9.24	92.44	3573.1	9.32	93.19
6	Aldrin	219.5	438.2	829.4	0.997	389.5	8.89	88.89	753.2	9.08	90.81
7	op-DDE	500.8	1059.3	2182.2	0.998	1234.2	11.65	116.51	1985.2	9.10	90.97
8	Endosulphan-I	300.8	685	1350.4	0.997	607.3	8.87	88.66	1156.2	8.56	85.62
9	pp-DDE	400.7	862.3	1756.2	0.998	825.3	9.57	95.71	1531.5	8.72	87.21
10	Dieldrin	509.5	1093.8	2065.1	0.997	989.2	9.04	90.44	1964.2	9.51	95.11
11	op-DDD	1056	2698	5295.1	0.992	2587.6	9.59	95.91	4862.1	91.88	91.82
12	Endosulphan-II	598.4	1062.3	2698.3	0.972	1055.2	9.93	99.33	2485.6	9.21	92.12
13	pp-DDD	305.8	705.2	1398.3	0.996	685.3	9.72	97.18	1245.8	8.91	89.09
14	op-DDT	150.9	299.8	623.5	0.998	318.6	10.63	106.27	589.2	9.45	94.50
15	Endosulphan Sulphate	150.6	293.1	623.7	0.997	286.2	9.76	97.65	597.2	9.58	95.75
16	pp-DDT	485.8	861.7	1724.8	0.996	824.6	9.57	95.69	1538.4	8.92	89.19

Table 18: Parameters of method validation of GC-ECD for detection and quantification of OCP residues in chicken eggs

Parameter	Observation	Validation Limits
System Suitability	Avg. RSD 14.2	<RSD 15.0%
Linearity	Avg. $R^2 = 0.960$	> $R^2 = 0.950$
LOD&LOQ	LOD-10ppb LOQ less than maximum residue limit	LOQ should be less than MRL (Maximum residue limit)
Method Precision	Avg RSD 7.57 %	< RSD 15.0%
Intermediate Precision	RSD 7.57 %	< RSD 15.0%
Accuracy	Avg $R^2 = 0.991$ Avg 86-113%	> $R^2 = 0.950$ Recovery between 70%-130%

Table 19: Gas chromatographic retention time and area for some organochlorine pesticides using electron capture detector in broiler meat, milk and chicken eggs

Pesticide	Retention time (min)	Area of the sample under curve
Alpha-BHC	8.43	2080.1
Lindane	10.87	1850.7
Beta-BHC	13.12	1679.5
Delta-BHC	15.28	703.9
Alachlor	17.86	12048.6
Aldrin	18.37	4385.5
op-DDE	18.59	8519.4
Endosulphan-I	19.02	7674.9
pp-DDE	20.19	6681.4
Dieldrin	20.96	8979.0
op-DDD	22.10	15252.4
Endosulphan-II	22.51	8288.3
pp-DDD	22.73	5859.7
op-DDT	22.98	3513.5
Endosulphan Sulphate	23.11	4107.9
pp-DDT	23.90	7964.7

Table 20: Residue concentration and frequency of some organochlorine pesticides detected in commercial broiler meat, milk and chicken eggs marketed in Bengaluru

Pesticide	Broiler meat (n=30)				Milk (n=30)				Eggs (n=30)			
	Frequency	%	Mean*	Range*	Frequency	%	Mean*	Range*	Frequency	%	Mean*	Range*
Alpha-BHC	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Lindane	ND	ND	ND	ND	1	3.33	86.60	86.60	ND	ND	ND	ND
Beta-BHC	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Delta-BHC	ND	ND	ND	ND	ND	ND	ND	ND	8	26.67	39.38	40.43 to 49.12
Alachlor	1	3.33	< LOD	< LOD	2	6.67	< LOD	ND	4	13.33	< LOD	< LOD
Aldrin	1	3.33	< LOD	< LOD	2	6.67	10.17	< LOD to 11.33	5	16.67	13.02	< LOD to 18.27
op-DDE	1	3.33	< LOD	< LOD	1	3.33	< LOD	< LOD	1	3.33	17.33	17.33
Endosulphan-I	2	6.67	< LOD	< LOD	5	16.67	13.79	< LOD to 24.61	ND	ND	ND	ND
pp-DDE	10	33.33	32.28	< LOD to 107.64	12	40	37.79	< LOD to 240.45	7	23.33	10.22	< LOD to 25.53
Dieldrin	1	3.33	22.84	22.84	3	10	13.64	< LOD to 21.40	5	16.67	17.25	< LOD to 47.67
op-DDD	ND	ND	ND	ND	3	10	116.70	47.01 to 152.29	1	3.33	94.26	94.26
Endosulphan-II	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
pp-DDD	1	3.33	< LOD	< LOD	1	3.33	17	17	1	3.33	24.05	24.05
op-DDT	ND	ND	ND	ND	ND	ND	ND	ND	1	3.33	26.02	26.02
Endosulphan Sulphate	1	3.33	< LOD	< LOD	ND	ND	ND	ND	2	6.67	21.66	< LOD to 39.71
pp-DDT	1	3.33	< LOD	< LOD	1	3.33	< LOD	< LOD	ND	ND	ND	ND

Note: ND- Not detected < LOD- Less than limit of detection ***-** values in ppb

Table 21: Residue concentration and frequency of some organochlorine pesticides detected in commercial broiler meat, milk and chicken eggs marketed in Shivamogga

Pesticide	Broiler meat (n=30)				Milk (n=30)				Eggs (n=30)			
	Frequency	%	Mean*	Range*	Frequency	%	Mean*	Range*	Frequency	%	Mean*	Range*
Alpha-BHC	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Lindane	ND	ND	ND	ND	3	10	98.04	56.33 to169.04	4	13.33	55	25.17 to 234.79
Beta-BHC	ND	ND	ND	ND	2	6.67	63.98	30.25 to 97.70	ND	ND	ND	ND
Delta-BHC	5	16.67	15.57	11 to 15.32	3	10	16.78	10.83 to 19.14	ND	ND	ND	ND
Alachlor	2	6.67	18.18	< LOD to 18.18	2	6.67	13.16	< LOD to 20.59	2	6.67	< LOD	< LOD to 11.26
Aldrin	3	10	< LOD	< LOD	3	10	15.91	11.89 to 20.00	1	3.33	< LOD	< LOD
op-DDE	ND	ND	ND	ND	2	6.67	< LOD	< LOD	1	3.33	< LOD	< LOD
Endosulphan-I	ND	ND	ND	ND	4	13.33	11.98	< LOD to 23.99	7	23.3	21.55	11.15 to 30.17
pp-DDE	9	30	84.22	< LOD to 239.06	12	40	46.66	< LOD to 129.04	11	36.67	94.31	33.81 to 205.68
Dieldrin	4	13.33	< LOD	< LOD	1	3.33	11.33	11.33	4	13.33	27.30	14.35 to 40.36
op-DDD	ND	ND	ND	ND	2	6.67	15.11	< LOD to 24.51	ND	ND	ND	ND
Endosulphan-II	ND	ND	ND	ND	ND	ND	ND	ND	3	10	45.17	17.78 to 67.82
pp-DDD	ND	ND	ND	ND	ND	ND	ND	ND	3	10	98.53	10.20 to 221.64
op-DDT	ND	ND	ND	ND	ND	ND	ND	ND	1	3.33	228.90	228.90
Endosulphan Sulphate	ND	ND	ND	ND	ND	ND	ND	ND	1	3.33	36.06	36.06
pp-DDT	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Note: ND- Not detected < LOD- Less than limit of detection ***-** values in ppb

Table 22: Residue concentration and frequency of some organochlorine pesticides detected in commercial broiler meat, milk and chicken eggs marketed in Mysuru

Pesticide	Broiler meat (n=30)				Milk (n=30)				Eggs (n=30)			
	Frequency	%	Mean*	Range*	Frequency	%	Mean*	Range*	Frequency	%	Mean*	Range*
Alpha-BHC	1	3.33	< LOD	< LOD	ND	ND	ND	ND	1	3.33	24.44	24.44
Lindane	ND	ND	ND	ND	1	3.33	45.37	45.37	1	3.33	10.95	10.95
Beta-BHC	ND	ND	ND	ND	3	10.00	33.94	16.82 – 43.34	ND	ND	ND	ND
Delta-BHC	ND	ND	ND	ND	ND	ND	ND	ND	1	3.33	15.23	15.23
Alachlor	1	3.33	< LOD	< LOD	ND	ND	ND	ND	ND	ND	ND	ND
Aldrin	1	3.33	11.33	11.33	ND	ND	ND	ND	ND	ND	ND	ND
op-DDE	2	6.67	< LOD	< LOD	2	6.67	< LOD	< LOD	4	13.33	< LOD	< LOD
Endosulphan-I	2	6.67	24.61	< LOD to 24.61	2	6.67	< LOD	< LOD	2	6.67	< LOD	< LOD
pp-DDE	ND	ND	ND	ND	4	13.33	< LOD	< LOD	ND	ND	ND	ND
Dieldrin	ND	ND	ND	ND	1	3.33	< LOD	< LOD	ND	ND	ND	ND
op-DDD	3	10	< LOD	< LOD	ND	ND	ND	ND	ND	ND	ND	ND
Endosulphan-II	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
pp-DDD	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
op-DDT	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Endosulphan Sulphate	1	3.33	< LOD	< LOD	ND	ND	ND	ND	ND	ND	ND	ND
pp-DDT	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Note: ND- Not detected < LOD- Less than limit of detection ***-** values in ppb

Table 23: Comparison of residues detected in broiler meat, milk and chicken egg samples collected from commercial outlets of Bengaluru with MRL values

Pesticide	MRL (ppb)	Broiler meat (ppb)	Milk (ppb)	Chicken eggs (ppb)
Alpha-BHC	200	ND	ND	ND
Lindane	200	ND	86.60	ND
Beta-BHC	200	ND	ND	ND
Delta- BHC	50	ND	ND	39.38
Alachlor	200	< LOD	< LOD	< LOD
Aldrin	200	< LOD	10.17	13.02
o.p-DDE	300	< LOD	< LOD	17.33
Endosulphan-I	50	< LOD	13.79	ND
pp-DDE	300	32.28	37.79	10.22
Dieldrin	200	22.84	13.64	17.25
o.p-DDD	300	< LOD	116.70	94.26
Endosulphan-II	50	< LOD	ND	ND
pp-DDD	300	ND	17.00	24.05
op-DDT	300	ND	< LOD	26.02
Endosulphan sulphate	50	< LOD	ND	21.66
pp-DDT	300	< LOD	ND	ND

Note: ND- Not detected < LOD- Less than limit of detection

Table 24: Comparison of residues detected in broiler meat, milk and chicken egg samples collected from commercial outlets of Shivamogga with MRL values

Pesticide	MRL (ppb)	Broiler meat (ppb)	Milk (ppb)	Chicken eggs (ppb)
Alpha-BHC	200	ND	ND	ND
Lindane	200	ND	98.04	55
Beta-BHC	200	ND	63.98	ND
Delta- BHC	50	157.57	196.78	ND
Alachlor	200	18.18	13.16	< LOD
Aldrin	200	< LOD	15.91	< LOD
o.p-DDE	300	ND	< LOD	< LOD
Endosulphan-I	50	ND	11.98	21.55
pp-DDE	300	84.22	46.66	94.31
Dieldrin	200	< LOD	11.33	27.30
o.p-DDD	300	ND	15.11	ND
Endosulphan-II	50	ND	98.04	45.17
pp-DDD	300	ND	63.98	98.53
op-DDT	300	ND	296.78	228.90
Endosulphan sulphate	50	ND	13.16	36.06
pp-DDT	300	ND	15.91	55

Note: ND- Not detected < LOD- Less than limit of detection

Table 25: Comparison of residues detected in broiler meat, milk and chicken egg samples collected from commercial outlets of Mysuru with MRL values

Pesticide	MRL (ppb)	Broiler meat (ppb)	Milk (ppb)	Chicken eggs (ppb)
Alpha-BHC	200	< LOD	ND	24.44
Lindane	200	ND	45.37	10.95
Beta-BHC	200	ND	33.94	ND
Delta- BHC	50	ND	ND	15.23
Alachlor	200	< LOD	ND	ND
Aldrin	200	11.33	ND	ND
o.p-DDE	300	< LOD	< LOD	< LOD
Endosulphan-I	50	24.61	< LOD	< LOD
pp-DDE	300	ND	< LOD	ND
Dieldrin	200	ND	< LOD	ND
o.p-DDD	300	< LOD	ND	ND
Endosulphan-II	50	ND	ND	ND
pp-DDD	300	ND	ND	ND
op-DDT	300	ND	ND	ND
Endosulphan sulphate	50	< LOD	ND	ND
pp-DDT	300	ND	ND	ND

Note: ND- Not detected < LOD- Less than limit of detection

Fig. 1: Standard calibration curve for linearity of alpha-BHC in broiler meat

Name of the Pesticide : Alpha-BHC	
Dilution in ppb	**Area**
10	481
20	1069
30	1338
40	2011
50	2541

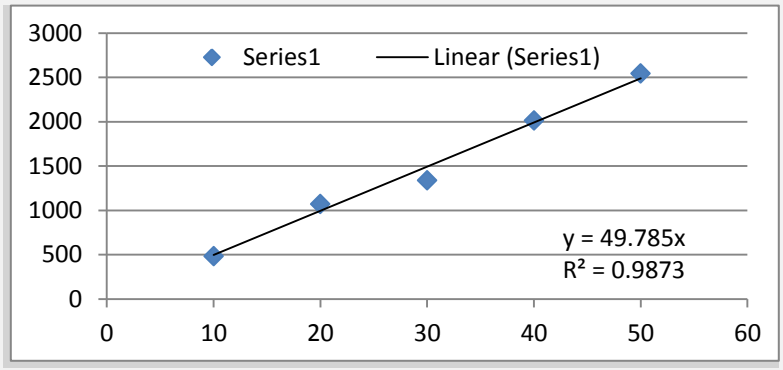


Fig. 2: Standard calibration curve for linearity of lindane in broiler meat

Name of the Pesticide : Lindane	
Dilution in ppb	**Area**
10	648
20	1107
30	1459
40	1846
50	2361

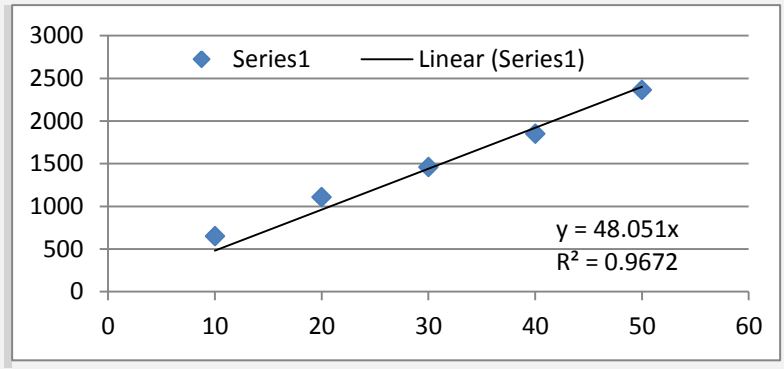


Fig. 3: Standard calibration curve for linearity of beta- BHC in broiler meat

Name of the Pesticide : beta-BHC	
Dilution in ppb	**Area**
10	452
20	931
30	1082
40	1602
50	2206

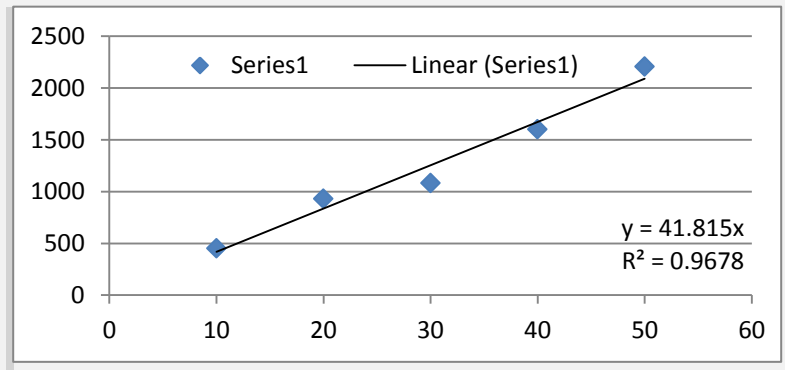


Fig. 4: Standard calibration curve for linearity of delta- BHC in broiler meat

Name of the Pesticide : delta-BHC	
Dilution in ppb	**Area**
10	321
20	844
30	1275
40	1596
50	1935

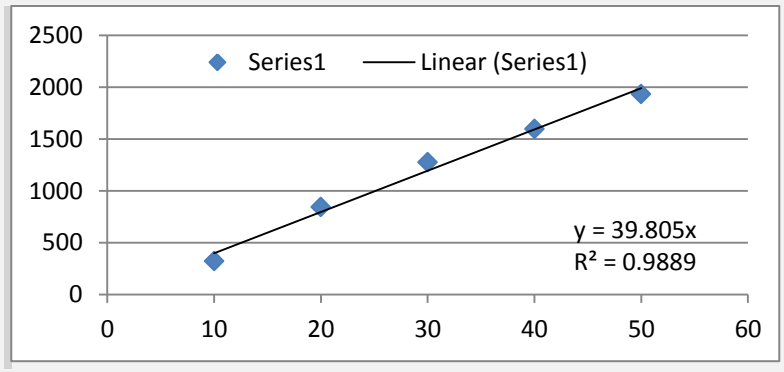


Fig. 5: Standard calibration curve for linearity of Alachlor in broiler meat

Name of the Pesticide : Alachlor	
Dilution in ppb	**Area**
10	1423
20	3379
30	6126
40	8254
50	9748

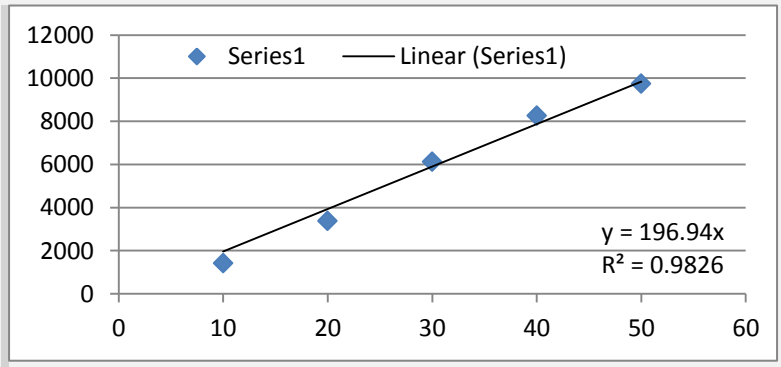


Fig. 6: Standard calibration curve for linearity of Aldrin in broiler meat

Name of the Pesticide : Aldrin	
Dilution in ppb	**Area**
10	450
20	986
30	2042
40	2967
50	3919

