UNIVERSITY OF NAIROBI

ASSESSMENT OF VETERINARY DRUGS AND PESTICIDE RESIDUE LEVELS IN COW AND CAMEL MILK FROM KIAMBU, LAIKIPIA AND ISIOLO COUNTIES, KENYA

BY

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DECLARATION

I declare that this thesis is my original work and has not been submitted elsewhere for award of a degree in any University. Where other people's work or my own work has been used, this has properly been acknowledged and referenced in accordance with the University of Nairobi's requirements.

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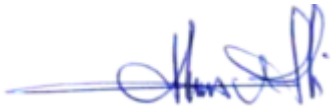
Signature… .  Date…….28/11/2021…….

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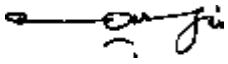
Signature…  ……. Date…26/11/2021….

DEDICATION

I dedicate this work to my beloved parents Mr. and Mrs. Jonathan Phillip Malombe, my son Shawn Mbithi, my siblings Rocky, Jackline, Sandra, Sharon and friends for their moral support and understanding during the period of carrying out this research work.

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ABSTRACT

Exposure to pesticides and veterinary drugs residues in foods of animal origin is a major public health concern due to increase usage of this chemicals. Humans are exposed to these chemicals through ingestion of contaminated food, skin absorption and inhalation. Among all food sources milk is one of the main sources of chemical contaminants. The current study aimed at evaluating organochlorine and organophosphate pesticides and veterinary drug residues levels in cow and camel milk collected from Kiambu, Isiolo, and Laikipia Counties between 2017 and 2018. Kiambu, Isiolo, and Laikipia Counties are of interest to this study as they are associated with the highest production of cow and camel milk in Kenya, however, little is known of the levels of pesticides and the effect of seasons on the pesticide levels in milk collected from these areas. A total of 82 camel milk and 90 cow milk samples were collected from 8 different wards of Kiambu, Isiolo and Laikipia Counties and a total of 18 organochlorines and 14 organophosphates were evaluated using gas chromatography-tandem mass spectroscopy (GCMS-TQ8040). Eleven veterinary drug residues were evaluated using ultra high-performance liquid chromatography-tandem mass spectroscopy (UHPLC-MS/MS). Variations in the pesticide and veterinary drug levels in milk collected from the different counties was evaluated using Analysis of variance and Tukey's post hoc test. Differences in the pesticide and veterinary drug levels in milk samples collected in the dry and wet seasons were evaluated using the independent sample student's t-test. $p< 0.05$ was considered significant in all cases. With the exception of heptachlor with mean concentration of 12.38 ng/mL for Kiambu, 4.62 ng/mL for Isiolo and 13.42 ng/mL for Laikipia county, all other pesticides in cow and camel milk were below the maximum residue limits. Chlorthiophos-1 had the highest mean concentration of 46.07 ng/mL in cow milk from Kiambu ranging between <0.2 to 387.47 ng/mL while camel milk from Isiolo and Laikipia had mean concentrations of 10.77 ng/mL and 14.30 ng/mL respectively. Multiple residues of veterinary drugs were below the maximum residue limits for sulphonamides of 100 ng/mL. The mean level of tetrachlorvinphos in camel milk was greater during the dry season than the wet season. The mean levels of γ-BHC, *o'p*-DDD, and cis-nonachlor in camel milk were greater during the wet season than during the dry season. Similarly, the mean levels of dieldrin, *o'p*-DDD, nonachlor, and endosulphan in cow milk were greater during the wet season than during the dry season. There was no difference in the mean levels of all the veterinary drug tested in camel milk during wet and dry season. However, the mean levels of sulfathiazole in camel milk during wet season was higher than in dry season. The mean levels of veterinary drug residues in cow milk were higher in dry season relative to wet season. Cow and camel milk collected from Isiolo, Kiambu, and Laikipia counties are contaminated with heptachlor which implies that consumers are exposed to the associated risk such as cancer. There is a need for government policy to mitigate the contamination of cow and camel milk in the study area by the organochlorine heptachlor. The season of sample collection appears to have an effect on the mean levels of some pesticides and veterinary drugs but not others. There is a need for studies to unravel the underlying causes of season dependent variations in the mean levels of some pesticides and veterinary drugs.

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LIST OF ABBREVIATIONS

ADI	Acceptable daily intake
AMR.	Antimicrobial resistance
ASAL	Arid and Semi-arid Lands
BDL.	Below detection limit
C18EC	Carbon 18 Ended- capped
CCPRF	Codex Commission on Pesticides Residues in Food
CCVDRF	Codex Commission on Veterinary drug residues in Food
DDT	Dichlorodiphenyltrichloroethane
d-SPE	Dispersive- Solid Phase Extraction
EFSA	European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization
FSSAI	Food Safety and Standards Authority of India
GC-MS/MS	Gas Chromatography Tandem Mass Spectroscopy
GDP	Gross Domestic Product
GoK	Government of Kenya
GPS	Global Position System
JECFA	Joint FAO/WHO Expert Committee on Food Additive
KEVEVAPI	Kenya Veterinary Vaccines Production Institute
KEBS	Kenya Bureau of Standards
KDB	Kenya Dairy Board
LOD	Limit of detection

LOQ	Limit of quantification
MRLs	Maximum Residue Limits
MRM	Multiple reaction monitoring
NEAP	National Environmental Action Plan
OCs	Organochlorines
OPs	Organophosphates
p'p-DDD	Dichlorodiphenyldichloroethane
p'p-DDE	Dichlorodiphenyldichloroethane
PSA	Primary Secondary Amine
PTFE	Polytetrafluoroethylene tubes
QuEChERS	Quick, easy, cheap, effective, rugged and safe
UHPLC-MS/MS	Ultra High-Performance Liquid Chromatography Tandem Mass Spectroscopy
WHO	World Health Organization

CHAPTER ONE

1. INTRODUCTION

1.1 Background information

Agriculture growth is among the highest key tools to end severe poverty, feeding estimated 9.7 billion persons by 2050 globally. Sustainable Development Goals (SDGs) aims in ending poverty, zero hunger, well-being and good health of citizens by 2030, therefore Agriculture plays a key role in achieving SDGs (Griggs *et al.*, 2017). Agriculture increases income among the poorest comparing with other sectors. In 2016 analyses on working adults was done and it was found that 65% made a living through agriculture (World Bank, 2021). Additionally, in terms of economic growth it reported a global gross domestic product (GDP) of four percent and accounted for above 25% of GDP in several developing countries by 2018.

Livestock production contribute 40% globally and 44% of total agricultural Gross Domestic Product (GDP) of African countries, improving livelihoods, combating food insecurity and livestock value chain benefits nearly 1.3 billion people (ILRI, 2013).