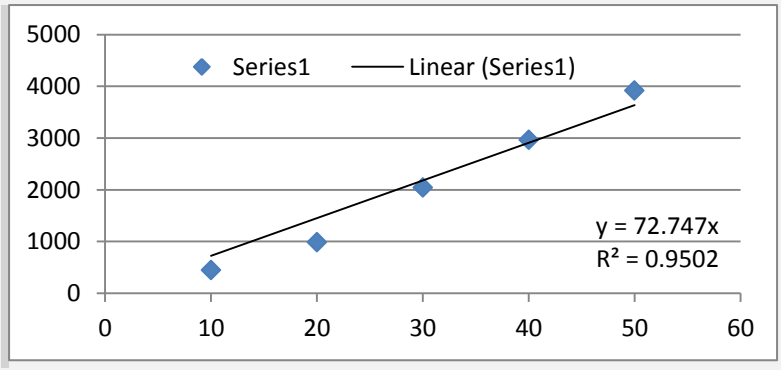


Fig. 7: Standard calibration curve for linearity of op-DDE in broiler meat

Name of the Pesticide : op-DDE	
Dilution in ppb	**Area**
10	1021
20	2122
30	4435
40	6203
50	7919

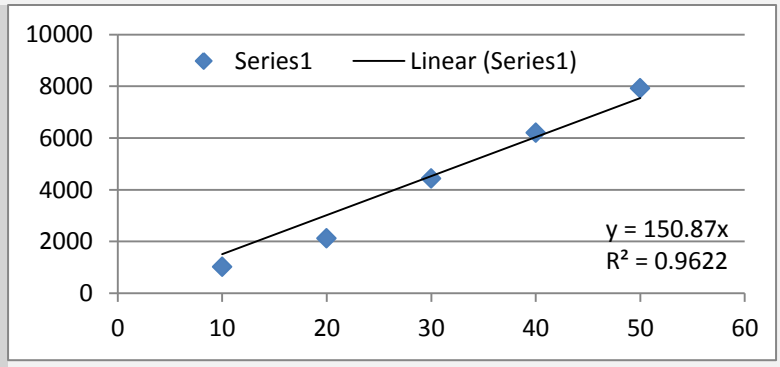


Fig. 8: Standard calibration curve for linearity of Endosulphan-I in broiler meat

Name of the Pesticide : Endosulphane-I	
Dilution in ppb	**Area**
10	653
20	1525
30	3401
40	4160
50	4926

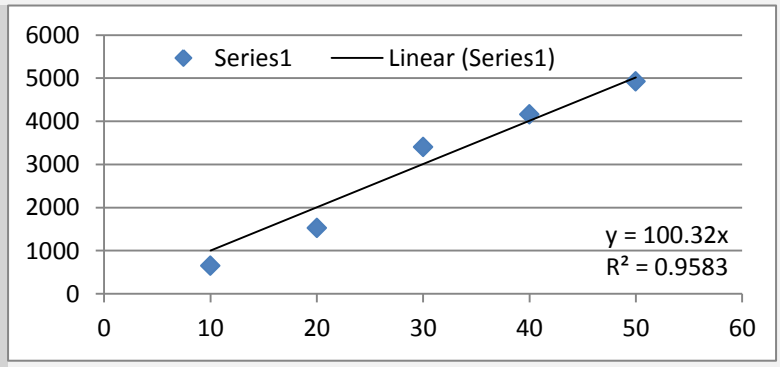


Fig. 9: Standard calibration curve for linearity of pp-DDE in broiler meat

Name of the Pesticide : pp-DDE	
Dilution in ppb	**Area**
10	853
20	1645
30	3265
40	4333
50	5682

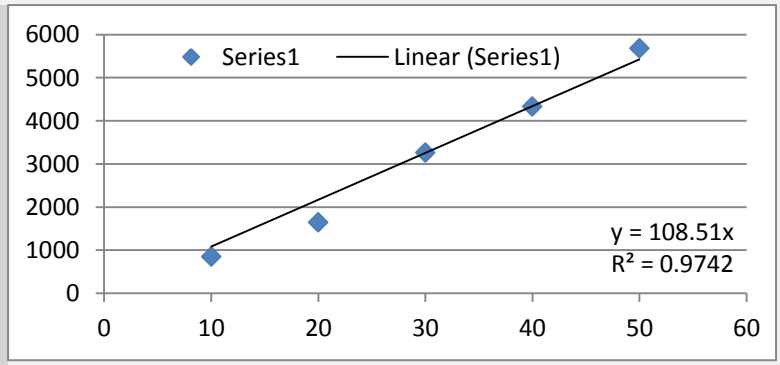


Fig. 10: Standard calibration curve for linearity of Dieldrin in broiler meat

Name of the Pesticide : Dieldrin	
Dilution in ppb	**Area**
10	1153
20	2297
30	4112
40	5935
50	6778

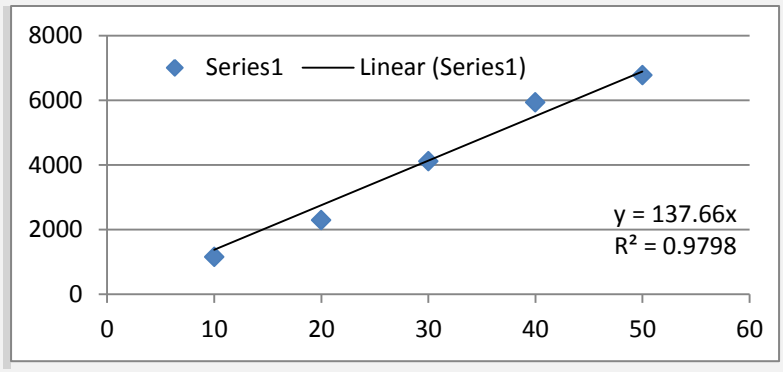


Fig. 11: Standard calibration curve for linearity of op-DDD in broiler meat

Name of the Pesticide : op-DDD	
Dilution in ppb	**Area**
10	2329
20	4217
30	7764
40	11261
50	13144

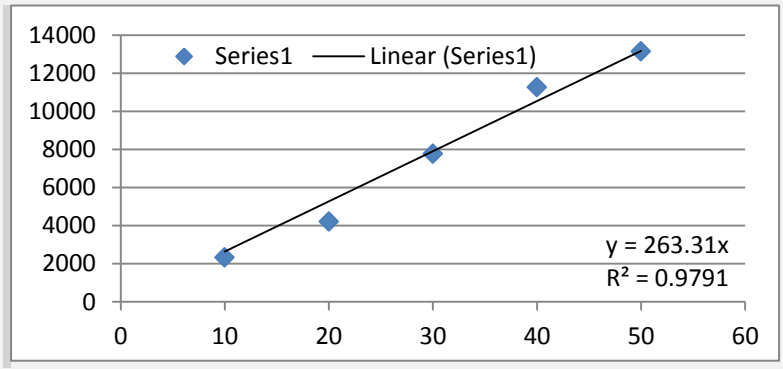


Fig. 12: Standard calibration curve for linearity of Endosulphan-II in broiler meat

Name of the Pesticide : Endosulphan-II	
Dilution in ppb	**Area**
10	1129
20	2080
30	3724
40	5766
50	7390

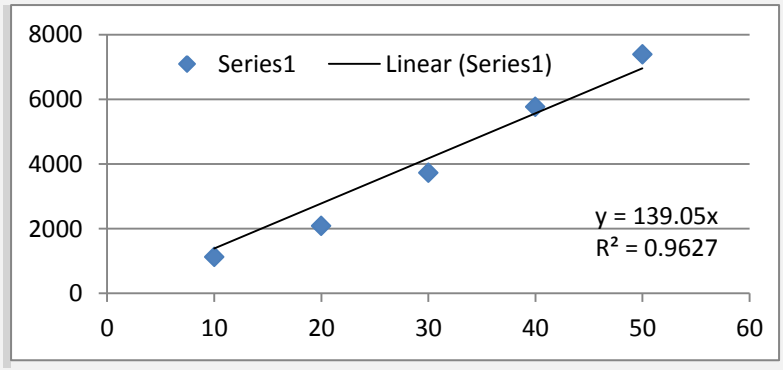


Fig. 13: Standard calibration curve for linearity of pp-DDD in broiler meat

Name of the Pesticide : pp-DDD	
Dilution in ppb	**Area**
10	728
20	1401
30	2567
40	3951
50	4486

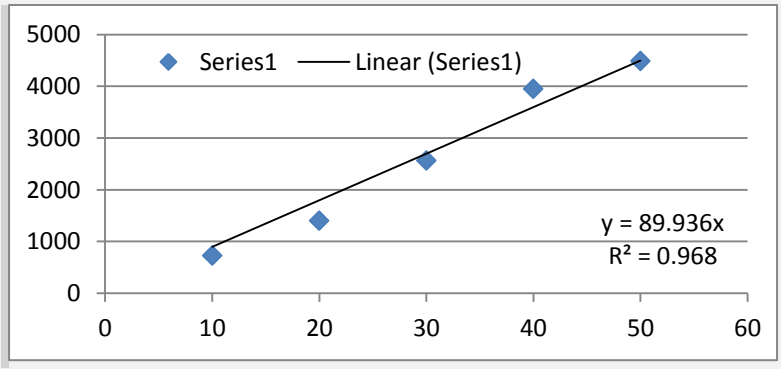


Fig. 14: Standard calibration curve for linearity of op-DDT in broiler meat

Name of the Pesticide : op-DDT	
Dilution in ppb	**Area**
10	321
20	749
30	1302
40	1762
50	2184

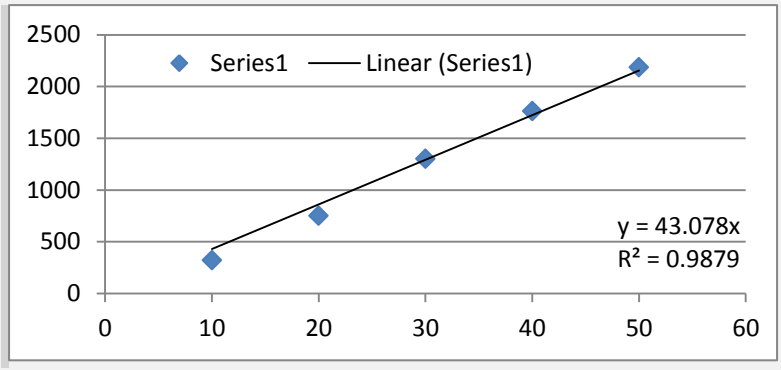


Fig. 15: Standard calibration curve for linearity of Endosulphan Sulphate in broiler meat

Name of the Pesticide : Endosulphan Sulphate	
Dilution in ppb	**Area**
10	357
20	820
30	1537
40	2451
50	2815

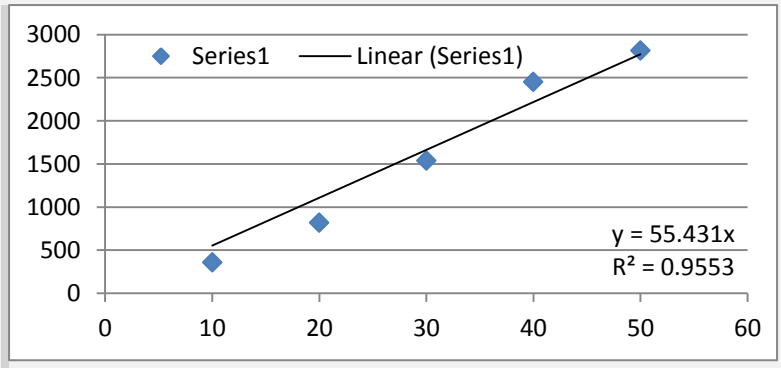


Fig. 16: Standard calibration curve for linearity of pp-DDT in broiler meat

Name of the Pesticide : pp-DDT	
Dilution in ppb	**Area**
10	853
20	1675
30	3062
40	4392
50	5566

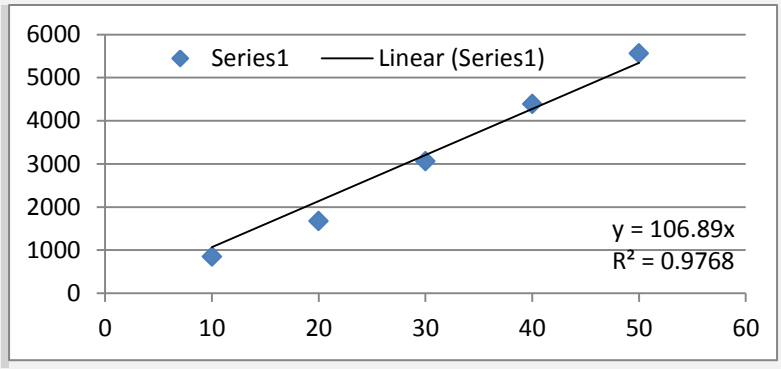


Fig. 17: Standard calibration curve for linearity of alpha-BHC in milk

Name of the Pesticide : Alpha-BHC	
Dilution in ppb	**Area**
10	481
20	1069
30	1338
40	2011
50	2541

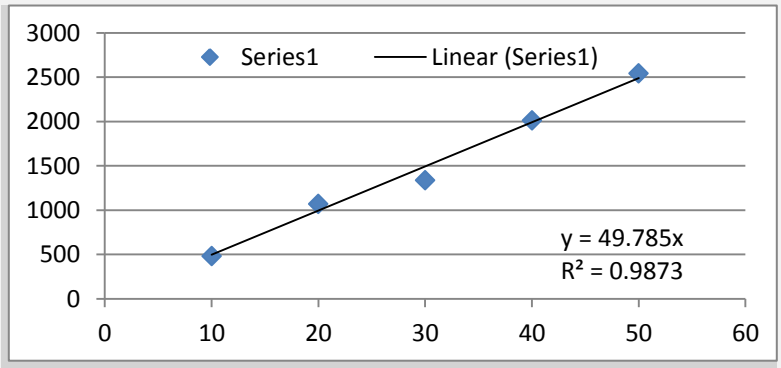


Fig. 18: Standard calibration curve for linearity of lindane in milk

Name of the Pesticide : Lindane	
Dilution in ppb	**Area**
10	648
20	1107
30	1459
40	1846
50	2361

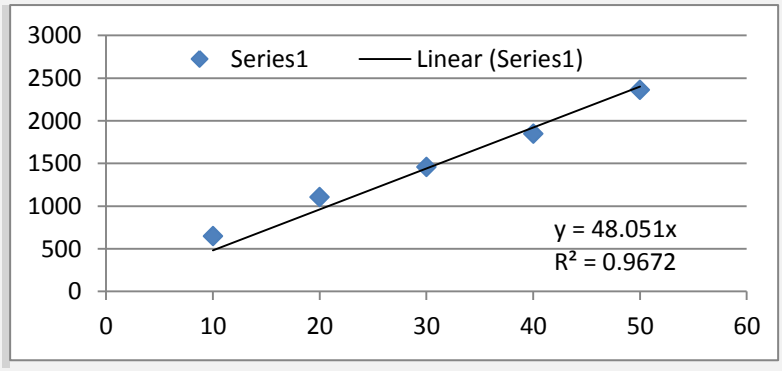


Fig. 19: Standard calibration curve for linearity of beta- BHC in milk

Name of the Pesticide : beta-BHC	
Dilution in ppb	**Area**
10	452
20	931
30	1082
40	1602
50	2206

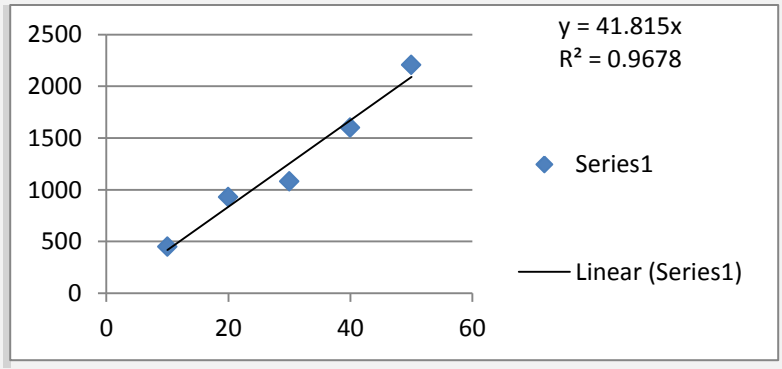


Fig. 20: Standard calibration curve for linearity of delta- BHC in milk

Name of the Pesticide : delta-BHC	
Dilution in ppb	**Area**
10	321
20	844
30	1275
40	1596
50	1935

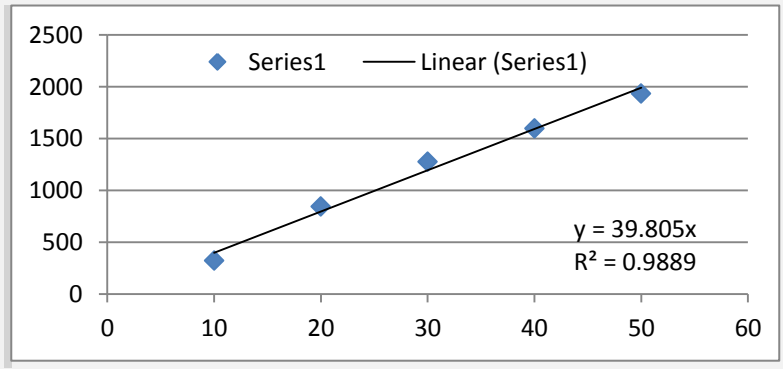


Fig. 21: Standard calibration curve for linearity of Alachlor in milk

Name of the Pesticide : Alachlor	
Dilution in ppb	**Area**
10	1423
20	3379
30	6126
40	8254
50	9748

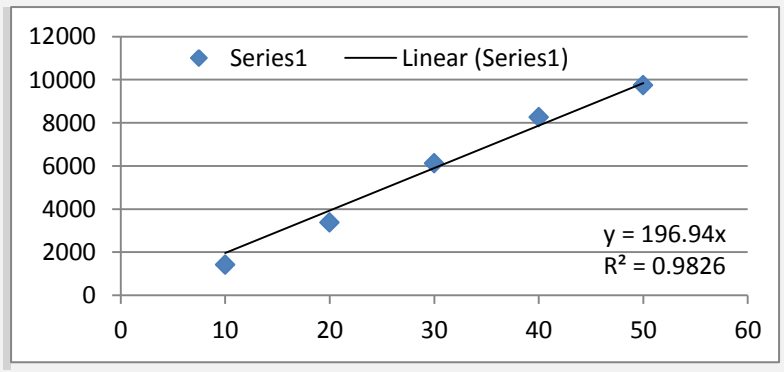


Fig. 22: Standard calibration curve for linearity of Aldrin in milk

Name of the Pesticide : Aldrin	
Dilution in ppb	**Area**
10	450
20	986
30	2042
40	2967
50	3919

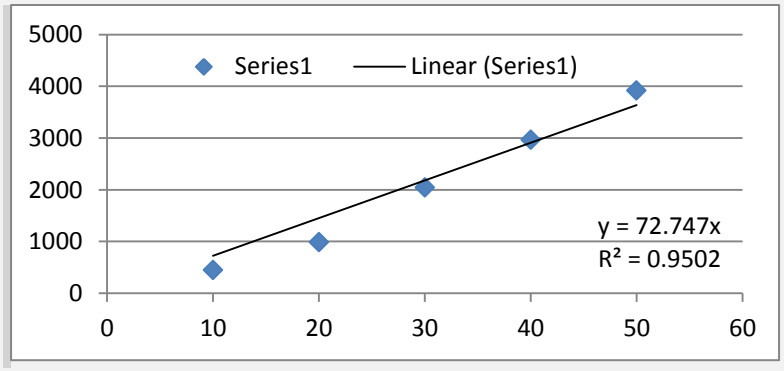


Fig. 23: Standard calibration curve for linearity of op-DDE in milk

Name of the Pesticide : op-DDE	
Dilution in ppb	**Area**
10	1021
20	2122
30	4435
40	6203
50	7919