Agriculture and livestock production is very important in Kenya because livestock sector contribute 4.4 % of the Gross Domestic Product (GDP) (USD 3.4 billion in 2017) or about 14.2% of agricultural value added (GoK, 2018). Livestock division in Kenya hires fifty percent of the agricultural workforce and produces an important figure of occupations in the value chain. Around 60% of the livestock population is located in the arid and semi-arid lands (ASAL) where ninety percent of the population rear animals both for beef and milk production. In areas with high rainfall, livestock sector offers income and employment through dairy, pig and poultry production (GoK, 2019). Animal population in Kenya encompasses 18.8 million cattle (4.5 million cows and

14.3 million beef cattle) 1.9 million donkeys, 3.2 million camels, 26.7 million goats, 0.5 million pigs, 18.9 million sheep, 44.6 million poultry and uncertain number of companions, game and aquatic animals (GoK, 2017b).

The main challenges in livestock farming in Kenya include high densities of human and animals especially in peri-urban areas, spread of zoonotic diseases, including re-emerging and emerging infectious diseases due to weak human and animal health services, livestock-driven antimicrobial resistance (AMR) due to misuse and incorrect usage of antibiotics by farmers, poor enforcement of rules and regulations which negatively affects livestock on the ecosystem, high occurrence of foodborne diseases, usage of counterfeit veterinary drugs, natural resources depletion and climate change (FAO, 2019).

The growing population in Kenya has led to higher consumption of milk and beef, However the share of families consuming milk has not changed considerably compared to today due to high-income inequality despite the thriving economy thus reducing the buying ability of the typical Kenyan. In addition, poor and weak food safety policies and regulations often lead to consumption of poor-quality animal source foods. To date cattle population has increased by 90%, beef and milk production has increased to 2,000 and 17,000 tonnes, respectively. Milk production is higher than consumption while the internal demand for beef is higher than its production in Kenya (FAO, 2019).

Milk makes a valuable contribution to Kenyans because of its nutritional importance as a source of protein having biological value that promotes growth and development of mammalian infants (Abdulkhaliq *et al.,* 2012). In addiction Milk is an important diet especially for children and the elderly people around the world because of its exceptional sources of many nutrients such as calcium, iodine, phosphorus, vitamins B2 and B12 (Abdelkhalek *et al.,* 2015).

Sources of protein for the average Kenyan include milk, when produced in line with World Health Organization/Food and Agriculture Organization (EFSA, 2010) concerning usage of antibiotic drugs including withholding periods after anti-biotic treatment and or exposure to other chemicals used on farms. These animal proteins make a significant contribution to the food balance sheet (WHO, 2002).

During production period animals may be exposed to medication from veterinary drug administration or application of acaricides to control ticks, herbicides to control weeds, usage of insecticides and fungicides which increases and intensifies agricultural activities (Meng, 2016). According to Meng (2016), these chemicals contaminate the environment through water, soil, feed or atmospheric pollution. Poor agricultural practice and not adhering with the withdrawal periods after administering veterinary drugs will lead to presence of chemicals in milk as residues which upon reaching certain levels, they result in public health hazard and environmental health risk. Veterinary drugs are made for prevention and treatment of diseases in animals such as brucellosis, arthritis, mastitis, respiratory diseases gastrointestinal diseases and many other diseases (Orwa *et al.*, 2017).

The use of antibiotics for non-therapeutic purposes in dairy rearing such as fattening and increasing growth rate may be significant in Kenya. WHO and FAO have put in place regulations and rules regarding usage of veterinary drugs including withholding periods after anti-biotic therapy but it is unlikely that these regulations are always adhered to (WHO, 2002). If the antibiotics are not used well according to the regulations large amounts of drugs will be carried over as residues to animal products such as milk and meat which will be passed through the milk value chain.

Various chemicals which can contaminate milk include organochlorine pesticides, organophosphate pesticides, veterinary drugs, herbicides, mycotoxins, detergents and heavy

metals (Aytenfsu *et al.,* 2016). These chemicals when they exceed maximum residue levels (MRLs) may be a problem of great magnitude in Kenya, particularly due to associated public health concerns that include long or short-term hypersensitivity reactions, toxicity, antibiotic resistance, teratogenic and carcinogenic effects. Therefore, the government should put regulations in place to protect consumers (Aytenfsu *et al.,* 2016).

Veterinary drugs and pesticides residues in milk can either be safe for human consumption or can lead to undesirable health effects to the consumer. This chemical residues in milk should be below the maximum residue limit (MRL's) as set by WHO/FAO Expert committee on Food Additives. Currently Kenya has adopted over 200 Codex Standards and KEBS is the Contact point. (JECFA, 2009). There are two standards on food animals pertaining to chemical residues: Codex Commission on veterinary Drug Residues in Food (CCVDRF) and Code Commission on Pesticides Residue in Food (CCPRF).

While some farmers adhere to prescribed regulations for animal husbandry a few unscrupulous ones employ unethical farming practices driven by the desire to shorten the rearing period, enhance weight, reduce costs and increase profits (WHO, 2002). This in turn is responsible for unhealthy products finding their way to consumer shelves. Worldwide most of the antibiotics produced half of them are used mostly in the farm not in the human medicine.

Antibiotic residues in cow milk are of a great concern, not only in developed countries with systematic residues detection programs, but also in developing countries where most of the milk bypasses official quality assurance channels creating a potential public health risk (Aboge, 2002). In addition to concerns on dangerous levels of antibiotic residue, inadequate pasture for cows due to conversion of pastureland for residential and commercial use has forced pastoralists to move into urban areas in search of fodder (Smith *et al*., 2005). Animals searching for fodder in urban

areas end up feeding on unhealthy grass and even from rubbish dumps where they get exposed to antibiotics and pesticides due to poor disposal and management of chemical waste (Smith *et al*., 2005).

Usage of Veterinary drugs in animal production worldwide is estimated to increase even by 67% between 2010 and 2030 and this increase will be even higher where production is shifting towards large scale farming (Van-Boeckel *et al.,* 2015). Antibiotic-resistant bacteria (ARB) and antibiotics and can be in the ecosystem through soil around farms, air, in surface and ground water (Smith *et al*., 2005).

The extent of environmental pollution brought by extensive and haphazard usage of pesticides in Kenya has not been well established. Accumulation of pesticides can occur in animals producing milk from, fodder, water, inhaling contaminated air, soil and contaminated feed among others (Deti *et al.,* 2014) which eventually leads to contamination of foods of animal origin consumed by human beings (Nag *et al.,* 2007). Along the food chains some pesticides especially organochlorine pesticides are persistent in the environment and have the ability of translocation and biomagnifications. The toxic and risky features of OCPs are because of their highly lipophilic property which makes their residues increase in fatty foods such as dairy products and milk. Higher levels of these chemicals can accumulate in the body which could be harmful to health especially human beings who are at the top of the most food chains. Pesticides are strong toxicant for both human health and ecosystem, monitoring of these residues should be done constantly in the environment and in all foods in order to prevent any possible health calamity (Bulut *et al*., 2010).

Pesticides have been widely utilized for veterinary and agricultural purposes in Kenya. Usage of these chemicals have considerably benefited this country in controlling serious livestock diseases and increasing food production highly required by the fast-growing population. To overcome

problems of food insecurity, food shortage and shrinking farm size, pesticide's usage is considered to be the better option (Deti *et al*., 2014). However, these compounds have been shown to leave pesticide residues in animal products in many countries where they have been used. Since these compounds are poisonous in nature presence of pesticide residues in foods intended for human consumption is considered undesirable.

In Kenya, Large-scale farmers about 33% mainly use pesticides. Small scale farmers mainly at substance-level farms have minimal usage of pesticides (Wandiga *et al.,* 1990) some of these same pesticides can act as toxins to plants, animals and human when found in sufficient concentrations as residue.

The general population can be exposed to these residues mostly through consumption of treated foods or being close to pesticides contaminated areas such as farms. Pesticide residues maybe within tolerance levels or above tolerance levels. If the residues are above the tolerance levels this is usually a public health hazard.

1.2 Statement of the problem

The presence of residues of banned substances or substances permitted but exceeding the recommended limits by regulatory authorities in the event of veterinary drugs, pharmaceutically active substances and pharmaceutical products in milk is a big concern for public health. The presence of these substances may lead to allergic reactions, acute or chronic toxic effects, antimicrobial resistances, teratogenic and carcinogenic effects depending on the compound (FSSAI, 2012). Pesticide residues in milk have been associated with a broad variety of human health hazards ranging from chronic impacts like reproductive harm, endocrine disruption, wide variety of cancers and impaired immune function (Deti *et al*., 2014) and short-term impacts such as nausea and headaches. In addition, food containing residues of these chemicals exceeding MRLs

or the default tolerance of 0.01ppm cannot be marketed. Levels of these residues should be monitored regularly using appropriate analytical tools to minimize adverse health effects of residues from food.

Due to increase in population growth, there is a high demand for food production thus increasing pressure to enhance agricultural production by use of pesticides and veterinary drugs to control pests and livestock diseases. However, lack of awareness on the usage and hazardous effects associated with these chemicals among majority of the farmers is widespread, leading to mishandling and misuse of these chemicals resulting in environmental contamination. The past studies on pesticide residues in Kenya have been on fish (Omwenga *et al.*, 2016) and selected riverine ecosystem and marine (Wandiga *et al.,* 2002 and Wandiga *et al.,* 2006; Getanga *et al.,* 2004). There is limited data on veterinary drug and pesticide residues in cow and camel milk. This study was conducted to address this gap and provide data which can be used to access the safety aspect of milk before it reaches the consumer.

1.3 Hypothesis

i. Cow and Camel milk from Kiambu, Isiolo and Laikipia contain pesticides and veterinary drug residues above the recommended Maximum Residue Levels.
ii. There is lack of knowledge on health risks associated with consumption of milk products containing pesticides and veterinary drug residues.

1.4 Objectives

1.4.1 Overall objective

The main objective of this study is to determine the extent of veterinary drugs and pesticide residue contamination in milk from cow and camel milk in Kenya.

1.4.2 Specific Objectives

1) To determine the levels of pesticide contamination in camel and cow milk from Laikipia, Isiolo and Kiambu Counties and make recommendations to address the residues problem.
2) To determine the levels of veterinary drugs contamination in camel and cow milk from Laikipia, Isiolo and Kiambu Counties and make recommendations to address the residues problem.
3) To determine the effect of seasonal variation on the levels of pesticides and veterinary drugs residues in milk in Laikipia, Isiolo and Kiambu Counties.

1.5 Justification of the Study

Veterinary drug and pesticide residues found in milk may cause harmful effects to humans once consumed. Of particular concern is the possibility of these residues to causing cancer, allergy and development of resistance leading to treatment failures. In this regard, Ministry of Agriculture, Livestock, Fisheries and Irrigation and Ministry of Health are tasked with monitoring of the undesirable Veterinary drug and Pesticide residues in animal food intended for human consumption. However, there is no data generated regularly by the ministries on the residue levels of these chemicals in the animal products The main purpose of this monitoring is to confirm that such residues do not surpass Maximum Residue Limits (MRL). The provision of consistent and reliable data is still needed in Kenya for monitoring as well as risk assessment of harmful effects of Veterinary drug and Pesticides residues in animal products. Therefore, results from this study may form a basic intervention to human health from potentially harmful residues in cow and camel milk consumed in Kenya.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Usage of veterinary drugs

The usage of antibiotics drugs in human and veterinary medicine started in mid-1940 after the discovery of benzyl-penicillin in 1929 by Alexander Fleming. There are seven major families of antibiotic drugs which include; Sulfa-drugs, beta-lactams, aminoglycosides, macrolides/lincosamides, tetracycline, amphenicols and fluoroquinolones. Other antibiotics include novobiocin and spectinomycin. The most common veterinary drugs used in Kenya for the treatment of livestock diseases are aminoglycosides, tetracyclines, sulfonamides and beta-lactams (Aboge, 2002). A study by Mitema *et al*. (2001) revealed that approximately every year 14,600 kg of antibiotics are used in animals which produce food in Kenya from which approximately 78% are sulfonamides and tetracyclines.

In order to increase milk production some drugs are applied for ectoparasites, endoparasites control and other diseases. Intra-mammary infusion of antibiotics to control mastitis is used mainly for veterinary treatment of camel and dairy cattle. Antibiotics are mostly administered to animals by many routes such as topical on the skin, orally in water and food, by intrauterine infusions and intra-mammary, intravenous, intramuscular injection routes (Aytenfsu *et al*., 2016). Theoretically these routes may lead to presence of residues emerging in dairy products and milk. For a few days detectible levels of veterinary drug residues are usually detectable in the milk whenever in a lactating cow after the last treatment. In addition, antibiotics residues may hinder milk normal microflora and have adverse effect during manufacturing processes of various dairy products such as cheese and yoghurt (Kurjogi *et al*., 2019). Lack of good knowledge and advice on the stability

of veterinary drug residues and withdrawal periods leads to failure in controlling excessive use of Veterinary drugs.

About eighty percent of all food producing animals gets treatment always (Lee *et al*., 2001; Pavlov *et al*., 2008; Darwish *et al*., 2013). Some farmers treat the whole group of livestock in the farm such as fish, birds and only few animals are affected. Therefore, the situation unnecessarily and unintentionally exposes healthy animals to veterinary drugs (Darwish *et al*., 2013). The occurrence of pathogens on a farm depends on a variety of factors which includes the ecological pressure on a farm, type of husbandry and the normal stockman ship (Sattar *et al*., 2014). When the management of the farm is not optimal usage of veterinary drugs in animals for therapy and prophylaxis tend to increase or when widespread diseases are not well controlled (Sirdar, 2010). However, to control diseases that can lead to heavy usage of veterinary drugs for control and treatment of diseases in the farm, caused by increased density of livestock operations, serious rearing requires an aggressive approach (Cheong *et al*., 2010; Sattar *et al*., 2014). In a bid to reduce the cost of veterinary services, some farmers in Nigeria buy drugs from the market for treatment of diseases without sound diagnostic advice from a veterinarian leading to misuse and abuse of the drugs (Olatoye *et al*., 2010).

2.2 Antimicrobial Resistance

Afar from the spread of antimicrobial resistance in hospital environment, improved understanding of veterinary and environmental health factors (for example improved hygiene and sanitation, antibiotic stewardship in agriculture) are needed to reverse or slow down the rapid global spread of antibiotic-resistant bacteria (Ashbolt, 2013).