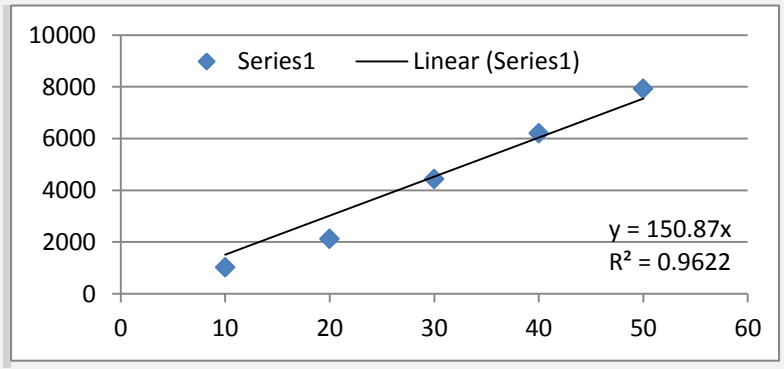


Fig. 24: Standard calibration curve for linearity of Endosulphan-I in milk

Name of the Pesticide : Endosulphan-I	
Dilution in ppb	**Area**
10	653
20	1525
30	3401
40	4160
50	4926

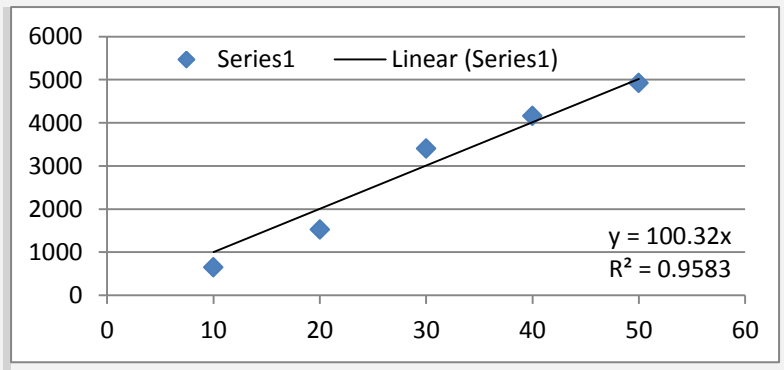


Fig. 25: Standard calibration curve for linearity of pp-DDE in milk

Name of the Pesticide : pp-DDE

Dilution in ppb	Area
10	853
20	1645
30	3265
40	4333
50	5682

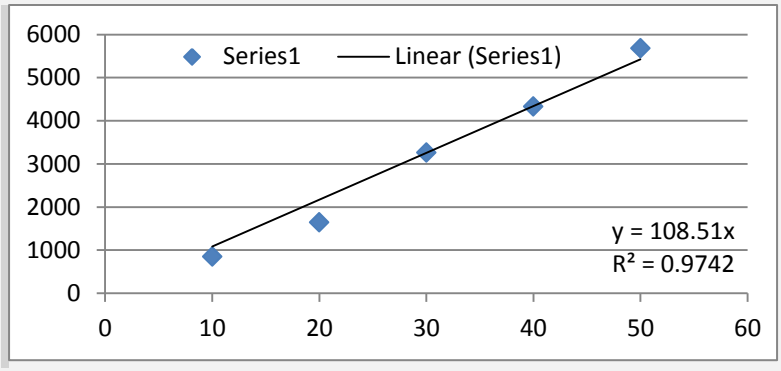


Fig. 26: Standard calibration curve for linearity of Dieldrin in milk

Name of the Pesticide : Dieldrin

Dilution in ppb	Area
10	1153
20	2297
30	4112
40	5935
50	6778

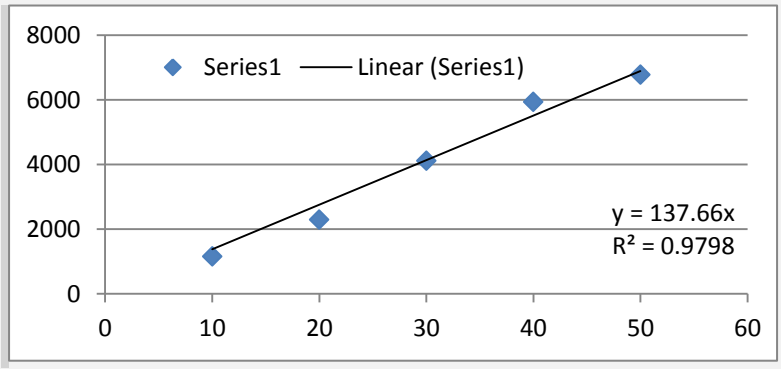


Fig. 27: Standard calibration curve for linearity of op-DDD in milk

Name of the Pesticide : op-DDD

Dilution in ppb	Area
10	2329
20	4217
30	7764
40	11261
50	13144

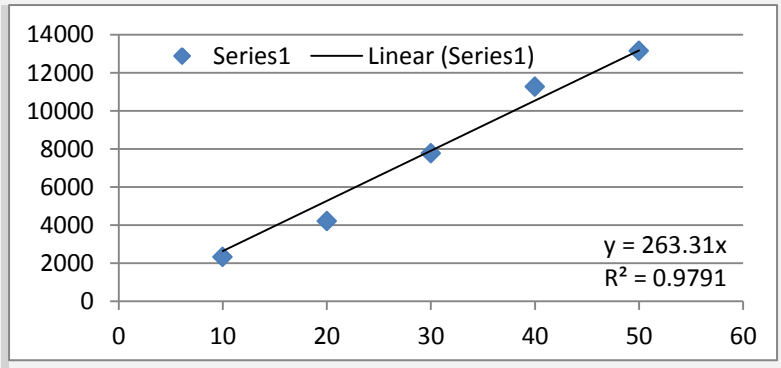


Fig. 28: Standard calibration curve for linearity of Endosulphan-II in milk

Name of the Pesticide : Endosulphan-II	
Dilution in ppb	**Area**
10	1129
20	2080
30	3724
40	5766
50	7390

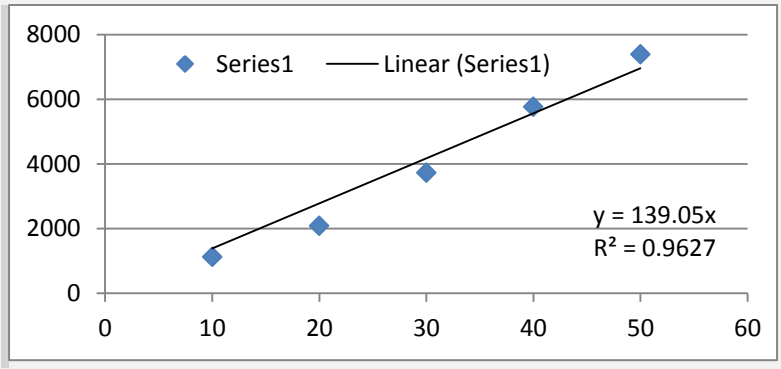


Fig. 29: Standard calibration curve for linearity of pp-DDD in milk

Name of the Pesticide : pp-DDD	
Dilution in ppb	**Area**
10	728
20	1401
30	2567
40	3951
50	4486

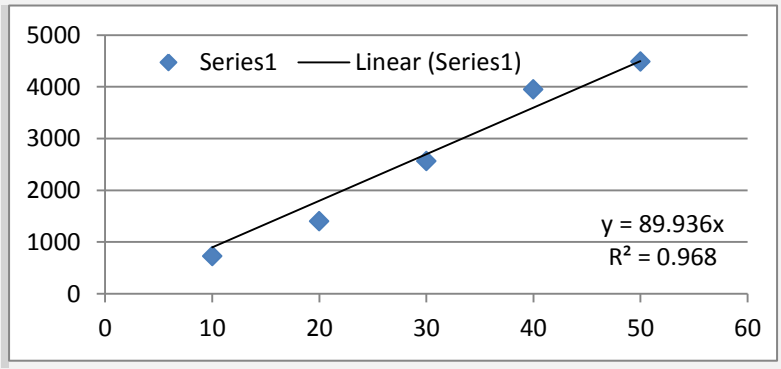


Fig. 30: Standard calibration curve for linearity of op-DDT in milk

Name of the Pesticide : op-DDT	
Dilution in ppb	**Area**
10	321
20	749
30	1302
40	1762
50	2184

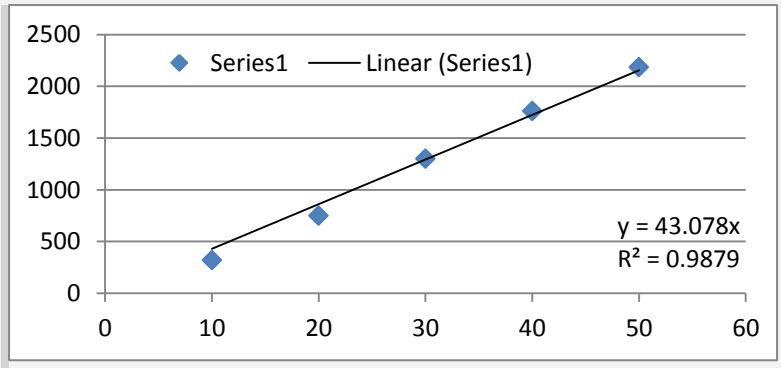


Fig. 31: Standard calibration curve for linearity of Endosulphan Sulphate in milk

Name of the Pesticide : Endosulphan Sulphate	
Dilution in ppb	**Area**
10	357
20	820
30	1537
40	2451
50	2815

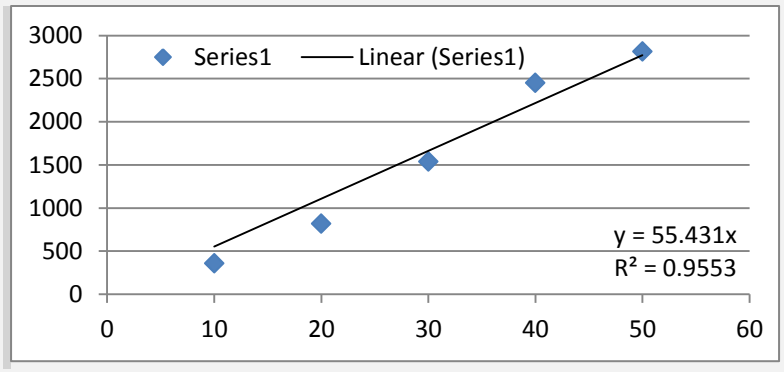


Fig. 32: Standard calibration curve for linearity of pp-DDT in milk

Name of the Pesticide : pp-DDT	
Dilution in ppb	**Area**
10	853
20	1675
30	3062
40	4392
50	5566

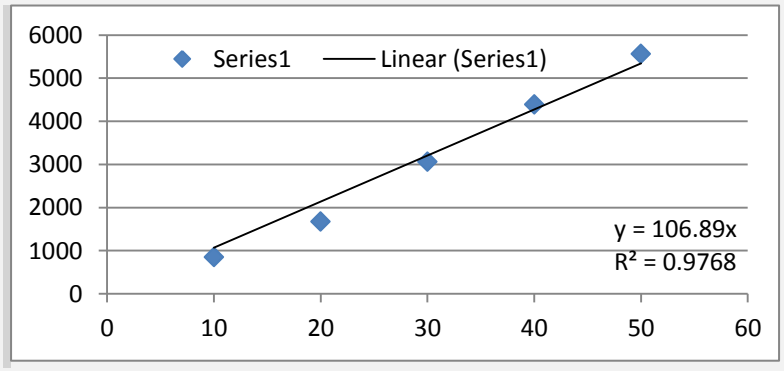


Fig. 33: Standard calibration curve for linearity of alpha-BHC in chicken eggs

Name of the Pesticide : Alpha-BHC	
Dilution in ppb	**Area**
10	481
20	1069
30	1338
40	2011
50	2541

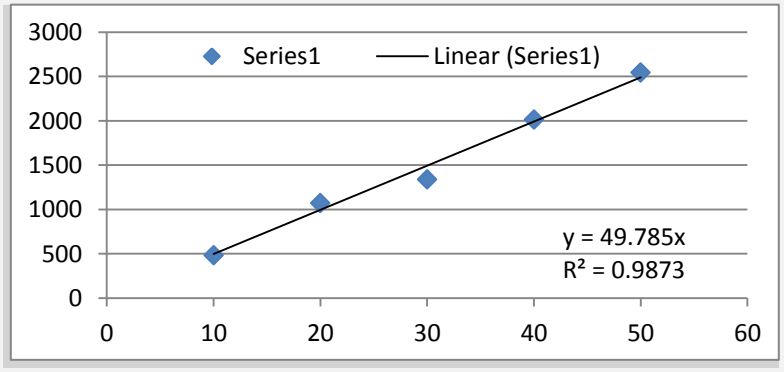


Fig. 34: Standard calibration curve for linearity of lindane in chicken eggs

Name of the Pesticide : Lindane	
Dilution in ppb	**Area**
10	648
20	1107
30	1459
40	1846
50	2361

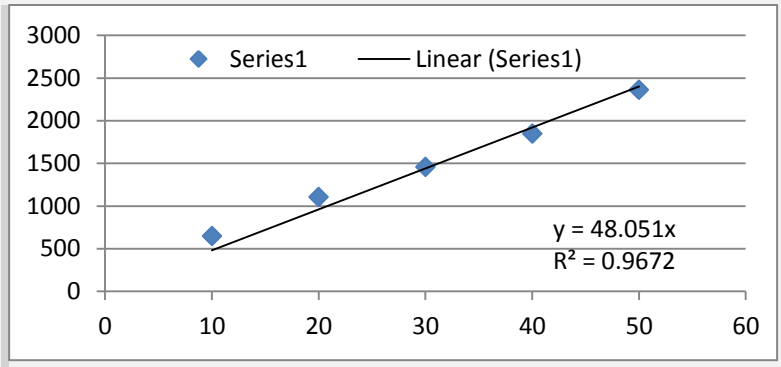


Fig. 35: Standard calibration curve for linearity of beta- BHC in chicken eggs

Name of the Pesticide : beta-BHC	
Dilution in ppb	**Area**
10	452
20	931
30	1082
40	1602
50	2206

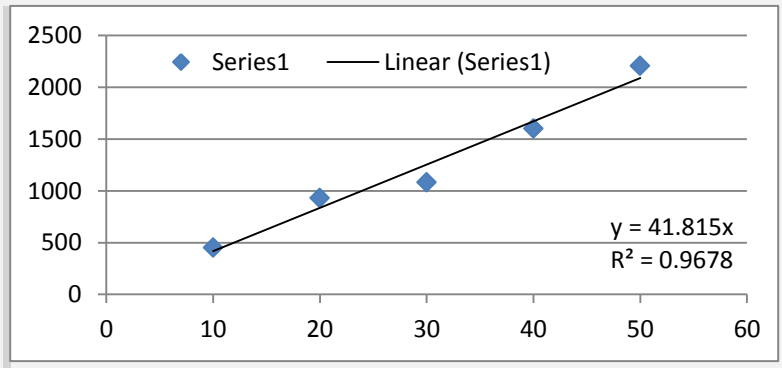


Fig. 36: Standard calibration curve for linearity of delta- BHC in chicken eggs

Name of the Pesticide : delta-BHC	
Dilution in ppb	**Area**
10	321
20	844
30	1275
40	1596
50	1935

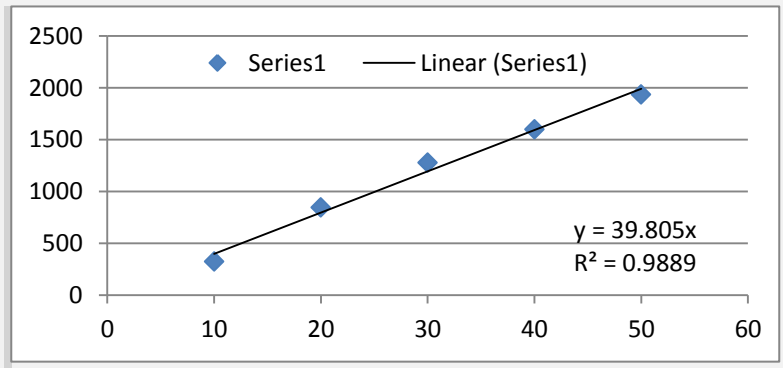


Fig. 37: Standard calibration curve for linearity of Alachlor in chicken eggs

Name of the Pesticide : Alachlor	
Dilution in ppb	**Area**
10	1423
20	3379
30	6126
40	8254
50	9748

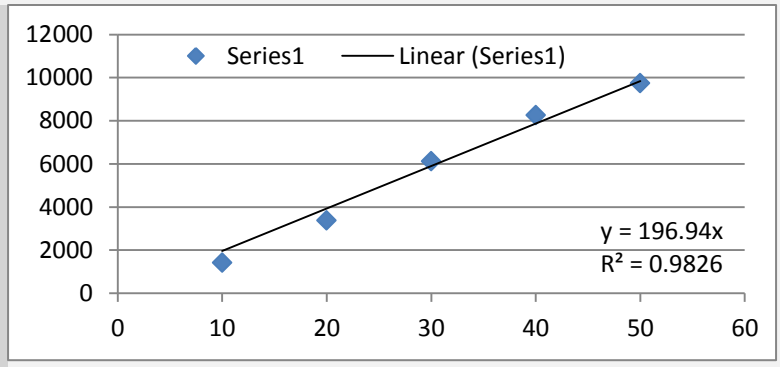


Fig. 38: Standard calibration curve for linearity of Aldrin in chicken eggs

Name of the Pesticide : Aldrin	
Dilution in ppb	**Area**
10	450
20	986
30	2042
40	2967
50	3919

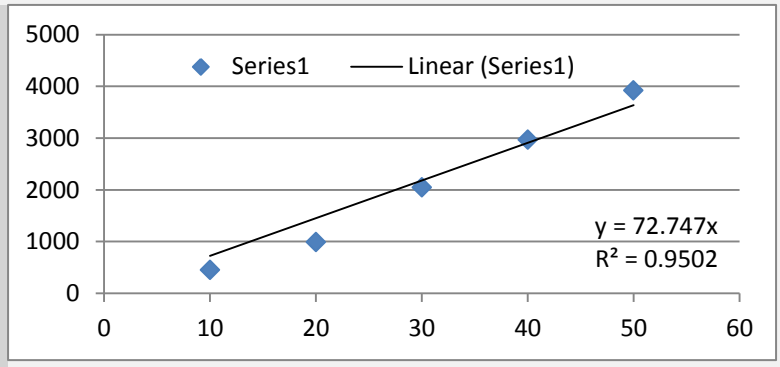


Fig. 39: Standard calibration curve for linearity of op-DDE in chicken eggs

Name of the Pesticide : op-DDE	
Dilution in ppb	**Area**
10	1021
20	2122
30	4435
40	6203
50	7919

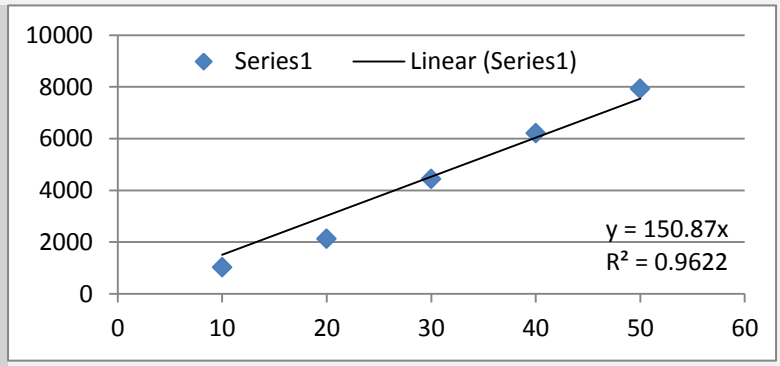


Fig. 40: Standard calibration curve for linearity of Endosulphan-I in chicken eggs