In low- and middle-income nations there are great concerns in development of antibiotic resistance, since science is missing important quantitative data from these high-risk areas for

antibiotic resistance. Globally, research requirements of antibiotic resistance are great in Africa, especially because of inadequate resources for discouraging antibiotic misuse in home, clinic and farm settings (Vlieghe *et al.,* 2009). Antibiotic resistance is a problem without borders as these genes can be transmitted quickly from animal-associated flora for example manure bacteria to pathogenic or nonpathogenic flora in the environment. These antibiotic resistance bacteria may then be acquired and shed by wildlife, livestock or humans that travel (WHO, 2012).

The role of cattle and cattle antibiotics for promoting human-acquired antibiotics in East Africa has gained a lot of attention among veterinary, medical and global public health communities (WHO, 2012). In peri-urban and rural areas of Kenya, livestock and humans are in close vicinity every day and may share drinking water sources. Animal-related fecal contamination of soil, water and food supplies with antibiotic resistance flora may be likely in the presence of antibiotics (Lupindu *et al.,* 2015). A recent U.K. report warned that if antimicrobial usage is unchecked, antibiotic resistance infections will result to being the world's leading killer by the year 2050 with the most important antibiotic resistance related mortality estimated to happen in Africa (Shallcross *et al.,*2015).

2.3 Studies done on Veterinary Drug Residues

A large study to assess public health hazards linked with marketed milk was carried out in Kenya, between January 1999 and January 2000. Samples were collected seasonally from raw (unpasteurized) milk consuming households and various cadres of informal market agents. The Charm-AIM screening test was used for screening samples and it showed that 5.7 % and 9.4 % of samples from market agents and consumer households had antimicrobial residues above EU MRLs, respectively (Aboge, 2002).

Another study carried out in Nandi and Makueni-Kenya out of 480 samples 114 samples were positive with antibiotic residues in cows' milk using Delvotest® screening test. 295 (61%) negative, 71 (15%) unclear. Group Specific Triensor test was further used which indicated that 9% of all the samples were positive, 2.5% sulfonamides,0.6% tetracyclines and 5 % beta-lactams. Positive sample were further tested with HPLC but antibiotics were not identified (Ahlberg *et al.,* 2016).

A study in milk testing antibiotic residues from milk vending machine in Eldoret-Kenya reported that out of 80 milk samples collected at least one antibiotic was positive using IDEXX SNAP® test for test for sulfamethazine, beta-lactams, tetracyclines and gentamicin (Kosgey *et al.,* 2018).

Orwa *et al.* (2017) reported that 72 samples out of 229 (31.4%) samples along the dairy sub value chain in the rural areas were positive with tetracyclines after screening although after confirmatory tests with HPLC-UV none of the samples was positive. Sulfonamides were detected in 60% of the samples with HPLC-UV, out of which 71% were above the maximum residue limits.

A study for antibiotic residues in marketed milk for human consumption in Kibera, Nairobi reported tetracyclines and beta-lactam residues as 3.2 % and 7.4 % of all the ninety-five samples of milk collected, respectively using IDEXX SNAP® kit (Brown *et al.,* 2020).

Shitandi and Sternesjo (2001) reported that out of 1109 milk samples from Nakuru, Kenya 229 (21%) samples were positive with antibiotics using improved Dutch tube diffusion test. 165 samples (14.9 %) were detected with penicillin G, 118 samples had levels above the EU MRL for penicillin (4 ppb).

In China Hebei province residues of lincomycin, trimethoprim, sulfacetamide and Penicillin G were detected in 12 samples though the latter were within the maximum residue levels regulated by China, United states, Codex Alimentarius Commission and European Union (Han *et al.,* 2017).

A study carried out in Kamataka, India, detected antibiotic residues in milk by microbiological assay, azithromycin and tetracycline antibiotics were detected at high concentration of 9,708.7 and 5,460 μg/kg, respectively (Kurjogi *et al.,* 2019). In Switzerland the level of non-compliant samples is small considering for many years they have implemented plans for national residue monitoring in food which are efficient (Ortelli *et al.,* 2018).

A few research reports in Ethiopia show the presence of chemical residue contamination in meat and milk such as Penicillin G and Oxytetracycline antibiotic residue. Organochlorine pesticide residue in cow milk and in human, tetracycline residue levels in slaughtered beef cattle were some of the studies which highlighted the importance of the problematic chemical residues in Ethiopia. (Aytenfsu *et al.,* 2016).

2.4 Pesticides and Insecticides

The usage of pesticides by farmers started in 1939 when Paul Müller, a Swiss entomologist discovered DDT. During World War II DDT played a major important role in the health and welfare of soldiers using it to control mosquitoes and body lice which transmitted dangerous diseases. By mid-1950s DDT became the most widely used pesticide worldwide replacing arsenic. However, the earliest usage of chemical to control pests dates back 2500BC (Hock *et al.,* 1991). Pesticides were derived from plants or inorganic products, for example burning Sulphur to control mites and insects. In the early years a variety of insects were controlled by the usage of insecticides such as pyrithrin, nicotine to control aphids, hellebore to control body lice (Hock *et al.,* 1991). In the year 1892 lead arsenate was used as an orchard spray and in the same year a mixture of copper sulphate and lime was accidentally discovered for controlling downy mildew, a dangerous fungal disease of grapes, which is still widely used as a fungicide (Hock *et al.,* 1991). These early

chemicals had disadvantages including high toxicity, damaging the crops they are meant to protect, very persistent and posing a threat to the ecosystem (Hock *et al.,* 1991).

Currently pesticides are sophisticated and carefully researched to ensure that they are efficient and effective against the target organisms by targeting specific biochemical reactions within the target organism for example an enzyme important for photosynthesis within a plant or a hormone necessary for normal insect development (Mwenda, 2011).

To control crop pests and animal ectoparasites Organochlorine pesticides (OCs) and Organophosphates (OPs) have been used in livestock and agriculture for a very long time (Aytenfsu *et al.,* 2016). To fight mosquitoes causing malaria and some other deadly diseases of human beings these pesticides are also employed. Lately several chemicals belonging to OCs like heptachlor, HCH, DDT, dieldrin, Aldrin etc. which are also significant components of the toxic group known as persistent organic pollutants (POPs), have been restricted or banned (Aytenfsu *et al.,* 2016).

Feeds can be contaminated in the store or field where they are treated with pesticides. OCs can enter dairy products and milk when the cow consumes contaminated feeds. The OCs are very persistent, stable, endocrine disrupting agents, bioaccumulating and toxic compounds that are widely distributed. They will find their way into the food chain mostly through their use in controlling and preventing environmental or animal pests (Aytenfsu *et al.,* 2016).

The high persistence nature of OCs due to their lipophilic character and chemical stability accumulate in different environment sections and also in food chains hence causing high health effects in human body. The OCs reduces at a very slow rate even after sources of contamination have been eliminated (Subir *et al.,* 2008). In the animal body, consumption of contaminated fodder and feed is the core source of entry of pesticides which ultimately results in the contamination of

meat, milk etc. consumed by human being. Consequently, human body also gets contaminated (Subir *et al.,* 2008).

2.5 Pesticides in Kenya

The usage of pesticides in Kenya dates back in the early 1900s. Sodium arsenates were the first pesticides to be introduced as acaricides between 1912 and 1949 for controlling vectors which transmitted East Coast Fever disease in livestock (Keating, 1983). In 1949 Hexachlorohexane (HCH) and Benzene Hexachloride (BHC) were introduced for vector control. In 1950 toxaphene was introduced due to development of resistance by strains of ticks caused by continuous usage of previous acaricides. National Environmental Action plan (NEAP, 1994) banned toxaphene, a chlorinated camphene acaricide which was more stable in dip washes and residual effect (Keating, 1983).

DDT and dieldrin were introduced in 1956 and 1961 respectively. Other acaricides introduced the same period include tetraethylprophosphate (TEPP), dinitroeresol (DNC), dioxathion, schradan and organophosphorus compounds. Organochlorines were banned for tick control due to strains of ticks developing resistance in 1986 (Keating, 1983).

Currently over 1,000 pesticides are used in the modern agricultural practices; in 2018 Kenya imported approximately 17,803 tonnes of pesticides valued at 128 million dollars of pesticides: herbicides, insecticides, fungicides, fumigants, rodenticides, proteins, surfactants, defoliators, growth regulators and wetting agents. Of the total pesticide imports, fungicides, herbicides and insecticides accounts for about 87% in terms of volume and 88% of the total cost of pesticides imported. The volume of imported herbicides, fungicides and insecticides have increased within four years from 6400 tons in 2015 to 15600 tons in 2018 with a growth rate of 144% (GoK, 2020)

2.6 Studies done on Pesticide residues

A study on pesticide residues in cow and human milk, fruits, flowers, vegetables, fish, beef, was done in different areas of Kenya. Chlorfenvinphos residues was reported in cow milk ranging from 1.58 to 10.69 mg/kg during the wet season and 0.52 to 3.90 mg/kg during the dry season. Dairy cow which was occasionally plunged in cattle dip wash for tick control had the highest pesticides residue levels (Kituyi *et al.*, 1997). γ-HCH was reported in human milk ranging concentration from 9×10^{-6} to 1.0 mg/kg from a Nairobi Hospital (Wandiga *et al.*, 1988). A study by Kanja (1988) earlier had reported 13 organochlorine residues in human milk from 8 different areas in Kenya. The study reported that breasting mothers living in urban areas had lower levels of organochlorines residues in their breast milk than their counter parts living in rural areas.

Levels of heptachlor, aldrin heptachlor epoxide, endrin, diedrin, methoxychlor, endosulphan, lindane, α-BHC, β-BHC were reported by Getenga *et al.* (2004) from a river draining the sugarcane fields and soils in the same fields with residue levels ranging from 0.219 to 0.691mg/L). Wandiga *et al.* (2002) reported pesticide residues in sediments, seaweeds, sea water and fish including endrin, dieldrin, aldrin, α-endosulphan, lindane, *p,p'*-DDD, *p,p'*-DDT and *p,p'*-DDE. The residues ranged from 0.584 to 59.00 ng/g in sediments, 0.503 to 9.025 ng/g in sea water and concentration of 418 ng/g, 1011 ng/g of *p,p'*-DDD and *p,p'*-DDT in fish, respectively). Kanja *et al.* (1999) studied 41 samples of milk subcutaneous fat, maternal blood and umbilical blood from women giving birth by caesarean operation at a Hospital in Nairobi. The presence of lindane, dieldrin, β-HCH, transnonachlor, *o,p'*-DDT, *p,p'*-DDE, *p,p'*-DDT was reported with mean levels of 4.86 mg/g in mothers' milk, 2.75 mg/kg in maternal serum 1.9 mg/kg in umbilical cord serum and total DDT 5.9 mg/kg in subcutaneous fat. Residues of β-HCH in milk fat and subcutaneous fat were 0.26 mg/Kg, 0.034 mg/Kg respectively. Mwenda (2011) studied a total of 17 Organochlorines

pesticide residues in cow milk and reported a mean concentration ranging between 0.0011 to 4361 ng/Kg, *p,p'*-DDE with the highest concentration of 4861.19ng/Kg. Abong'o *et al.* (2018) reported residues of methoxychlor as 8.817 ±0.020 μgL^{-1}in water, sediments (92.893±3.039 µg Kg^{-1}) and weeds (39.641 ±3.045 µg Kg^{-1}) from the river Nyando catchment, Lake Victoria, Kenya. Ndunda *et al.* (2018) reported organochlorines residues in water and sediment ranging from below the detection limit to 39.7 ngL^{-1} and 0.01 to 41.9 µg Kg^{-1}, respectively from the Nairobi River. Madadi (2005) reported Organophosphorus and organochlorine residues levels ranging from BDL-10.07 µg/Kg in weeds, BDL-65.48 µg/Kg in soil, BDL-481.18 µg/Kg in fish and BDL-0.44 µg/Kg in water from river Sio and Nzoia, Lake Victoria catchment area.

A study was done in India on pesticides residues on cows, 206 (63.38%) samples were contaminated out of 325 samples with residues of different organochlorines (Nag & Raikwar, 2008).

2.7 Metabolism and excretion

Metabolism and excretion of various antibiotics have been studied. FAO/WHO (1996) reported that a beta-lactam drug, ceftiofur was rapidly metabolized to desfuroyl-certiofur and excreted in faeces and urine following intra-muscular administration in cattle. The other metabolite of ceftiofur is furic acid. The residue of the metabolites was also demonstrated in milk of a lactating cow (Aboge, 2002).

In another study, FAO/WHO (1996) reported that tetracycline and chlortetracycline undergo minimal metabolism. They are eliminated in both faeces and urine, either in microbiologically inactive form after administration in animals or unchanged. Following intra-mammalian and intrauterine administration Chlortetracycline residues were also detected in milk of lactating dairy cow.

The metabolism of sulphonamides takes place with glucuronidation, acetylation and oxidation occurring to varying degrees. Acetylation route is the most significant route. The unbound sulphonamide is excreted in the kidney by the glomerulus and also excreted actively in the proximal tubules as ionized molecules (Roudaut & Moretain, 1990). Demonstration of residues of sulphonamide in milk of lactating cow following intravenous injection means the drug is also excreted in milk. Other elimination routes of antibiotics drugs include biliary excretion and sweat. Ingestion, inhalation and dermal exposure are the main routes of exposure in human to pesticides and other chemicals (Roudaut & Moretain, 1990). The presence of sulphonamide residues in milk is of great concern because of its ability of allergic reactions in hypersensitive individuals and producing antibiotic-resistant strains. Additionally, the carcinogenic potential of sulphonamide residues causes effects to the liver, urinary tract and hematopoietic disorders (Armentano *et al.*, 2018).

2.8 Public Health Hazards

Usage of antibiotics and pesticides for treatment of animal diseases and parasites has led to much concern about the possible health hazards to human caused by their use. Some of the drugs are excreted in milk when withdrawal periods are not observed and are likely to cause some health problems when human consumes milk containing the residues.