Name of the Pesticide : Endosulphan-I	
Dilution in ppb	**Area**
10	653
20	1525
30	3401
40	4160
50	4926

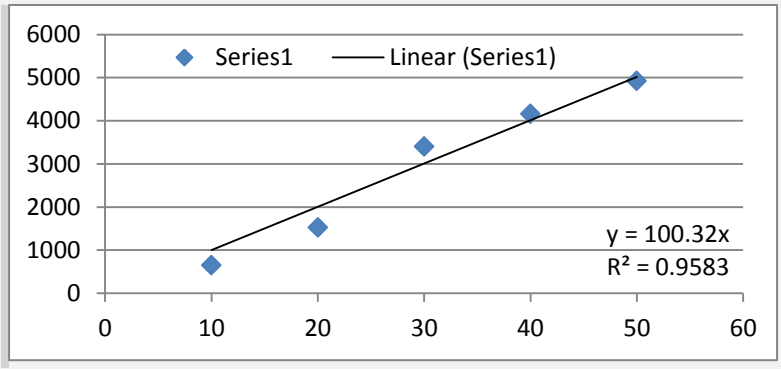


Fig. 41: Standard calibration curve for linearity of pp-DDE in chicken eggs

Name of the Pesticide : pp-DDE	
Dilution in ppb	**Area**
10	853
20	1645
30	3265
40	4333
50	5682

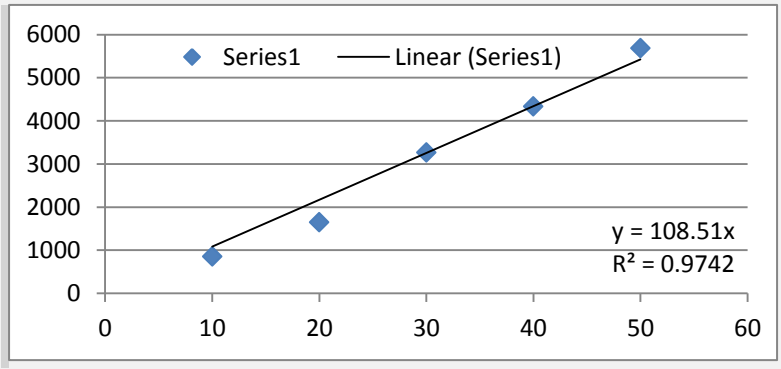


Fig. 42: Standard calibration curve for linearity of Dieldrin in chicken eggs

Name of the Pesticide : Dieldrin	
Dilution in ppb	**Area**
10	1153
20	2297
30	4112
40	5935
50	6778

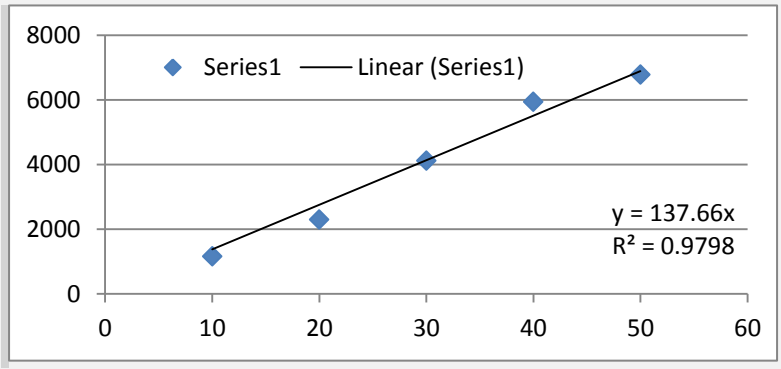


Fig. 43: Standard calibration curve for linearity of op-DDD in chicken eggs

Name of the Pesticide : op-DDD	
Dilution in ppb	**Area**
10	2329
20	4217
30	7764
40	11261
50	13144

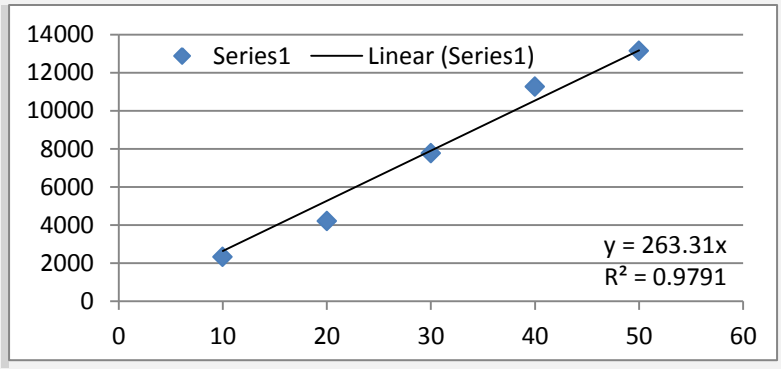


Fig. 44: Standard calibration curve for linearity of Endosulphan-II in chicken eggs

Name of the Pesticide : Endosulphan-II	
Dilution in ppb	**Area**
10	1129
20	2080
30	3724
40	5766
50	7390

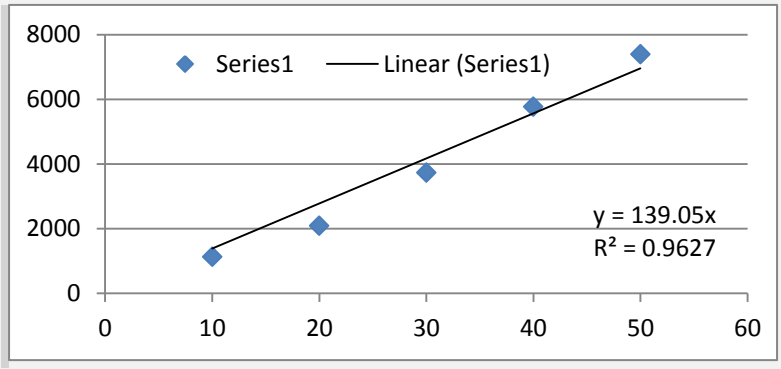


Fig. 45: Standard calibration curve for linearity of pp-DDD in chicken eggs

Name of the Pesticide : pp-DDD	
Dilution in ppb	**Area**
10	728
20	1401
30	2567
40	3951
50	4486

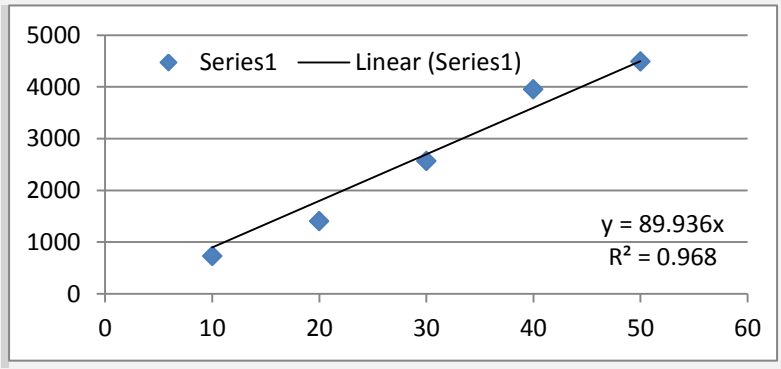


Fig. 46: Standard calibration curve for linearity of op-DDT in chicken eggs

Name of the Pesticide : op-DDT	
Dilution in ppb	**Area**
10	321
20	749
30	1302
40	1762
50	2184

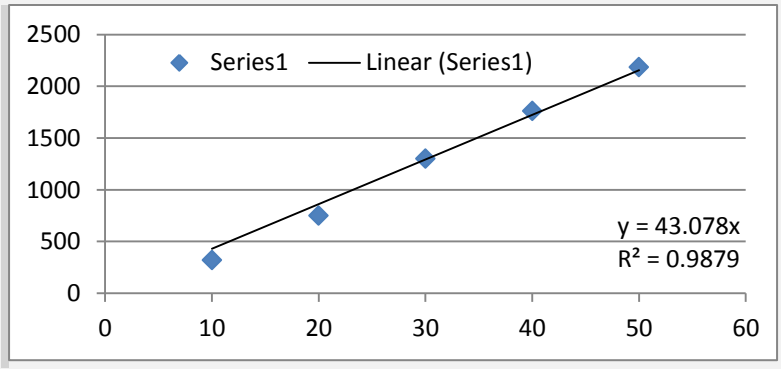


Fig. 47: Standard calibration curve for linearity of Endosulphan Sulphate in chicken eggs

Name of the Pesticide : Endosulphan Sulphate	
Dilution in ppb	**Area**
10	357
20	820
30	1537
40	2451
50	2815

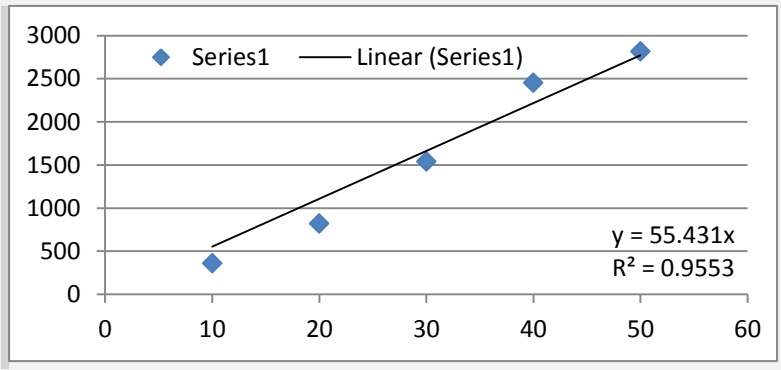


Fig. 48: Standard calibration curve for linearity of pp-DDT in chicken eggs

Name of the Pesticide : pp-DDT	
Dilution in ppb	**Area**
10	853
20	1675
30	3062
40	4392
50	5566

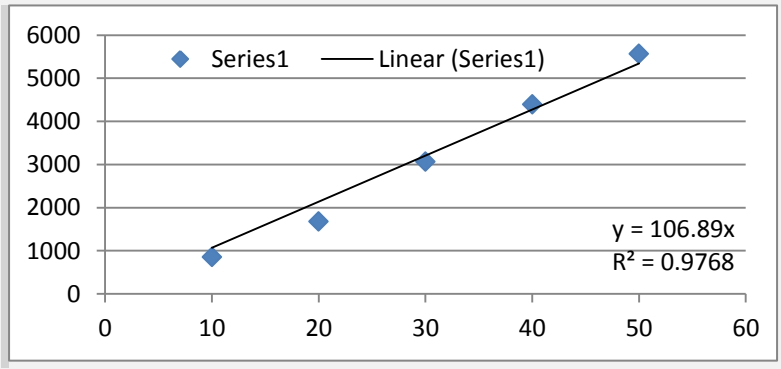
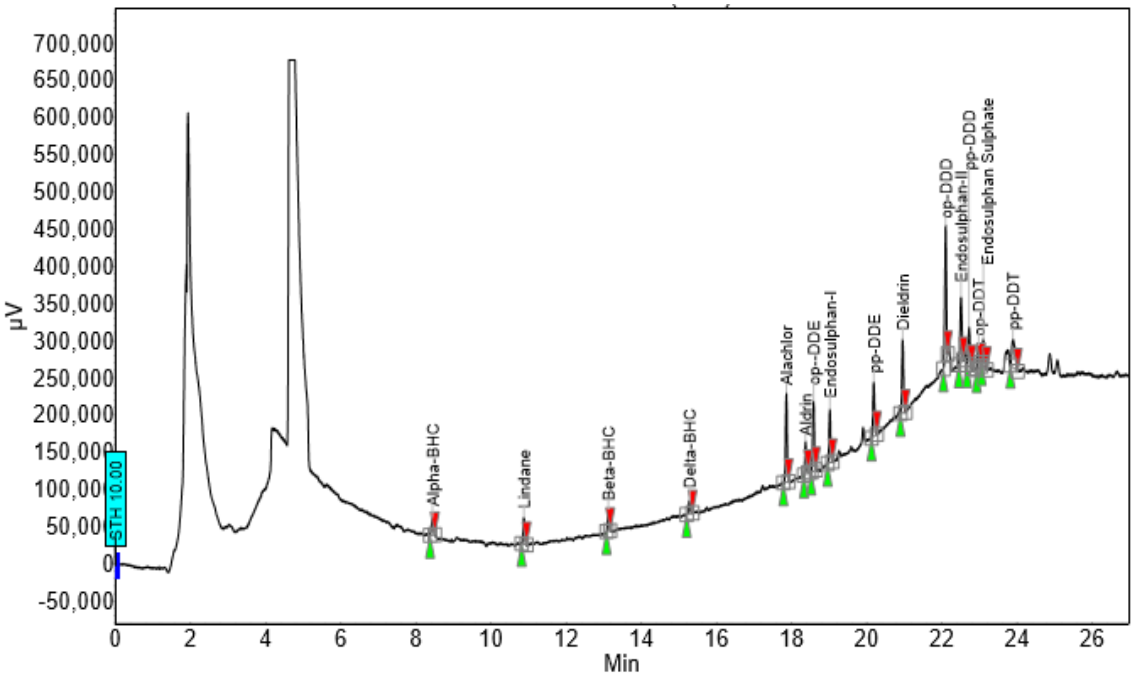
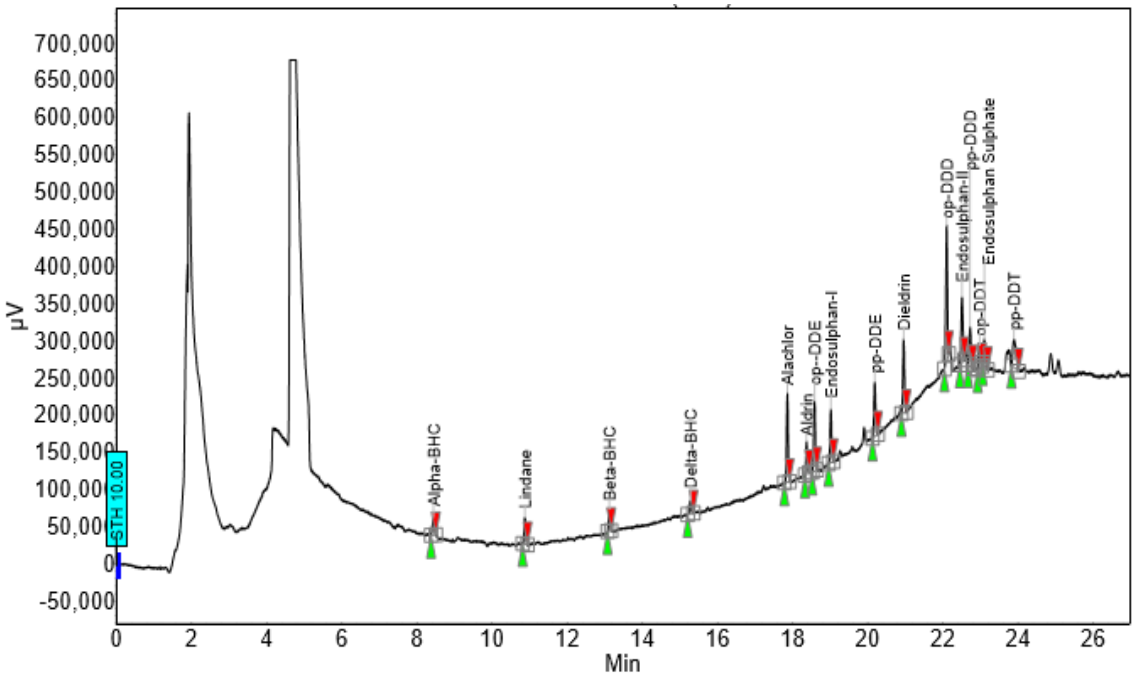


Fig. 49: Standard chromatogram of some organochlorine pesticides after method validation in broiler meat



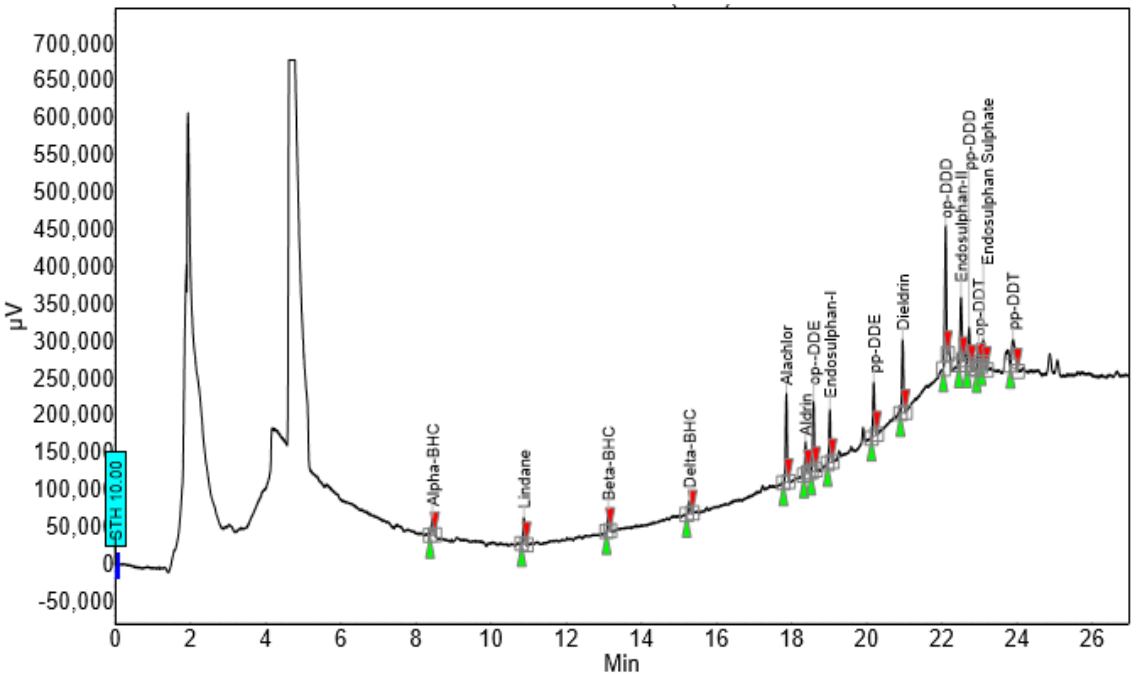
Index	Name	Time [Min]	Quantity [% Area]	Height [µV]	Area [µV.Min]	Area % [%]
1	Alpha-BHC	8.44	2.66	23909.2	1439.7	2.656
2	Lindane	10.87	3.29	35704.0	1784.1	3.291
3	Beta-BHC	13.12	2.61	29248.4	1412.3	2.605
4	Delta-BHC	15.28	1.68	18137.9	909.5	1.678
5	Alachlor	17.86	10.53	120337.4	5709.3	10.532
6	Aldrin	18.37	3.76	44063.6	2040.4	3.764
7	op--DDE	18.58	8.20	94289.4	4447.8	8.205
8	Endosulphan-I	19.02	6.14	71934.1	3328.1	6.139
9	pp-DDE	20.18	7.04	73746.4	3814.9	7.037
10	Dieldrin	20.95	9.29	98949.5	5035.7	9.289
11	op-DDD	22.10	16.52	182483.7	8954.1	16.518
12	Endosulphan-II	22.51	8.96	88181.0	4858.2	8.962
13	pp-DDD	22.72	5.42	53390.3	2936.6	5.417
14	op-DDT	22.98	3.08	30442.1	1670.1	3.081
15	Endosulphan Sulphate	23.10	3.62	33568.5	1961.4	3.618
16	pp-DDT	23.89	7.21	38236.0	3907.4	7.208

Fig. 50: Standard chromatogram of some organochlorine pesticides after method validation in milk



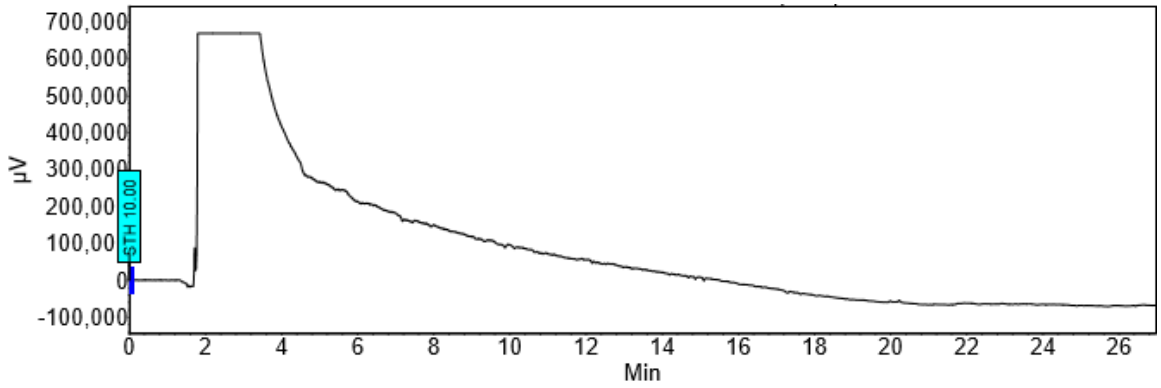
Index	Name	Time [Min]	Quantity [% Area]	Height [µV]	Area [µV.Min]	Area % [%]
1	Alpha-BHC	8.44	2.66	23909.2	1439.7	2.656
2	Lindane	10.87	3.29	35704.0	1784.1	3.291
3	Beta-BHC	13.12	2.61	29248.4	1412.3	2.605
4	Delta-BHC	15.28	1.68	18137.9	909.5	1.678
5	Alachlor	17.86	10.53	120337.4	5709.3	10.532
6	Aldrin	18.37	3.76	44063.6	2040.4	3.764
7	op--DDE	18.58	8.20	94289.4	4447.8	8.205
8	Endosulphan-I	19.02	6.14	71934.1	3328.1	6.139
9	pp-DDE	20.18	7.04	73746.4	3814.9	7.037
10	Dieldrin	20.95	9.29	98949.5	5035.7	9.289
11	op-DDD	22.10	16.52	182483.7	8954.1	16.518
12	Endosulphan-II	22.51	8.96	88181.0	4858.2	8.962
13	pp-DDD	22.72	5.42	53390.3	2936.6	5.417
14	op-DDT	22.98	3.08	30442.1	1670.1	3.081
15	Endosulphan Sulphate	23.10	3.62	33568.5	1961.4	3.618
16	pp-DDT	23.89	7.21	38236.0	3907.4	7.208

Fig. 51: Standard chromatogram of some organochlorine pesticides after method validation in chicken eggs



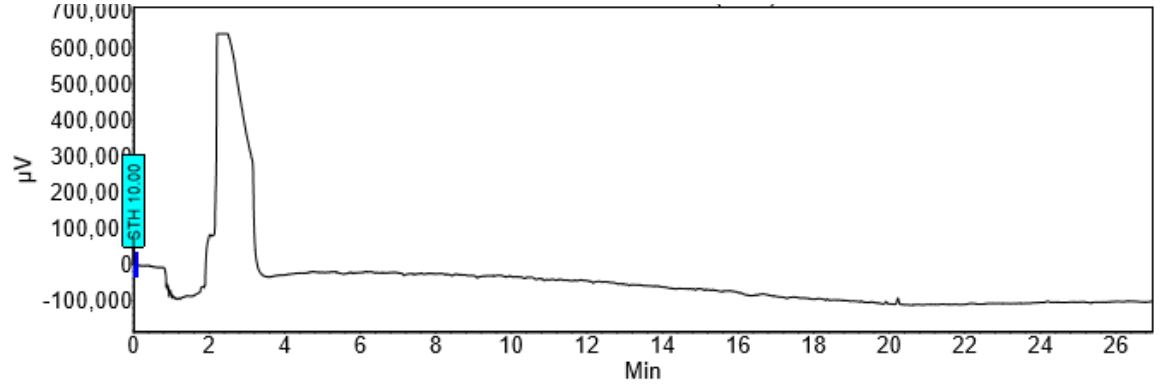
Index	Name	Time [Min]	Quantity [% Area]	Height [µV]	Area [µV.Min]	Area % [%]
1	Alpha-BHC	8.44	2.66	23909.2	1439.7	2.656
2	Lindane	10.87	3.29	35704.0	1784.1	3.291
3	Beta-BHC	13.12	2.61	29248.4	1412.3	2.605
4	Delta-BHC	15.28	1.68	18137.9	909.5	1.678
5	Alachlor	17.86	10.53	120337.4	5709.3	10.532
6	Aldrin	18.37	3.76	44063.6	2040.4	3.764
7	op--DDE	18.58	8.20	94289.4	4447.8	8.205
8	Endosulphan-I	19.02	6.14	71934.1	3328.1	6.139
9	pp-DDE	20.18	7.04	73746.4	3814.9	7.037
10	Dieldrin	20.95	9.29	98949.5	5035.7	9.289
11	op-DDD	22.10	16.52	182483.7	8954.1	16.518
12	Endosulphan-II	22.51	8.96	88181.0	4858.2	8.962
13	pp-DDD	22.72	5.42	53390.3	2936.6	5.417
14	op-DDT	22.98	3.08	30442.1	1670.1	3.081
15	Endosulphan Sulphate	23.10	3.62	33568.5	1961.4	3.618
16	pp-DDT	23.89	7.21	38236.0	3907.4	7.208

Fig. 52: Chromatogram of extracted blank broiler meat sample



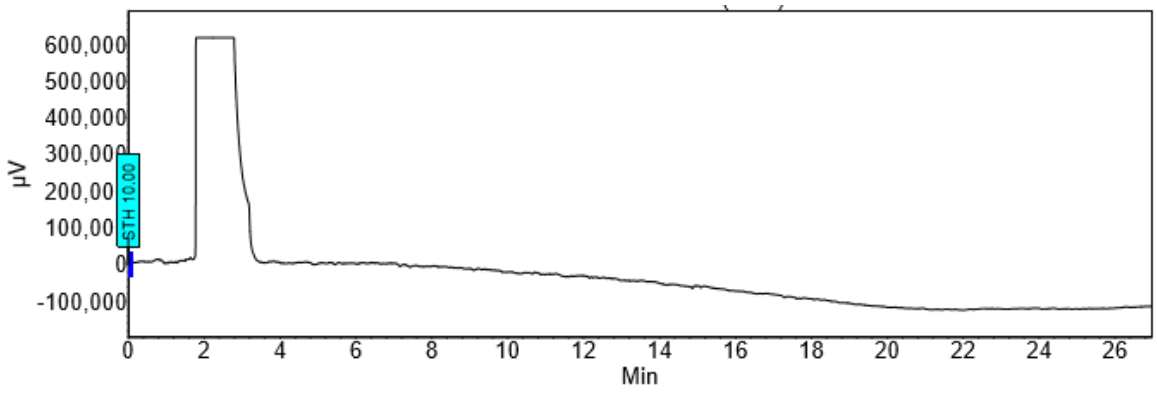
Index	Name	Time [Min]	Quantity [% Area]	Height [µV]	Area [µV.Min]	Area % [%]
Total			0.00	0.0	0.0	0.000

Fig. 53: Chromatogram of extracted blank milk sample



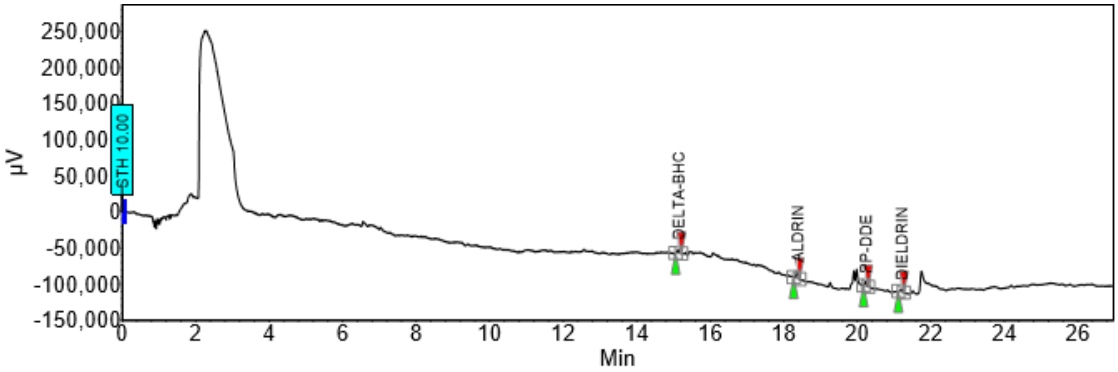
Index	Name	Time [Min]	Quantity [% Area]	Height [µV]	Area [µV.Min]	Area % [%]
Total			0.00	0.0	0.0	0.000

Fig. 54: Chromatogram of extracted blank egg sample

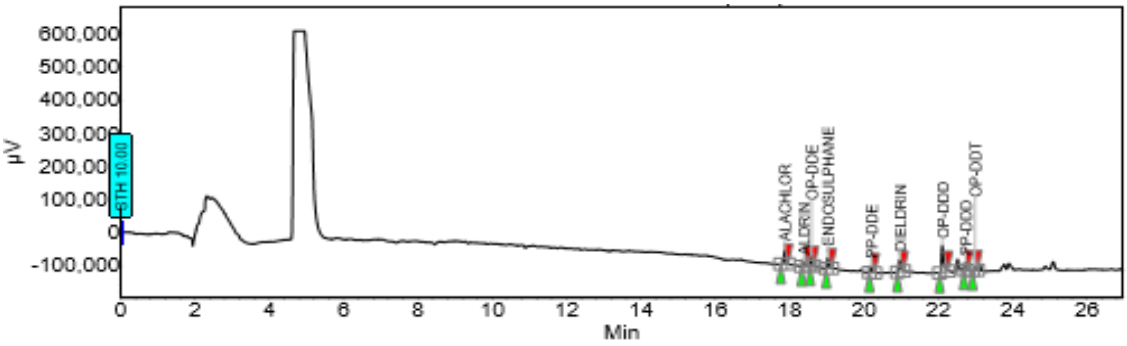


Index	Name	Time [Min]	Quantity [% Area]	Height [µV]	Area [µV.Min]	Area % [%]
Total			0.00	0.0	0.0	0.000

Fig. 55: Chromatograms of some OCP residues in broiler meat samples collected from Bengaluru

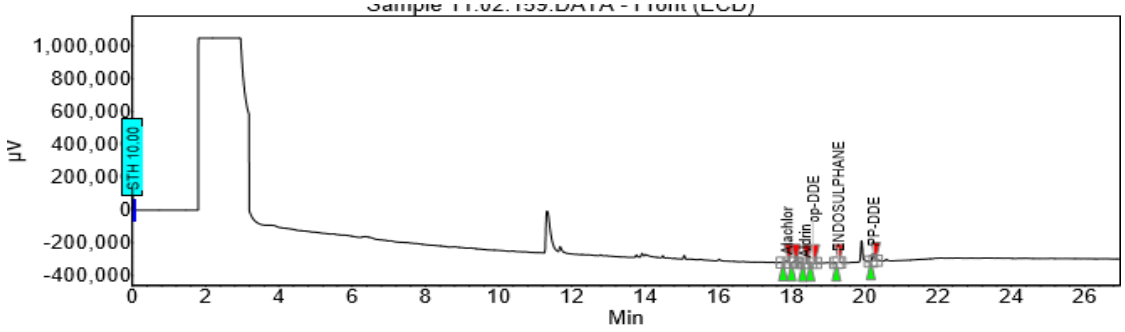


Index	Name	Time [Min]	Quantity [% Area]	Height [µV]	Area [µV.Min]	Area % [%]
1	DELTA-BHC	15.13	20.54	5612.2	284.6	20.544
2	ALDRIN	18.36	39.15	11611.9	542.3	39.155
3	PP-DDE	20.22	25.95	7896.9	359.4	25.948
4	DIELDRIN	21.20	14.35	4280.3	198.8	14.354
Total			100.00	29401.3	1385.1	100.000

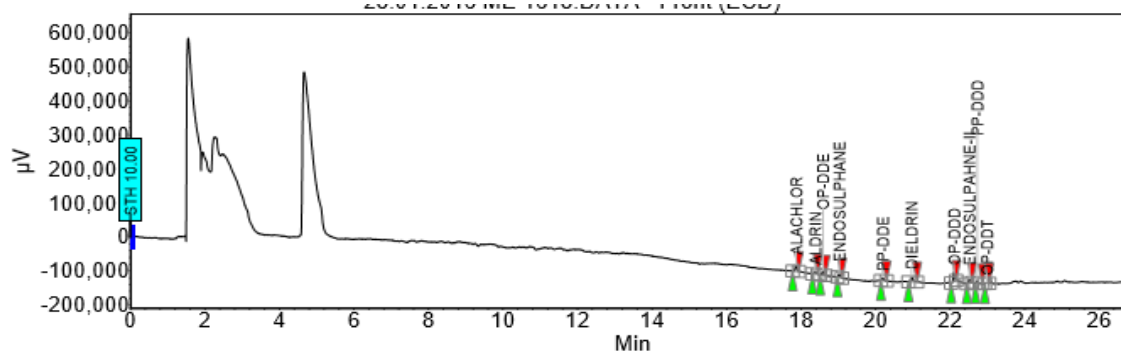


Index	Name	Time [Min]	Quantity [% Area]	Height [µV]	Area [µV.Min]	Area % [%]
1	ALACHLOR	17.87	12.69	43327.3	2190.9	12.686
2	ALDRIN	18.38	4.61	15410.3	795.8	4.608
3	OP-DDE	18.59	8.55	31360.9	1476.3	8.548
4	ENDOSULPHANE	19.03	9.44	32788.1	1631.1	9.445
5	PP-DDE	20.19	6.64	22007.7	1146.4	6.638
9	DIELDRIN	20.96	12.64	37551.3	2182.6	12.638
6	OP-DDD	22.11	31.98	81374.2	5523.5	31.983
7	PP-DDD	22.74	8.16	25263.7	1409.4	8.161
8	OP-DDT	22.99	5.29	16166.5	914.3	5.294
Total			100.00	305250.0	17270.3	100.000

Fig. 56: Chromatograms of some OCP residues in milk samples collected from Bengaluru

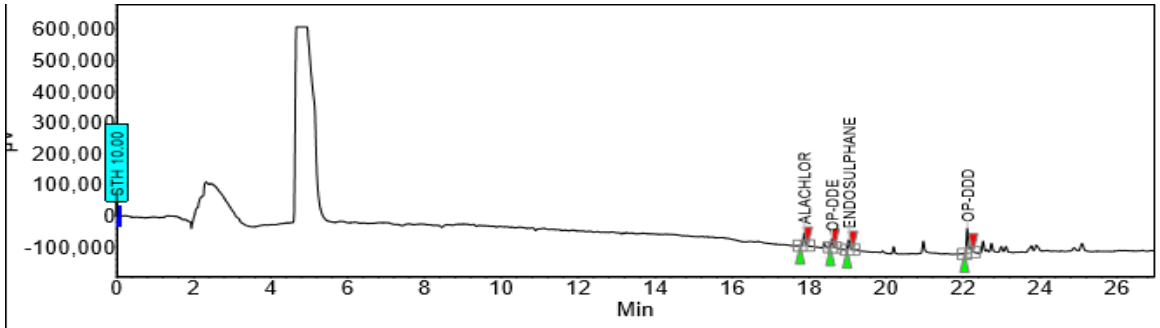


Index	Name	Time [Min]	Quantity [% Area]	Height [µV]	Area [µV.Min]	Area % [%]
1	Alachlor	17.86	18.56	14529.9	826.6	18.556
2	UNKNOWN	18.08	10.22	10753.2	455.3	10.222
3	Aldrin	18.35	6.46	5580.3	287.7	6.460
4	op-DDE	18.58	3.25	2815.2	144.9	3.254
5	ENDOSULPHANE	19.27	2.17	2653.9	96.7	2.170
6	PP-DDE	20.23	59.34	49777.3	2643.2	59.339
Total			100.00	86109.8	4454.5	100.000

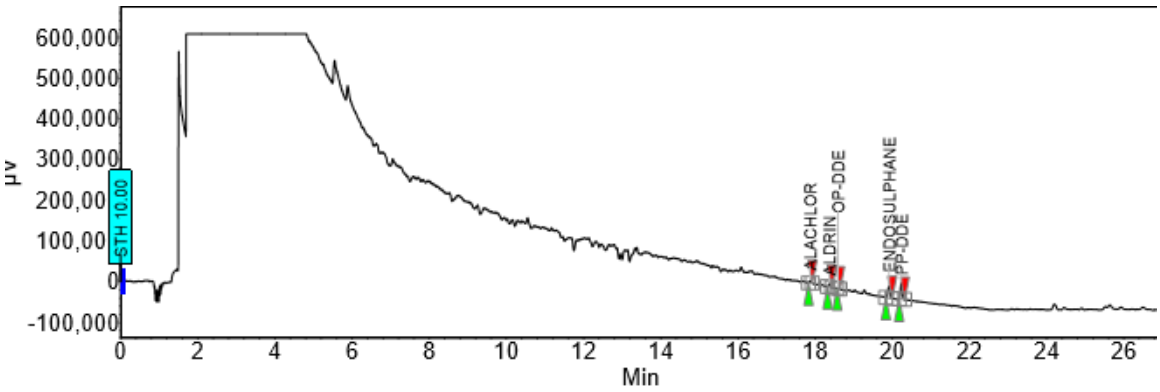


Index	Name	Time [Min]	Quantity [% Area]	Height [µV]	Area [µV.Min]	Area % [%]
1	ALACHLOR	17.87	14.11	19987.0	996.3	14.111
2	ALDRIN	18.38	6.38	8435.2	450.6	6.382
3	OP-DDE	18.59	9.47	13959.7	668.8	9.473
4	ENDOSULPHANE	19.02	7.38	10800.4	520.9	7.378
5	PP-DDE	20.19	6.25	8444.3	441.5	6.253
6	DIELDRIN	20.96	15.91	15371.7	1123.7	15.915
7	OP-DDD	22.10	18.88	27786.5	1333.2	18.883
8	ENDOSULPAHNE-II	22.51	10.30	12623.4	727.4	10.302
9	PP-DDD	22.73	8.39	9637.3	592.5	8.392
10	OP-DDT	22.98	2.91	3752.3	205.5	2.911
Total			100.00	130797.7	7060.5	100.000