Hypersensitivity – Lon and short-term Hypersensitivity reactions have been reported for many antibiotics including tetracycline group. However, they are not as common as those of penicillin. These effects can be lethal if they are very severe (Anderson, 1968; Olson & Sanders, 1975).

Antibiotic Resistance Problems-Resistance to antibiotics drugs develops following exposure to sub-therapeutic levels of these drugs after consumption of animal food products including milk containing the drug residues. Within a group of tetracyclines antibiotics, studies have shown

existence of cross-resistance. This means microorganisms that are resistant to one of the tetracyclines are often resistant to other compounds in this class (Brown, 1988; Kapusnik-Uner *et al.,* 2001; Chopra *et al.,*1992 and Roberts,1996).

2.8.1 Gastrointestinal Disturbances

Tetracyclines can cause irritation of gastrointestinal tract in human after oral administration. The seriousness depends on the type of tetracyclines and the dosage. The clinical signs include vomiting, abdominal discomfort, nausea and epigastric burning (Kapusnik-Uner *et al.,* 2001).

2.8.2 Miscellaneous effects

Following use of tetracyclines, liver damage in pregnant women was reported by Schultz *et al.* (1963). Tetracyclines particularly oxytetracyclines have been reported to cause dental hypoplasia and yellowing of teeth.

2.8.3 Toxic Effects of Pesticides

Health effects of pesticides may be acute or chronic in those who are exposed. Some of the health problems include hypertrophy of hepatocytes, hepatic tumors, hyper-susceptibility to external stimuli (light, touch and sound), irritability, tremor, vertigo, paresthesia of tongue, lips and face, dizziness and convulsions.

2.9 Maximum Residue Limit (MRL)

Maximum residue limit is the concentration in tissue below which a marker residue for the chemical or drug must fall in the target tissue before that animal edible tissues are considered safe for human consumption (Seri, 2013). Maximum Residue Limit is established using Acceptable Daily Intake (ADI) entirely on the basis of safety to the consumer of the product and does not have pharmacodynamic relevance in the animal to which the drug has been administered (Seri, 2013).

ADI is the amount of a food additive, expressed as mg/kg body weight that can be ingested daily over a lifetime without incurring any appreciable health risk. MRL is calculated by back calculation from the acceptable daily intake taking into account the maximum amounts of edible animal food that would be consumed per day for residues of veterinary medicine and livestock feed additives (Benford, 2000).

Many countries have established maximum residue limits to protect consumers from risks related to drug residues. The main reason of MRL is to limit the exposure of consumer to residues of the chemicals or drugs used in foods of animal origin, to concentrations that do not cause human health risk (Kennedy *et al*., 2000; Sasanya *et al*., 2005). In Europe, Canada and the U.S.A, the MRL for total sulfonamide concentration in edible tissues is 0.1μg/g (Zhang *et al*., 2009). Some sulphonamides have a single MRL unlike other veterinary drugs which may lead to serious health risks to consumers of animal products especially when sulfonamides are misused. The risk is higher if sulfonamides are not recommended for use in certain foods of animal origin (Sasanya *et al*., 2005). African countries have adopted MRLs from Codex limits or that of the importing country.

2.10 Laws and regulations governing Veterinary drug and Pesticides use.

To safeguard human health, the regulatory authorities need to conduct regular surveillance and monitor antimicrobial levels (Shahid *et al*., 2007). It is for this reason that internationally recognized human health agencies such as World Health Organization (WHO), World Organization for Animal Health (OIE), Food and Agriculture Organization of United Nation (FAO) and European Commission (EC) have emphasized the importance of prudent and rational use of antibiotics in animals. This is to minimize the possible impact of antimicrobial use in animals on public health (Magalhães *et al*., 2012). Various health agencies have established MRLs

for different classes of antimicrobials for various target animal species and target tissues to safeguard against veterinary drug residues exceeding the acceptable levels.

Food stakeholder and industries need to ensure that food complies with all the legal requirements and regulations at all areas of the food chain. Although regulatory bodies like Codex, EU and US have set, reviewed and harmonized pesticides MRLs, globally these limits remain variable (Zikankuba *et al*., 2019). Developed countries have more strict regulations than developing countries, with the developing countries lacking commitment, expertise, resources and willingness to impose legislation on pesticide residues.

There is need for establishment of national residue avoidance and control program in Kenya in accordance with national and international regulation by the Ministry of Livestock Development (Azegele, 2010). Table 2.1 shows the recommended MRLs for veterinary drugs in milk.

Table 2.1: Maximum Residue Limit (MRLs)(ng/mL) for Veterinary Drug Residues

Antimicrobial	**MRL**
Benzypencillin	**4**
Ampicillin	4
Amoxycillin	4
Oxacillin	30
Cloxacillin	30
Dicloxacillin	30
Tetracycline	100
Oxytetracycline	100
Streptomycin	200

Dihydrostreptomycin	200
Gentamycine	200
Neomycin	100
Sulphonamides	100
Trimethoprim	50
Spiramycin	200
Tylosine	50
Erythromycin	40
Quinalones	75
Polymixine	50
Centiofur	100
Cefquinome	20
Nitrofurans	0.000
Nitromidazoles	0.000
Other chemotherapeutics (Chloramphenicols Novobiocine)	0.000

Source: Commission Regulation (EU) NO. 37/2010

Table 2.2 shows the MRLs for pesticides in milk.

Table 2.2: Maximum Residue Limit (MRL)(ng/ml) for Pesticide Residues

Antimicrobial	**MRL**
Methacrifos	10
Disulfoton	10
Terbufos	10
Tolclofos methyl	10
Malathion	20
Fenamiphos	5
Profenofos	10
DDT	40
Hexachlorobenzene	6
Lindane (Gamma, Alpha, Beta, Delta-BHC)	10
Heptachlor	4
Trans & Cis Chlordane	2
Endosulfan	50
Dieldrin	6
Chlorfenson	50
Endrin	0.8

Source: EU Pesticide database

2.11 Analytical methods for Pesticide and Veterinary drug residues

Residue analysis in foods of animal origin is important worldwide for maintaining compliance with export regulations and evaluating food safety (Lake and Kahler,2012). The European Union through Decision 2002/657/EC, Decision 93/257/EC and Decision 93/256/EC provides criteria for identification, confirmation for monitoring compliance and analytical methods for testing official samples (Shankar *et al*., 2007).

2.11.1 Microbiological methods for screening veterinary drug residues

Microbiological or bio-assay techniques are widely used as screening methods and do not require high skilled manpower and expensive machines. However, they are limited only to those analytes that either inhibit or promote microbiological growth (Wang *et al*., 2006). They are the earliest methods used to detect veterinary drug residues in foods and were based on detection of growth inhibition of various bacterial strains. (Sattar *et al*., 2014). Microbiological methods estimate the total residues detected semi-quantitatively but do not distinguish between members of different classes of antimicrobials (Stolker *et al*., 2008).

2.11.2 Enzyme Linked Immunosorbent Assay (ELISA)

The use Enzyme Linked Immunosorbent Assay to detect several sulfonamides has been reported in the past (Dixon-Holland and Kartz, 1988; Jackman *et al*., 1993). ELISA is an immunological method based on the interaction of antigen-antibody which is very specific for a particular residue (Shankar *et al*., 2010).