Fig. 57: Chromatograms of some OCP residues in egg samples collected from Bengaluru

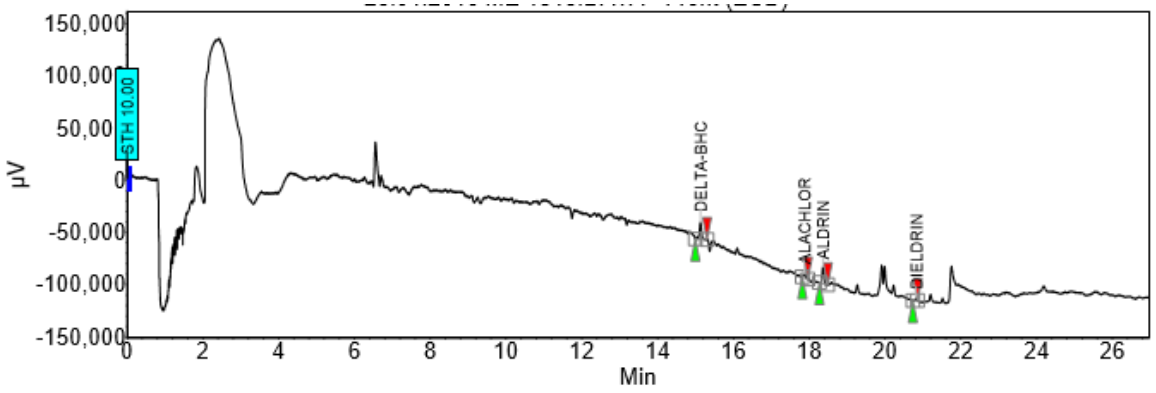


Index	Name	Time [Min]	Quantity [% Area]	Height [μV]	Area [μV.Min]	Area % [%]
1	ALACHLOR	17.87	20.25	43327.3	2190.9	20.245
2	OP-DDE	18.59	13.64	31360.9	1476.3	13.642
3	ENDOSULPHANE	19.03	15.07	32788.1	1631.1	15.072
4	OP-DDD	22.11	51.04	81374.2	5523.5	51.041
Total			100.00	188850.5	10821.8	100.000

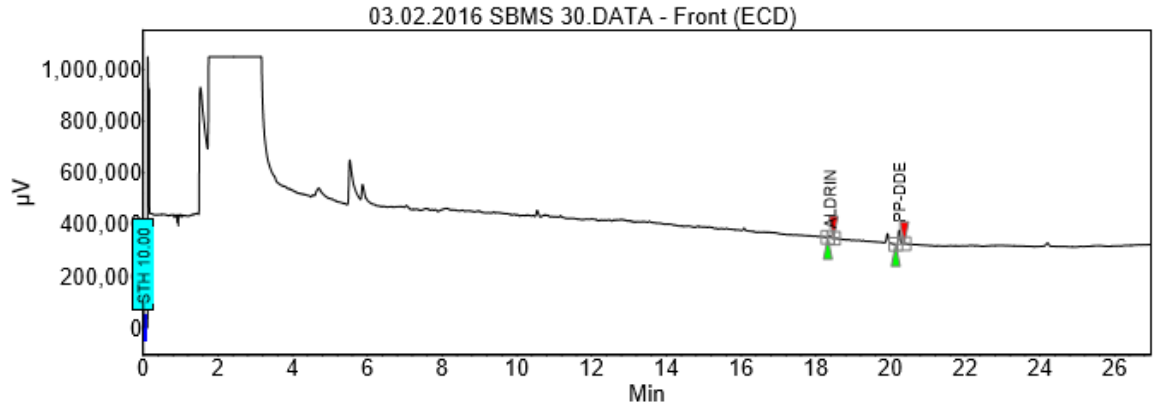


Index	Name	Time [Min]	Quantity [% Area]	Height [μV]	Area [μV.Min]	Area % [%]
1	ALACHLOR	17.87	5.72	7362.2	290.7	5.722
2	ALDRIN	18.37	9.78	10234.6	496.8	9.778
3	OP-DDE	18.59	2.86	3519.3	145.5	2.864
5	ENDOSULPHANE	19.91	37.17	30572.1	1888.5	37.169
4	PP-DDE	20.23	44.47	41366.9	2259.3	44.467
Total			100.00	93055.1	5080.8	100.000

Fig. 58: Chromatograms of some OCP residues in broiler meat samples collected from Shivamogga

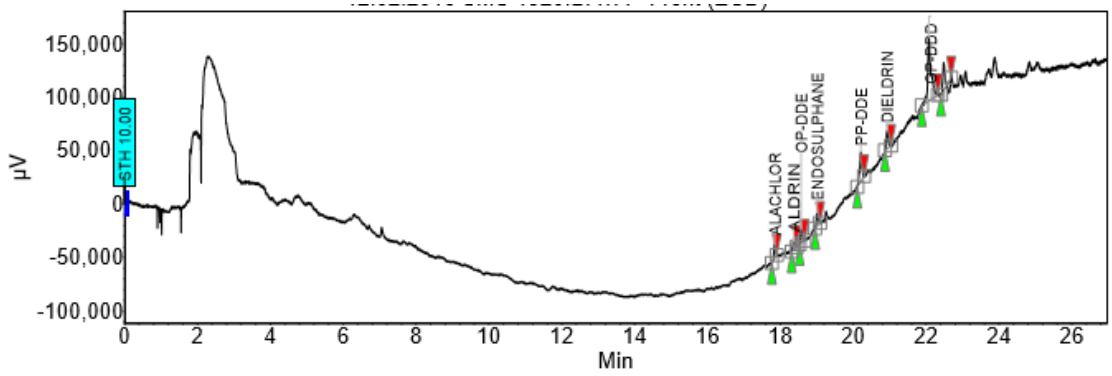


Index	Name	Time [Min]	Quantity [% Area]	Height [µV]	Area [µV.Min]	Area % [%]
1	DELTA-BHC	15.13	45.56	16409.0	976.5	45.563
2	ALACHLOR	17.87	12.26	3966.8	262.8	12.263
3	ALDRIN	18.36	37.39	16187.8	801.4	37.393
4	DIELDRIN	20.82	4.78	2794.3	102.5	4.781
Total			100.00	39357.8	2143.1	100.000

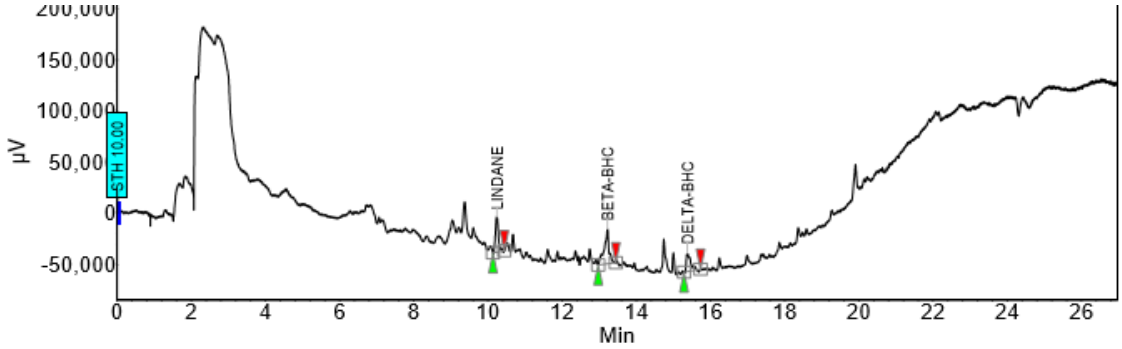


Index	Name	Time [Min]	Quantity [% Area]	Height [µV]	Area [µV.Min]	Area % [%]
1	ALDRIN	18.37	11.62	9450.6	395.0	11.620
2	PP-DDE	20.23	88.38	55975.3	3004.5	88.380
Total			100.00	65425.9	3399.5	100.000

Fig. 59: Chromatograms of some organochlorine pesticides in milk samples collected from Shivamogga

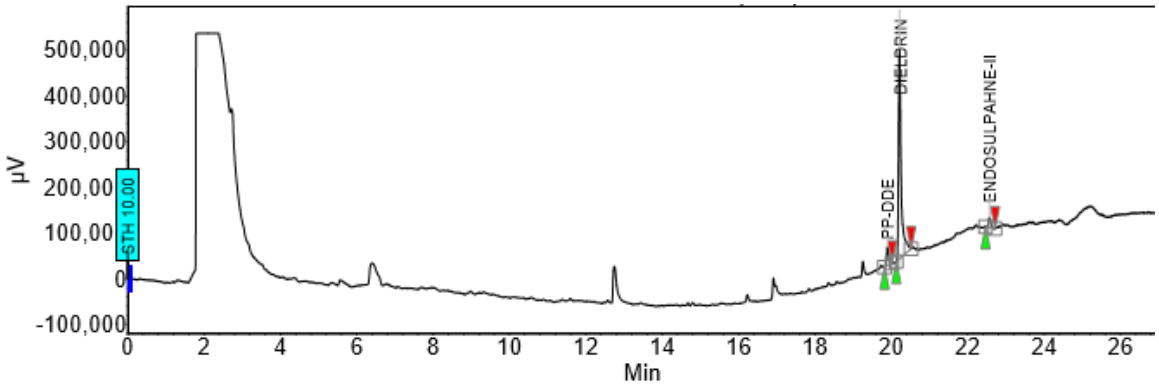


Index	Name	Time [Min]	Quantity [% Area]	Height [µV]	Area [µV.Min]	Area % [%]
1	ALACHLOR	17.84	5.44	13057.7	689.3	5.444
2	ALDRIN	18.37	4.14	8072.5	524.3	4.141
3	OP-DDE	18.57	5.96	13859.3	754.6	5.960
4	ENDOSULPHANE	19.01	5.56	12301.4	704.5	5.564
5	PP-DDE	20.21	15.69	26879.1	1986.7	15.692
6	DIELDRIN	20.93	8.03	16526.7	1017.0	8.033
7	OP-DDD	22.08	48.29	59415.8	6113.4	48.286
8	UNKNOWN	22.48	6.88	24834.9	871.0	6.879
Total			100.00	174947.3	12660.8	100.000

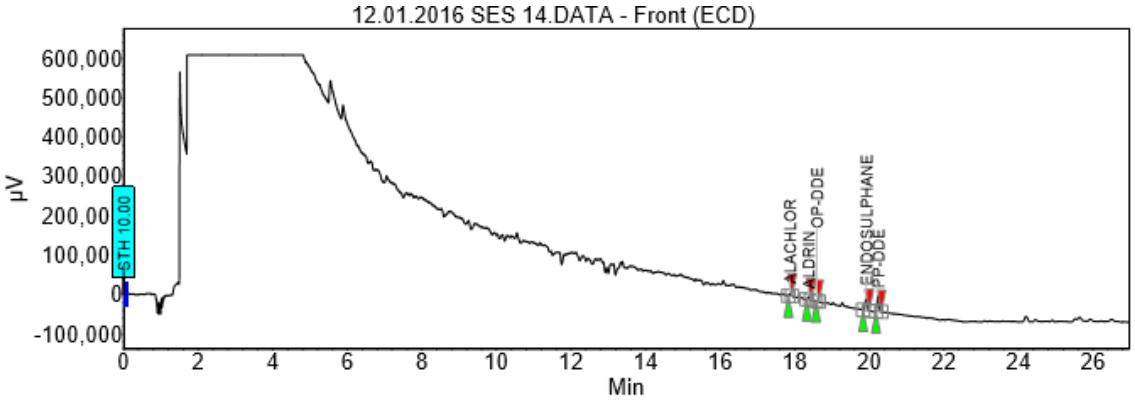


Index	Name	Time [Min]	Quantity [% Area]	Height [µV]	Area [µV.Min]	Area % [%]
1	LINDANE	10.24	30.11	34317.5	3128.5	30.115
2	BETA-BHC	13.22	48.31	34026.4	5019.0	48.313
3	DELTA-BHC	15.38	21.57	17758.1	2241.1	21.573
Total			100.00	86102.0	10388.6	100.000

Fig. 60: Chromatograms of some OCP residues in egg samples collected from Shivamogga

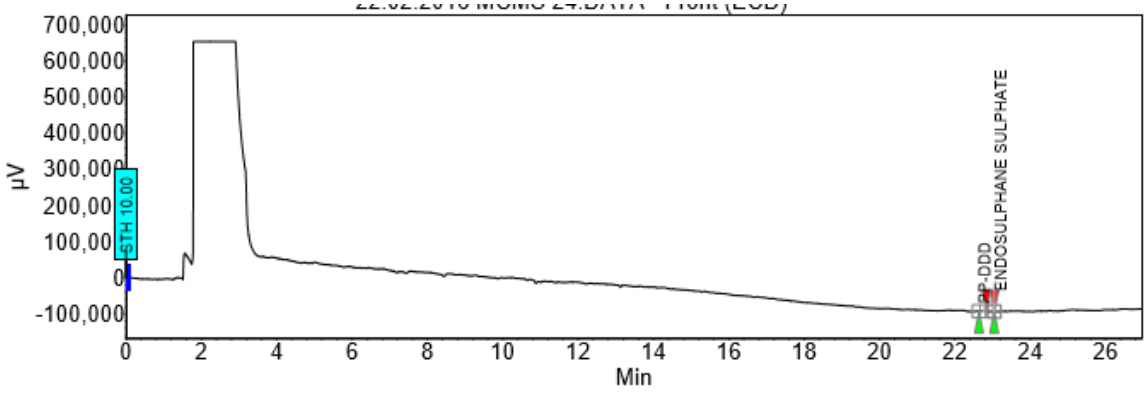


Index	Name	Time [Min]	Quantity [% Area]	Height [µV]	Area [µV.Min]	Area % [%]
1	PP-DDE	19.90	6.17	41179.0	2477.7	6.165
2	DIELDRIN	20.21	90.17	460259.3	36238.7	90.169
3	ENDOSULPAHNE-II	22.57	3.67	21886.5	1473.5	3.666
Total			100.00	523324.8	40189.9	100.000

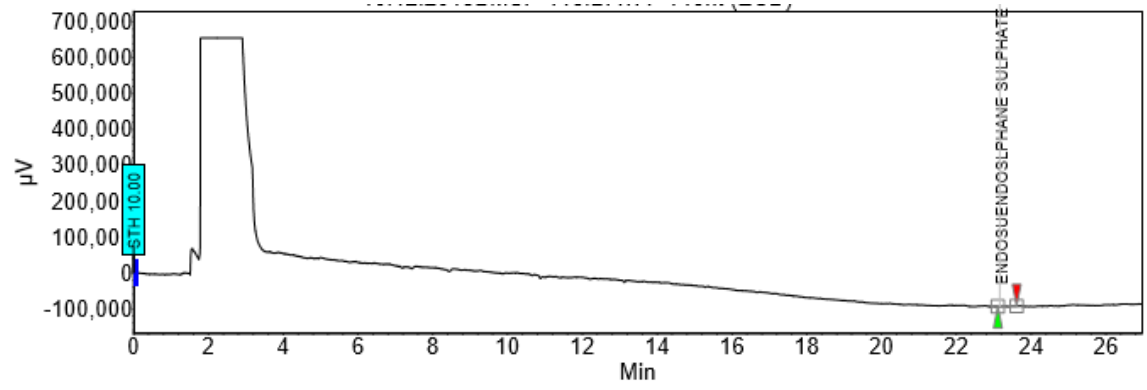


Index	Name	Time [Min]	Quantity [% Area]	Height [µV]	Area [µV.Min]	Area % [%]
1	ALACHLOR	17.87	5.72	7362.2	290.7	5.722
2	ALDRIN	18.37	9.78	10234.6	496.8	9.778
3	OP-DDE	18.59	2.86	3519.3	145.5	2.864
5	ENDOSULPHANE	19.91	37.17	30572.1	1888.5	37.169
4	PP-DDE	20.23	44.47	41366.9	2259.3	44.467
Total			100.00	93055.1	5080.8	100.000

Fig. 61: Chromatograms of some organochlorine pesticides in broiler meat samples collected from Mysuru

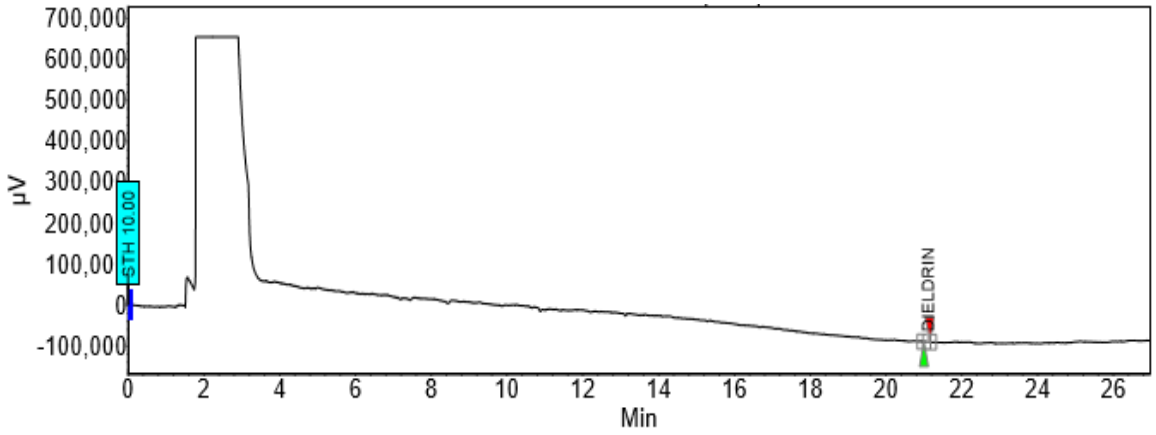


Index	Name	Time [Min]	Quantity [% Area]	Height [µV]	Area [µV.Min]	Area % [%]
1	PP-DDD	22.77	96.94	883.0	110.9	96.942
2	ENDOSULPHANE SULPHATE	23.05	3.06	5.5	3.5	3.058
Total			100.00	888.5	114.4	100.000

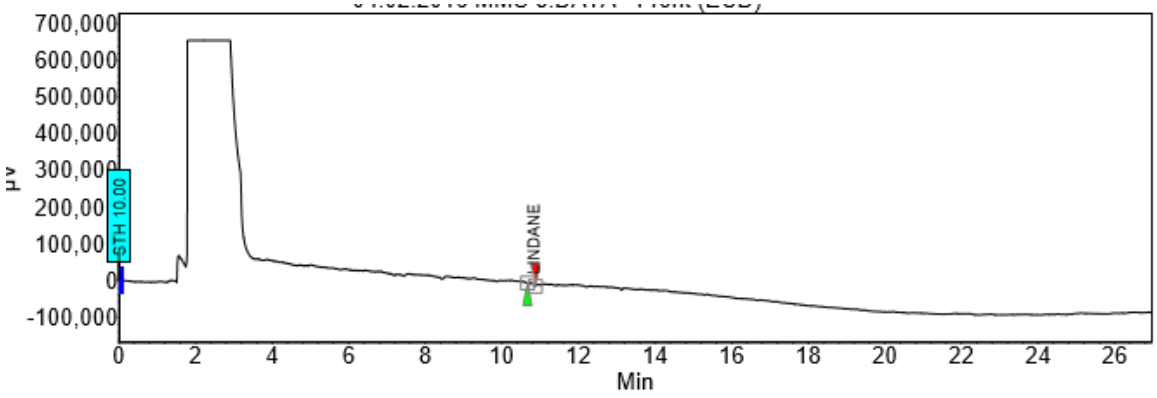


Index	Name	Time [Min]	Quantity [% Area]	Height [µV]	Area [µV.Min]
1	ENDOSUENDOSLPHANE SULPHATE	23.16	100.00	986.9	17.7
Total			100.00	986.9	17.7

Fig. 62: Chromatograma of some organochlorine pesticides in milk samples collected from Mysuru

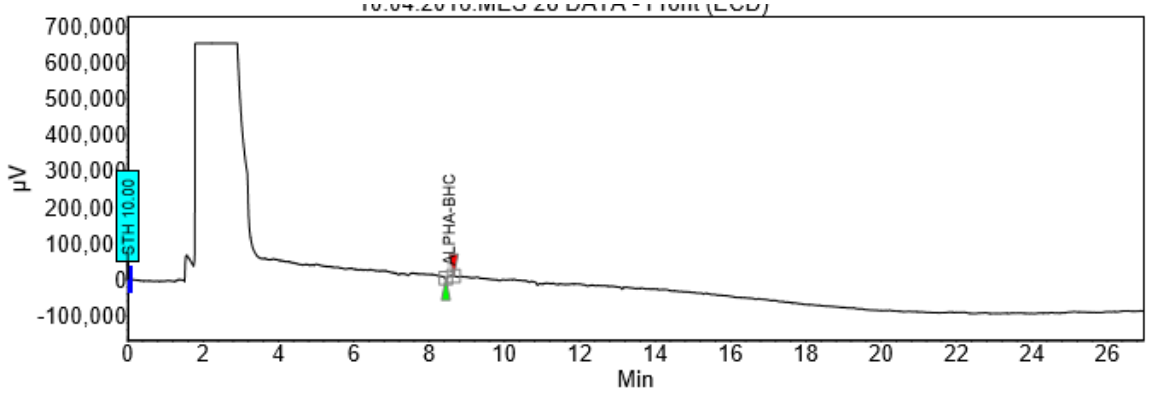


Index	Name	Time [Min]	Quantity [% Area]	Height [µV]	Area [µV.Min]	Area % [%]
1	DIELDRIN	21.10	100.00	660.8	55.4	100.000
Total			100.00	660.8	55.4	100.000

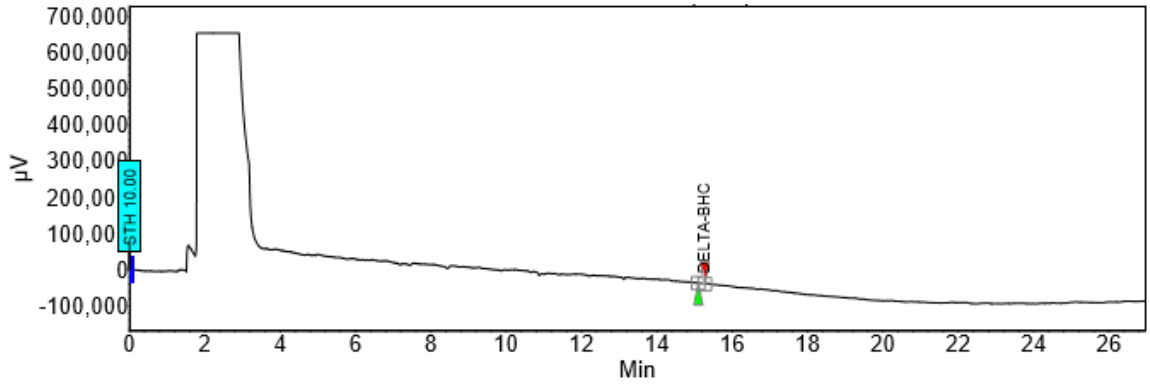


Index	Name	Time [Min]	Quantity [% Area]	Height [µV]	Area [µV.Min]	Area % [%]
1	LINDANE	10.82	100.00	6598.6	839.6	100.000
Total			100.00	6598.6	839.6	100.000

Fig. 63: Chromatograms of some organochlorine pesticides in egg samples collected from Mysuru



Index	Name	Time [Min]	Quantity [% Area]	Height [µV]	Area [µV.Min]	Area % [%]
1	ALPHA-BHC	8.52	100.00	5223.4	508.3	100.000
Total			100.00	5223.4	508.3	100.000



Index	Name	Time [Min]	Quantity [% Area]	Height [µV]	Area [µV.Min]	Area % [%]
1	DELTA-BHC	15.23	100.00	1376.8	107.2	100.000
Total			100.00	1376.8	107.2	100.000

Discussion

V. DISCUSSION

Present study was carried out to validate and standardize the analytical procedures for detection of some organochlorine pesticide residues using gas chromatography - electron capture detector in broiler meat, milk and chicken eggs collected from the commercial outlets of Bengaluru, Shivamogga and Mysuru. The residues of some organochlorine pesticides were compared with maximum residue limit values.

5.1 Validation of analytical procedures

5.1.1 Validation of analytical method for the detection of OCP residues in broiler meat, milk and chicken eggs

In the present study, the analytical method was validated for multiresidue analysis of Organochlorine pesticides, the validation parameters.

5.1.1.1 System suitability

The average relative standard deviation (RSD) for system suitability observed was 14.2 %.

The validation findings of this study are in accordance with the findings of several studies cited below.

Garrido *et al.* (2006) reported the relative standard deviation for system suitability was lower than 15% in validating the method for multiresidue analysis of organochlorine and organophosphorus pesticides in muscle of chicken, pork and lamb by using chromatography- triple quadruple mass spectrometry.

Pandit *et al.* (2002) obtained the average relative standard deviation of <15% in the recovery study on blank milk and dairy products spiked with pesticide standards.

Patrizia *et al.* (2009) reported the relative standard deviation (RSD %) turned out to be ranging from 2 to 15%.

Warangkana *et al*. (2016) obtained the relative standard deviation (RSD %) turned out to be < 7% in developing and validating the analytical method for measuring 11 OP pesticide residues in human plasma and breast milk.

It could be concluded that the findings of the present study are in accordance with the findings of the various studies conducted earlier in relation to system suitability and the values were within the validation limits, hence analytical method developed and validated was proper for some organochlorine pesticide residue detection in broiler meat, milk and chicken egg samples using gas chromatography-electron capture detector.

5.1.1.2 Linearity

The linearity (R2) for some of the organochlorine pesticides considered for validation was 0.96 %.

The validation findings of this parameter in the study are in accordance with the findings of several studies cited below

Jae *et al.* (2005) optimized the method by determining linearity determination coefficient. The correlation coefficients for the 22 extracted pesticide standard curves ranged from 0.998 to 1.000.

Patrizia *et al.* (2009) obtained the good linearity with correlation coefficient of higher than 0.98.

Warangkana *et al*. (2016) obtained good linearity, with the coefficients of determination of 0.996–0.999 in developing and validating the analytical method for measuring 11 OP pesticide residues in human plasma and breast milk.

It could be concluded that the findings of the present study are in accordance with the findings of the studies conducted earlier in relation to linearity and the values were within the validation limits, hence analytical method developed and validated was correct for some organochlorine pesticide residue detection in broiler meat, milk and chicken egg samples using gas chromatography-electron capture detector.

5.1.1.3 Method precision and intermediate method of precision

The precision of the method expressed in terms of co-efficient of variation and it was observed at 7.57 %. The intermediate method of precision observed after validating the analytical procedure was 7.57 %.

The validation findings of this parameter in the study are in accordance with the findings of several studies conducted earlier

Gonzalez *et al*. (2012) obtained the precision values lower than 12 % in developing the new analytical method to determine more than 40 multiclass pesticides in different kinds of processed and unprocessed milk samples using solid phase microextraction.

Garrido *et al.* (2006) obtained the method precision value of 15% in validating the method for multiresidue analysis of organochlorine and organophosphorus pesticides in muscle of chicken, pork and lamb by using chromatography- triple quadrupole mass spectrometry.

Xianyu *et al.* (2014) obtained the validation value for method precision was 12.4% in validating the method for quantification of current use and persistent pesticides in cow milk, human milk and baby formula using gas chromatography tandem mass spectrometry.

Warangkana *et al*. (2016) obtained method precision and intermediate method precision value in the range between 2.3–18.9% and 5.8–19.5%, respectively in developing and validating the analytical method for measuring 11 OP pesticide residues in human plasma and breast milk.

It could be concluded that the findings of the present study are in accordance with the findings of the studies conducted earlier in relation to method precision and intermediate method precision and the values were within the validation limits, hence analytical method developed and validated was correct for some organochlorine pesticide residue detection in broiler meat, milk and chicken egg samples using gas chromatography-electron capture detector.

5.1.1.4 Accuracy (recovery) rate

The accuracy (recovery) rate of the system for the selected organochlorine pesticides were between 86- 113%.

Gonzalez *et al*. (2012) obtained the mean recovery ranged from 81 to 110 % in developing the new analytical method to determine more than 40 multiclass pesticides in different kinds of processed and unprocessed milk samples using solid phase microextraction.

Garrido *et al.* (2006) reported the accuracy (recovery) rate ranging from 70-90% in validating the method for multiresidue analysis of organochlorine and organophosphorus pesticides in muscle of chicken, pork and lamb by using chromatography- triple quadrupole mass spectrometry.

Pandit *et al.* (2002) obtained the mean recovery ranged from 82.6-97.3% in validating the method performed for the recovery study on blank milk and dairy products spiked with pesticide standards.

Jae *et al.* (2005) obtained the accuracy rate (recovery rate) ranging from was 64.4 to 96.0%.

Patrizia *et al.* (2009) found the mean recoveries in the range between 70-110% and 84-99 % for OCPs and pyrethroids respectively.

Xianyu *et al.* (2014) obtained the accuracy (recovery) rate in the range between 80-120% in validating the method for quantification of current use and persistent pesticides in cow milk, human milk and baby formula using gas chromatography tandem mass spectrometry.

Warangkana *et al*. (2016) obtained the extraction recoveries from spiked plasma and breast milk samples ranged from 59.4% to 94.0% in developing and validating the analytical method for measuring 11 OP pesticide residues in human plasma and breast milk.

It could be concluded that the findings of the present study are in accordance with the findings of the studies conducted earlier in relation to accuracy (recovery rate), hence analytical method developed and validated was correct for some organochlorine pesticide residue detection in broiler meat, milk and chicken egg samples using gas chromatography-electron capture detector.

5.1.1.5 The limit of detection and limit of quantification (LOD &LOQ)

The limit of detection and limit of quantification (LOD &LOQ) for pesticide residues was calculated. The limit of detection for organochlorine pesticide residues in broiler meat, milk and chicken eggs was validated to 10 ppb and the limit of quantification of OCPs' was fixed at levels, less than maximum residue limit for residue analysis.

Garrido *et al.* (2006) fixed the limit of detection at 2 ppb/kg and limit of quantification at 5 ppb/kg fat basis in validating the method for multiresidue analysis of organochlorine and organophosphorus pesticides in muscle of chicken, pork and lamb by using chromatography- triple quadrupole mass spectrometry.

Patrizia *et al.* (2009) fixed the limit of quantification in the range of 0.005-0.1mg/kg for organochlorine class of compounds.

Xianyu *et al.* (2014) reported validation values for limit of detection was 1600 pg/ml in cow milk, human milk and baby formula using gas chromatography tandem mass spectrometry.

Warangkana *et al*. (2016) fixed the detection limits ranged from 0.18–1.36 and 0.09–2.66 ng/ml in plasma and breast milk respectively in developing and validating the analytical method for measuring 11 OP pesticide residues in human plasma and breast milk. Method detection limits of plasma and breast milk ranged from 0.18–1.36 and 0.09–2.66 ng/ml respectively.

It could be concluded that the findings of the present study are in accordance with the findings of the various studies conducted earlier in relation to limit of detection and limit of quantification (LOD & LOQ) and the values were within the validation limits.

5.1.1.6 The retention time

The gas chromatographic retention times for organochlorine pesticide using rtx5 column and electron capture detector are presented here

The retention time for various organochlorine pesticides after method validation for alpha-BHC was 8.43 min, lindane was 10.87 min, beta- BHC was 13.12 min, delta-BHC was 15.28, alachlor was 17.86 min, aldrin was 18.37, *o,p'* DDE was 18.59 min, endosulphan-I was 19.02, *o,p'* DDE was 20.19, dieldrin was 20.96, *o,p'* DDD was 20.19, endosulphan-II was 22.51, *p,p'* DDD was 22.73, *o,p'*-DDT was 22.98, for endosulphan sulphate was 23.11 and *p,p'*-DDT was 23.90 min.

Garrido *et al* (2006) reported the retention time for lindane was 6.32 min, hexachlorobenzene was 6.19 min, aldrin was 6.96 min, dieldrin was 7.52 min, α-endosulphan was 7.23, β- endosulphan 7.35, endosulphan sulphate was 7.43 min, *p,p'* DDD was 7.49 min, *p,p'* DDT was 7.49 min *o,p'* DDT was 7.49 min and for *p,p'* DDE was 7.24 min in validating the method for multiresidue analysis of organochlorine and organophosphorus pesticides in muscle of chicken, pork and lamb by using chromatography- triple quadrupole mass spectrometry.

The findings of the present study are not in accordance with the findings of Garrido *et al* (2006). This might be attributed to the change in the column conditions, temperature programming of the system and extraction and clean up procedure involved in the sample preparation.

Xianyu *et al*. (2014) reported the retention time for hexachlorobenzene was 11.75 min, dieldrin was 12.65 min, α-endosulphan was 17.38 min, β- endosulphan was 18.93 min in validating the method for the quantification of current use and persistent pesticides in cow milk, human milk and baby formula using gas chromatography tandem mass spectrometry.

The findings of the study are alomost in accordance with the findings of Xianyu *et al*., 2014.

5.2 Residue analysis of OCPs in broiler meat, milk and chicken eggs collected from the commercial outlets of Bengaluru, Shivamogga and Mysuru

In the present study, residue concentrations of some organochlorine pesticides were analyzed in broiler meat, milk and chicken eggs collected from the commercial outlets of Bengaluru, Shivamogga and Mysuru. The presence of residues of some organochlorine pesticides in broiler meat, milk and chicken egg samples were found to be good indicators for their presence in these samples and suitable marker for the human consumption of broiler meat, milk and chicken eggs marketed in Bengaluru, Shivamogga and Mysuru.

5.2.1 Residue concentrations in broiler meat, milk and chicken eggs of Bengaluru, Shivamogga and Mysuru

5.2.1.1 DDT and its metabolite residue concentrations in broiler meat, milk and chicken eggs of Bengaluru, Shivamogga and Mysuru

In the present study, high residual concentration of DDT and its metabolites were detected in broiler meat, milk and chicken eggs.

The mean concentration of *pp'*-DDE in broiler meat, milk and chicken eggs samples was 32.28, 37.79, 10.22 ppb respectively.

op'- DDD residue in broiler meat was less than the limit of detection and in case of milk and chicken egg samples was 116.70 and 94.26 ppb respectively.

op'-DDT and *pp'*-DDD residues in chicken egg samples with mean residue concentration levels was 24.05 and 26.02 ppb respectively.

Other metabolites of DDTs' such as *pp'*-DDT, *op'*-DDE, *op'*-DDT and *pp'*-DDD residues were detected but their levels at less than the limit of detection.

High residual concentration of DDT and its metabolites was detected in boiler meat, milk and chicken eggs collected from the commercial outlets of Shivamogga. The mean concentration of *pp'*-DDE residue which was most detected DDT metabolite observed in broiler meat, milk and chicken eggs samples were 84.22, 46.66, 36.67 ppb respectively.

The mean concentration of *op'*- DDE was detected in milk and chicken eggs but at the levels, less than limit of detection. Whereas *op*'-DDD in milk samples with mean concentration of 15.11 ppb and *op*'-DDT with mean residue concentration 228.90 ppb in chicken egg samples.

High residue concentration of DDT and its metabolites were detected in broiler meat, milk and chicken egg samples collected from commercial outlets of Mysuru. The mean concentration of *pp'*-DDE and *op*'- DDD was observed at less than the limit of detection in broiler meat, milk and chicken egg samples.

Rafat *et al.* (2010) had reported that, out of 519 samples obtained from different areas of Jordan during 2001-2007, about 37% samples were found to be contaminated with OCP residues, 2.9 % samples exceeded the maximum residue limits. Out of 519 samples analyzed, 20 (15%), 8 (7%) and 74 (27%) of eggs, chicken and meat samples analyzed respectively were positive for *pp*'-DDE, with an overall detection of 19.7% (102/519) among the analyzed samples. The *op*'-DDD and *op*'-DDT were the only

metabolites among the DDTs' that were not detected in levels higher than the detection limit. Among the analyzed samples *pp*'-DDE was the most dominant.

The mean values of the residual concentrations of *pp*'-DDE in the examined eggs, chicken and meat samples were 0.031 (31 ppb), 0.032 (32ppb) and 0.038 (38ppb) mg/kg fat respectively.

In one of the egg samples the concentration of *pp*'-DDE exceeded the MRL for DDT. The *pp*'-DDT was detected in eggs, chicken and meat with mean concentration of 0.142 (142ppb), 0.018 (18 ppb) and 0.064 (64ppb) mg/kg fat respectively. In five egg samples the detected concentration exceeded the MRL.

The *pp*'-DDD was found to be present in six meat samples analyzed and the concentration ranged between 0.01- 0.15 (10-150ppb) mg/kg fat.

Pandit *et al.* (2001) subjected 22 milk, 18 curd, 8 milk powder and 6 butter samples for residue analysis. The total DDT in milk samples varied from 0.016 (16 ppb) to 0.338 (338 ppb) mg/kg. The mean concentration of DDT was more in all the samples. The residues levels of DDT exceed MRL values prescribed by FAO (1993).

Ahmed *et al.*(2010) reported the presence of residues of OCPs in imported chicken meat samples. The concentration of DDE was the least frequent one, but none of these exceeded the maximum residue limits given by FAO (Codex alimentarius).

The imported bovine meat samples contained residues of OCPs. *pp*'-DDE and *pp*'-DDT were the most frequently found pesticides in these samples. The concentration

of *pp'*-DDD residues found in the range of 0.010-0.150 ppb, while *pp*'- DDE residues was 0.005- 0.050 ppb and *pp*'-DDT residues found in the range of 0.010-0.500 ppb.

Waliszewski *et al*. (2003) reported that γ-HCH [0.106 to 0.087 mg/kg (106 to 87 ppb) on fat basis] was one of the main contaminant followed by *pp*'-DDT [0.078 (78 ppb) mg/kg], *pp*'-DDE - 0.051 (51 ppb) mg/kg. HCB and op'-DDT were detected in lower quantities at the rate of 0.008 (8 ppb) and 0.031(31 ppb) mg/kg on fat basis respectively in 150 milk samples subjected for analysis.

Weiki *et al*. (2003) determined OCPs and their metabolite residues in milk from supermarkets in Beijing, People Republic of China. The average concentrations of total HCH and DDT were 0.038 (38 ppb) and 0.046 (46 ppb) mg/kg respectively. Of the 72 milk samples analyzed, three from south China contained the higher levels of DDT and HCH residues which exceeded the FAO/WHO accepted tolerance level, in spite of ban on DDT and HCH in China since 1983.

The residues of such compounds still exist in the environment and cause food contamination; it's likely attributed to short prohibition period and illegal use for agricultural purposes at present.