Due to allergic or toxic reactions and the widespread use of sulfamethazine, many ELISA methods have been developed for its detection (Wang *et al*., 2006). Currently, ELISA kits of different types are commercially available for a large number of antimicrobials.

2.11.3 Chromatography Methods-Confirmatory methods

Gas Chromatography-Tandem Mass Spectrometry (GC-MS/MS)

GC method are widely used for the analysis of pesticide of residues in fatty food commodities due to their hydrophobic and volatile properties. Separation of pesticides happens in the capillary columns with different stationary phase (Madej *et al*., 2018). GC coupled with tandem mass spectrometry has been employed most often to determine pesticide residues at trace levels (Dallegrave *et al*., 2016). GC with double mass spectrometry attains high levels of sensitivity and lower detection limits for pesticide multi-residue analysis (Dallegrave *et al*., 2016; Deme *et al*., 2014; Han *et al*., 2016; Peng *et al*., 2015).

Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

Liquid chromatography is an analytical technique used for determination of veterinary drug and pesticides residues in matrices with high lipids. It is mostly appropriate for analysis of non-volatile, polar and or thermally labile pesticides (Madej *et al*., 2018). LC in combination with MS(MS) has a high sensitivity and selectivity in analysis of veterinary drug residues (Anagnostopoulos & Miliadis, 2013; Chung & Chan, 2010; Gomez-Almenar *et al*., 2015). Numerous reversed phases have been employed in the LC separation of veterinary drugs and pesticides residues such as C18, C8 or C12 with a preference for octadecyl-bonded silica (C18). Acetonitrile, methanol with formic acid have been used as mobile phase in a gradient flow (Madej *et al*., 2018). LC-MS/MS methods with triple quadrupole (QQQ) or ion-trap instruments in multiple reaction monitoring (MRM) mode have been the most powerful techniques for veterinary drugs and pesticides analysis in complex fatty matrices.

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Study area

The study area was in Kiambu, Laikipia (Nanyuki) and Isiolo Counties. Kiambu County (Figure 3.1) sampling was done in four Wards namely; Githunguri Latitude: -1.056522 Longitude: 36.778349, Komothai Latitude: -1.992171 Longitude:36.782721, Kiamathae Latitude: -1.047311 Longitude:36.791812 and Ngewa ward Latitude: -1.067521 Longitude: 36.768351(GPS). The area is 1,679 m above sea level, characterized by high elevation plains, hills and plateaus. The area is generally a dairy and tea zone area though other activities such as sheep farming, horticultural crops and maize activities are being practiced. Kiambu has a population of 1,623,282, and an area of 2450 km². It's divided into four broad topographical zones lower midland zone, upper midland, lower highland and upper highland. The climate in Kiambu is warm and temperate, there is a lot of rain even during the driest month. The rainy months are between April, May and June and between October, November and December. The dry months are between July, August and September and between January, February and March. Kiambu County currently lead in cow milk production in Kenya with approximately 350 million Kgs annually (Karanja, 2003; Ong'aro, 2012). In Nanyuki County samples were collected from Doldol Ward Latitude: 0.400260 Longitude:37. 166502 (GPS). Nanyuki has a population of 49,233 and an area of 8,696.1km². The Equator passes through the southern part of Nanyuki. Physical features include Mount Kenya National Park and Ol Pejeta Conservancy. The rainy months are between April, May and June and between October, November and December. The dry months are between June, July and September and between January, February and March. In Isiolo County sampling was done in three wards namely; Kashuru Latitude: 1.006060 Longitude: 38.750045, Kina Latitude: 1.015050

Longitude 38.740124 and Mulango Latitude: 1.007050 Longitude: 38.731040 (GPS). Isiolo has a population of 143,294 and an area of 25,336.1km². The rainy months are between March, April and May and between October, November and December. The dry months are between July, August and September and January, February and March. the local topography is arid or semi-arid low plains. Livestock production forms the backbone of the county's economy with nomadic pastoralism being the major lifestyle of Isiolo people. The map of the study area is shown in Figure 3.1 below.

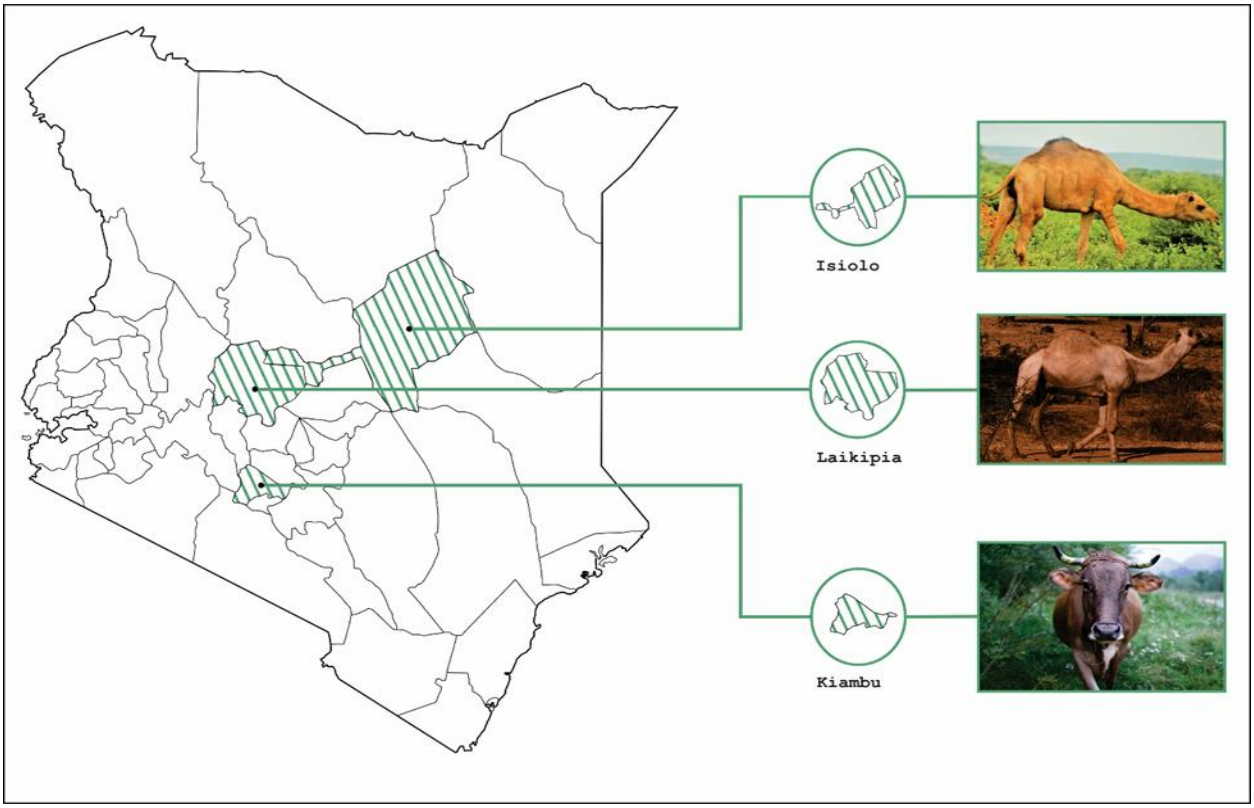

Figure 3.1: Map of Kenya showing the Cow and Camel milk sample collection areas. Images of Camel (s) and Cow adapted from Wikimedia commons

3.2 Study design and Sample collection

For each of county raw milk was collected seasonally at collection centers between 2017 and 2018. Cow raw milk samples were randomly collected in Kiambu county from 4 different wards; Githunguri, Kiamathae, Komothai & Ngewa wards during the wet season of October, November and December and dry season of January, February and March. Camel raw milk samples were randomly collected in Isiolo county from three wards; Kashuru, Kina & Mulango wards and Doldol ward in Nanyuki, Laikipia county during the wet season of October, November and December and dry season of January, February and March.