Among the various OCPs examined in the present study, DDTs are the most prominently noticed compounds, as they were detected at 30% (27/90), 46% (41/90) and 32% (31/90) of the broiler meat, milk and chicken egg samples collected from commercial outlets of Bengaluru, Shivamogga and Mysuru respectively.

The presence / existence of trace levels of organochlorine pesticide (DDTs' and its metabolites) residues in broiler meat, milk and chicken eggs owing to their usage pattern of pesticides in agriculture practices and various sanitary programmes, intake of pesticides by the animals and may also be attributed to way of nutrition and continuous exposure to spraying with insecticides to control external parasites.

These findings in accordance with the findings of Hassouba *et al.,* (2007) revealed that the persistence of DDTs' in the environment attributed to their use in control of insect borne diseases and elimination of agricultural pests which contaminates soil, water and air.

5.2.1.2 Hexachlorocyclohexane (HCH) and its isomers residue concentrations in broiler meat, milk and chicken eggs of Bengaluru, Shivamogga and Mysuru

The mean concentration of lindane detected in milk sample from Bengaluru was 86.60 ppb and the mean concentration of delta-BHC residues were detected in chicken eggs from Bengaluru was 39.38 ppb.

The mean residue concentration of lindane detected in milk and chicken egg samples from Shivamogga was 98.04 and 55 ppb respectively.

The mean concentration of delta-BHC detected in broiler meat and milk from Shivamogga was 15.57 and 16.78 ppb respectively. Beta-BHC concentration detected in milk sample was 63.98 ppb.

The mean residue concentration of lindane in milk and chicken egg samples from Mysuru was 45.37 and 10.95 ppb respectively.

The mean residue concentration of delta-BHC detected in chicken egg samples from Mysuru was 15.23 ppb and beta-BHC residues in milk was 33.94 ppb, whereas mean residue concentration of alpha-BHC detected in chicken egg samples from Mysuru was 24.44 ppb.

The present findings are in agreement with findings of Rafat *et al.* (2010) who reported that, out of 519 samples obtained from different areas of Jordan during 2001-2007, the predominantly accumulating and most active isomer of HCH was lindane was found to be present in three egg samples, one chicken sample and 37 meat samples analyzed. The mean values of the residual concentrations in the examined samples of eggs, chicken and meat were 0.019 (19 ppb), 0.050 (50 ppb) and 0.208 (208 ppb) mg/kg fat respectively. Meat has highest concentration of lindane compared to chicken and eggs. In the meat samples imported from India, the concentration of the lindane exceeded the MRL values.

The most persistent and metabolically stable beta isomer of HCH was identified in eggs, chicken and meat with mean concentration of 0.238 (238 ppb), 0.038 (38 ppb) and 0.028 (28 ppb) mg/kg fat basis. The alpha isomer of HCH was detected in eggs, chicken and meat with mean concentration of 0.197 (197 ppb), 0.029 (29 ppb) and 0.053 (53 ppb) mg/kg fat basis.

Pandit *et al.* (2001) subjected 22 milk, 18 curd, 8 milk powder and 6 butter samples for residue analysis. The residues of OCPs like α, β and γ isomers of HCH were detected in all the milk samples analyzed. The concentrations of total HCH varied from 0.009 (9 ppb) to 0.169 (169 ppb) mg/kg. The total DDT in milk samples varied from

0.016 (16 ppb) to 0.338 (338 ppb) mg/kg, which was twice the variation of concentration of total HCH

Ashnagar *et al.* (2009) reported the lindane concentration in 28 samples out of 35 samples of milk marketed in Ahwaz city of Iran. The maximum residue concentration detected was 42 ppb/kg fat basis and minimum residue detected was zero.

Ahmed *et al.* (2010) reported the residues of OCPs in imported chicken meat samples. The imported bovine meat samples contained residues of OCPs Endosulfan-II, dieldrin, aldrin, pp-DDE and pp-DDT were the most frequently found pesticides in these samples while least frequent one was γ isomers of HCH. The residue concentration of α-isomers of HCH in the range of 0.006-0.500 ppb, while β- isomers of HCH with concentration range of 0.009- 0.050 ppb.

Waliszewski *et al.* (2003) reported that γ-HCH [0.106 to 0.087 mg/kg (106 to 87 ppb) on fat basis] was one of the main contaminant followed by pp'-DDT [0.078 (78 ppb) mg/kg], pp'-DDE - 0.051 (51 ppb) mg/kg. HCB and op'-DDT were detected in lower quantities at the rate of 0.008 (8 ppb) and 0.031(31 ppb) mg/kg on fat basis respectively in 150 milk samples subjected for analysis.

Weiki *et al.* (2003) determined OCPs and their metabolite residues in milk from supermarkets in Beijing, P.R.China. The average concentrations of total HCH and DDT were 0.038 (38 ppb) and 0.046 (46 ppb) mg/kg respectively. Aldrin was detected in nine samples with a mean concentration of 0.035 (35 ppb) mg/kg. Heptachlor and its epoxides were not found in any milk samples.

Of the 72 milk samples analyzed, three from South China contained the higher levels of DDT and HCH residues which exceeded the FAO/WHO accepted tolerance level, in spite of ban on DDT and HCH in China since 1983. The residues of such compounds still exist in the environment and cause food contamination, its likely attributed to short prohibition period and illegal use for agricultural purposes at present.

Alpha-BHC, beta-BHC, delta-BHC and lindane were detected at low incidence but their presence in the samples surprising and this is attributed to limited or no control over the use and/or control of pesticide residues in foods. The presence / existence of trace levels of organochlorine pesticide (DDTs' and its metabolites) residues in broiler meat, milk and chicken eggs owing to their usage pattern of pesticides in agriculture practices and various sanitary programmes, intake of pesticides by the animals and may also be attributed to way of nutrition and continuous exposure to spraying with insecticides to control external parasites.

The presence of lindane and BHCs in the broiler meat, milk and chicken eggs indicates the need for concern from the public health point of view because of its much higher toxicity than other OCPs.

5.2.1.3 Endosulphan and its metabolites residue concentrations in broiler meat, milk and chicken eggs of Bengaluru, Shivamogga and Mysuru

The mean residue concentration of endosulphan-I detected in the milk sample from Bengaluru was 13.79 ppb and endosulphan sulphate concentration in chicken egg sample from Bengaluru was 21.66 ppb.

The mean residue concentration of endosulphan-I detected in the milk sample and chicken eggs from Shivamogga were 11.98 ppb and 27.30 ppb respectively. The mean residue concentration of endosulphan sulphate in chicken egg sample from Shivamogga was 36.06 ppb.

The mean residue concentration of endosulphan-I detected in milk samples from Mysuru was 24.61 ppb. It was also detected in broiler meat and chicken eggs but their levels below the limit of detection. Endosulphan sulphate detected only in broiler meat sample but their concentration was less than the limit of detection.

The present findings are in agreement with findings of Rafat *et al.*(2010) who reported that, out of 519 samples obtained from different areas of Jordan during 2001-2007, residues of endosulphan have not been detected in levels higher than the detection limit in any of the samples.

Ahmed *et al*. (2010) reported the residues of OCPs in imported chicken meat samples. Aldrin, endosulfan-II and dieldrin were frequently detected residues in chicken meat but none of these exceeded the maximum residue limits given by FAO (Codex alimentarius). The imported bovine meat samples contained residues of OCPs. Endosulfan-II, dieldrin, aldrin, pp-DDE and pp-DDT were the most frequently found pesticides in these samples while least frequent one was γ isomers of HCH.

Endosulphan and its isomers were other OCPs detected frequently next to DDTs, they were detected at 6.67% (6/90), 12.2% (11/90) and 16.67 % (15/90) in broiler meat,

milk and chicken egg (30 each) samples collected from Bengaluru, Shivamogga and Mysuru respectively.

5.2.1.4 Dieldrin, aldrin and alachlor residue concentrations in broiler meat, milk and chicken eggs of Bengaluru, Shivamogga and Mysuru

The mean residue concentration of aldrin detected in milk and chicken egg samples from Bengaluru was 10.17 and 13.02 ppb respectively.

The mean residue concentration of aldrin residue detected in broiler meat and chicken eggs from Shivamogga was at the levels less than the limit of detection.

The mean residue levels of aldrin detected in broiler meat and chicken egg samples of Mysuru was at levels less than limit of detection but in milk samples the detected levels of aldrin was 15.91 ppb.

The mean residue concentration of dieldrin detected in broiler meat from Bengaluru was 22.84 ppb, in milk was 13.64 and chicken eggs was 16.67 ppb.

The mean residue concentration of dieldrin detected from Shivamogga was at levels less than limit of detection in broiler meat, where in milk and chicken egg samples the detected mean concentrations were 11.00 and 27.30 ppb respectively.

The residues of alachlor was detected in broiler meat, milk and chicken eggs samples from Bengaluru but their levels were less than the limit of detection.

The residues of alachlor was detected in broiler meat, milk and chicken eggs samples from Shivamogga and their mean concentrations were detected in broiler meat and milk samples was 18.18 and 11.26 ppb respectively.

The alachlor residue was detected in chicken egg samples from Mysuru but their residue levels were below the level of limit of detection.

The present findings are in agreement with findings of

Rafat *et al.* (2010) who reported that, out of 519 samples obtained from different areas of Jordan during 2001-2007. None of the samples subjected for analysis found positive for the residues of organochlorine peticides except aldrin which was detected in only one meat sample with a mean concentration of 0.47 (47 ppb) mg/kg fat.

The residues of dieldrin have not been detected in levels higher than the detection limit in any of the samples.

Ashnagar *et al.* (2009) reported the lindane concentration in 18 samples out of 35 samples of milk marketed in Ahwaz city of Iran. The maximum residue concentration detected was 95 ppb/kg fat basis and minimum residue detected was zero.

Ahmed *et al.* (2010) reported the residues of OCPs in imported chicken meat samples. Aldrin and dieldrin were frequently detected residues in chicken meat and pp-DDE was the least frequent one, but none of these exceeded the maximum residue limits given by FAO (Codex alimentarius). Dieldrin, aldrin along with *pp'*-DDE and *pp'*-DDT

were the most frequently found pesticides in these samples. The concentration range of aldrin residue was 0.470 ppb.

Weiki *et al.* (2003) determined OCPs and their metabolite residues in milk from supermarkets in Beijing, P.R.China. The average concentrations of total HCH and DDT were 0.038 (38 ppb) and 0.046 (46 ppb) mg/kg respectively. Aldrin was detected in nine samples with a mean concentration of 0.035 (35 ppb) mg/kg. Heptachlor and its epoxides were not found in any milk samples.

Aldrin, dieldrin and alachlor were detected at low incidence but their presence in the samples were surprising and raised questions about pesticides banned for more than 2 decades, this is attributed to limited or no control over the use and/or control of pesticide residues in foods.

The presence of aldrin, dieldrin, lindane and BHCs in the broiler meat, milk and chicken eggs indicates the need for concern from the public health point of view because of their much higher toxicity than other OCPs.

The presence / existence of the organochlorine pesticides with varying concentrations reflects the intake of pesticides by the animals and may also be attributed to way of nutrition and continuous exposure to spraying with insecticides to control external parasites.

The results confirmed that the broiler meat, milk and chicken egg samples collected from commercial outlets of Bengaluru, Shivamogga and Mysuru indicates organochlorine pesticide residues in all the samples, which indicated human exposure

through these food products. However, the levels of organochlorine pesticide residues in all the samples were well below the MRL values. None of the samples, positive for the presence of OCP residues exceeded the maximum residue levels obtained from FAO/WHO food standards, codex alimentarius maximum residue limit of pesticide in food and European Union –MRL regulations.

Summary

VI. SUMMARY

Present study was carried out to validate and standardise the analytical procedures for detection of some organochlorine pesticide residues using gas chromatography – electron capture detector in broiler meat, milk and chicken eggs collected from the commercial outlets of Bengaluru, Shivamogga and Mysuru. The detected residues of some organochlorine pesticides were compared with maximum residue limit values.

A simple, sensitive and accurate method was developed for validation of analytical method for the detection of some organochlorine pesticide residues *viz*: alpha-BHC, beta-BHC, lindane, delta-BHC, alachlor, aldrin, *op'*-DDE, endosulphan-I, *pp'*-DDE, dieldrin, *op'*-DDD, endosulphan-II, *pp'*-DDD, *op'*-DDT, endosulphan sulphate, *pp'*-DDT. The analyzed validation parameters were the average relative standard deviation for system suitability - 14.2%, linearity (R^2) - 0.96%, limit of detection and limit of quantification (LOD & LOQ) values observed -10 ppb and less than maximum residue limit, method precision and intermediate method precision -7.57% and accuracy (recovery rate) - 86- 113%.

Residue concentrations of some organochlorine pesticides were analyzed in 30 samples each of broiler meat, milk and chicken eggs. Total of 90 samples from commercial outlets of Bengaluru, Shivamogga and Mysuru (total of 270 samples) have been collected and they were transported to the laboratory in cold chain and they were stored at -20^{O}C/refrigeration condition until further analysis.

The samples collected were subjected for extraction and clean up procedures for extraction of target analyte.

The samples collected from commercial outlets of Bengaluru, after subjecting them for analysis found that the samples were contaminated with high residual concentration of DDT and its metabolites. The mean concentrations of *pp'*-DDE observed were 32.28, 37.79, 10.22 ppb in broiler meat, milk and chicken egg samples respectively.

The mean residue concentrations of *op'*- DDD in milk and chicken egg samples were 116.70 and 94.26 ppb respectively. It was also detected in broiler meat but at less than limit of detection.

The mean residue concentrations of other metabolites in broiler and milk samples of DDTs' such as *pp'*-DDT, *op'*-DDE, *op'*-DDT and *pp'*-DDD were at levels, less than the limit of detection.

The mean residue concentrations of *pp'*-DDD in chicken egg sample was 24.05 ppb.

The mean residue concentrations of dieldrin broiler meat, milk and chicken egg samples were 22.84, 13.64 and 16.67 ppb respectively.

The mean residue concentrations of endosulphan-I in milk sample was 13.79 ppb and residue of endosulphan sulphate in chicken sample was 21.66 ppb.

The mean residue concentrations of aldrin in milk and chicken egg samples were 10.17 and 13.02 ppb respectively. Mean residue concentration of lindane in milk samples was 86.60 ppb and delta-BHC residues in milk was 79.38 ppb.

None of the OCP residues detected exceeded the maximum residue limit values.

Out of the 90 broiler meat, milk and chicken egg samples (30 each) from commercial outlets of Shivamogga, the analysis of samples found that high residual concentration of DDT and its metabolites detected in all the three samples.

The mean concentrations of *pp'*-DDE were observed at 84.22, 46.66, 36.67 ppb in broiler meat, milk and chicken eggs respectively.

Other metabolites of DDTs such as *op'*-DDD has mean concentration in milk was 15.11 ppb, *op'*-DDE, *pp'*-DDD residues were detected at less than the limit of detection, except *op'*-DDT residue concentration detected in chicken egg samples was 228.90 ppb.

The mean residue concentration of dieldrin in broiler meat was level less than limit of detection, in milk samples it was 11 ppb and in chicken egg samples the residue detected was 27.30 ppb.

The mean residue concentration of endosulphan-I in the milk sample was 11.98 ppb and chicken eggs was 27.30 ppb. The mean residue concentration of aldrin residue in milk was 15.91 ppb and in broiler meat, chicken eggs it was detected but their levels were less than the limit of detection.

The mean residue concentration of endosulphan sulphate in chicken egg samples was 36.06 ppb. Lindane residues detected in milk and chicken egg samples were 98.04 and 55 ppb respectively

The mean residue concentrations of delta-BHC in broiler meat and milk detected were 157.57 and 196.78 ppb respectively, where in the residue concentration of beta-BHC detected in milk sample was 63.98 ppb.

The mean residue concentrations of alachlor in all the three samples with their mean concentrations were 18.18 and 13.16 ppb in broiler meat and milk respectively. The concentration of alachlor in chicken eggs detected at less than the limit of detection.

The OCP residues in broiler meat, milk and chicken egg samples collected from commercial outlets of Mysuru has high residual concentrations of DDT and its metabolites in all the three samples.

The mean concentrations of *pp'*-DDE and *op'*- DDD were observed at less than the limit of detection in broiler meat, milk and chicken egg samples respectively. Endosulphan-I residue detected in the milk sample was 24.61ppb and it was also detected in broiler meat and chicken eggs but their levels were below the detection limits. Endosulphan sulphate detected in broiler meat sample was less than the limit of detection.

The mean residue concentrations of lindane in milk and chicken egg samples were 45.37 and 10.95 ppb respectively.

The mean residue concentration of delta-BHC in chicken egg sample was 15.23 ppb and beta- BHC detected in milk samples with residue concentration was 33.94 ppb, where in alpha-BHC detected in chicken eggs with the mean concentration was 24.44 ppb. The remaining residues detected were less than the limit of detection. None of the OCP residues detected exceeded the maximum residue limit values.

In conclusion the gas chromatography-electron capture detector (GC-ECD) is a good instrumentation technique for the detection of organochlorine pesticide residues in broiler meat, milk and chicken egg samples. Further, the residues of organochlorine pesticides and its metabolites detected in the samples collected from three different regions of Karnataka were below the maximum residue limit values.

Waste water from irrigation after spraying the crops, contaminated animal feeds, water bodies and polluted air etc, contaminated with pesticides could be the potential sources of residues in animal products like broiler meat, milk and chicken egg samples.

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Abstract

VIII. ABSTRACT

Present study was carried out to validate and standardize the analytical procedures for detection of some organochlorine pesticide residues using gas chromatography – electron capture detector in broiler meat, milk and chicken eggs collected from the commercial outlets of Bengaluru, Shivamogga and Mysuru of Kranataka, India. The comparison of the detected residues with maximum residue limits values was made. A simple, sensitive and accurate method was developed for validation of analytical method for the detection of some organochlorine pesticide residues *viz*: α-BHC, β-BHC, lindane, δ-BHC, alachlor, aldrin, *op'*-DDE, endosulphan-I, *pp'*-DDE, dieldrin, *op'*-DDD, endosulphan-II, *pp'*-DDD, *op'*-DDT, endosulphan sulphate and *pp'*-DDT. The validation parameters were , the average relative standard deviation for system suitability (14.2%), linearity (R^2) (0.96%), limit of detection and limit of quantification (LOD & LOQ) values (10 ppb and less than maximum residue limit), method precision and intermediate method precision (7.57%) and accuracy (recovery rate) (86- 113%.). Residues of some organochlorine pesticides were analyzed in thirty samples each of broiler meat, chicken eggs and milk (total of 90) samples from commercial outlets of Bengaluru, Shivamogga and Mysuru (total of 270 samples) which have been collected and were transported to the laboratory in cold chain and were stored at -20^{O} C untill further analysis. The samples collected were subjected for extraction and clean up procedures for extraction of target analyte. All the samples were analyzed for residues, DDT and its metabolites (88/270) are the most detected, followed by endosulphan and its isomers (32/270), dieldrin (19/270), aldrin (16/270), delta-BHC (15/270), alachlor (15/270), lindane (10/270), alpha-BHC (7/270) and beta-BHC(5/270) residues in broiler meat, milk and chicken eggs. The residues of organochlorine pesticides and their metabolites detected in the samples were below the maximum residue limit values. None of the detected residues exceeded the maximum residue limits.

Key words: Analysis, Extraction, Organochlorine pesticide, Residue and Validation.