Milk was collected in disposable plastic 50 mL falcon centrifuge tubes. A total of 82 camel milk and 90 cow milk samples were collected in duplicate. The samples were labelled with respect to date, location, time and season then packed into ice-bags. The samples collected were transported the same day in a cool box containing ice packs to the Analytical Chemistry and Food Safety Laboratory-Directorate of Veterinary Services, Kabete for storage at -20 °C in the deep freezer before analysis.

3.3 Laboratory Analysis of Pesticide Residues in Milk

3.3.1 Chemicals and reagents

Acetonitrile and isooctane were of the highest purity available (99.9%) purchased from Merck. The Organophosphates standards (methacrifos, disulfoton, terbufos, tolclofos methyl, fenchlorphos, malathion, tetrachlorvinfos, fenamiphos, chlorthiophos, profenofos, leptophos, coumaphos and Organochlorine standard (alpha-BHC, beta-BHC, hexachlorobenzene, gamma-BHC (lindane), delta-BHC, heptachlor, cis-chlordane, trans-chlordane, *p'p*-DDE, alpha-endosulfan, dieldrin, *o,p'*-DDD, trans-nonachlor, chlorfenson, *p,p'*-DDD, endrin, beta-endosulfan, *p,p'*-DDT, *o,p'*-DDT, cis-Nonachlor, mirex were purchased from Restec company as GC

multiresidue pesticide kit(100 μg/mL each in toluene, 1mL/ampul). QuEChERS extraction tubes AOAC method and pre-weighed salts (6 g magnesium Sulphate, 1.5g sodium acetate) and clean up by dispersive solid phase extraction(d-SPE)15 mL fatty samples AOAC tubes with ready to use salts (400 mg PSA, 400 mg C18EC, 1200 mg magnesium sulphate) were all purchased from Agilent Technologies through Nesvax Innovations Ltd Nairobi, Kenya.

3.3.2 Preparation of Standard Solution

Stock solution (1 μg/mL) was prepared by weighing 200 μL of the multiresidue pesticide standard into a 20 mL volumetric flask and topping up with isooctane. The standard stock solution was used to prepare various working concentrations through serial dilutions. The standard stock solution and other working solutions were stored in a refrigerator at -20 °C.

The standard for spiking experiment was obtained by transferring predefined volumes of family mixes from 150 -1500 μL.

3.3.3 Extraction of Pesticide Residues in Milk

Milk samples were extracted using QuEChERS technique by weighing 15 mL of whole milk into a 50 mL polypropylene centrifuge tube. To determine the accuracy of the method an appropriate amount of the pesticide spiking standard was added to the fortified blank sample. The blank milk sample was analyzed before spiking to confirm the absence of pesticide residues, vortexed for 60 seconds, allowing for 30 minutes for the interaction of the matrix and the standard before extraction.

An aliquot of 15 mL of acetonitrile was added to each milk sample, capped and vortexed for 1-minute, pre-weighed salts (6 g magnesium sulfate, 1.5 g sodium acetate) were added and immediately vigorously shaken by hand for 1 minute to prevent the agglomeration of salts and then centrifuged at 5000 rpm for 10 min at 4 °C.

An aliquot of 6 mL of the supernatant layer was transferred into a 15 mL tube containing 400 mg PSA, 400 mg C18EC, 1,200 mg magnesium sulfate, capped and vortexed for 1 minute then centrifuged at 5000 rpm for 10 minutes. An aliquot 1 mL of extract was transferred into another tube and dried by nitrogen flow at 40 °C and reconstituted with 1 mL of isooctane, filtered with disposable filters 0.45 μm membrane into GC-MS/MS autosampler vails for GC-MS/MS analysis.

3.3.4 Gas Chromatography Tandem Mass Spectroscopy

The pesticide residues were detected using a GC-MS-TQ8040 Shimadzu, Japan equipped with AOC-20s autosampler and AOC-20i autoinjector all from Shamadzu, Japan. The analytical capillary column was a ZB-5ms with thickness of 0.25 μm length 30.0 m and diameter of 0.25 mm.

The column temperature was maintained at 50 °C for 1 minute then programmed at 25 °C/min up to 125 °C then finally 10 °C up to 300 °C for 3.50 minutes which was held for 20 minutes. Helium (99.999% purity) was used as the carrier gas at flow rate of 1.69 mL/min. The injection port temperature was 250 °C and 1 μL as the injection volume with spitless as the injection mode and purge flow at 5 mL/min. The ion source temperature was at 200 °C. Interface temperature at 250 °C, solvent cut at 1.5 minute and detector voltage 0.5 KV. Multiple Reaction Monitoring (MRM) was used for conversion from MS1 (parent ion) to MS2 (daughter ion). Retention time windows and base peak ions were designed for OP and OC pesticides.

All pesticides were identified with specific ions, retention time and quantified using the external standard method. Data acquisition and quantification was performed using Lab Solutions software GCMS (Release 4.42) Shimadzu.

3.3.5 Validation of a modified GC-MS/MS Method.

To obtain reliable data and determine the quality of the method it was important to validate a modified GC-MS/MS method. The following parameters were used to validate the method before it was used for analysis of milk; accuracy, linearity, selectivity, limit of detection (LOD), and limit of quantification (LOQ).

3.3.6 Determination of accuracy of the method.

To determine the accuracy of the method recovery studies were carried out using milk blank samples spiked at 10 ppb, 50 ppb and 100 ppb with the pesticide standards. The blank milk sample was analyzed before spiking to confirm the absence of pesticides residues The amount of analyte recovered after complete sample extraction and processing step was determined. The average recoveries of spiked OCs samples ranged from 73% to 97% and 78% to 100% for OPs. All samples were treated and analyzed using GC-MS/MS-MRM procedure described above. (Table A1 is for OCs recoveries, Table A2 is for OPs recoveries).

3.3.7 Determination of linearity of the method

To determine the linearity of the GC-MS/MS method, a standard curve was constructed. The mixture of OPs and OCs pesticides calibration standards were prepared using concentrations of 20 ppb, 30 ppb, 50 ppb,100 ppb and 200 ppb. The calibration standards were prepared from the mixed (OP & OC) stock solution of 1 ppm through serial dilution. Linearity was then determined by regression analysis of the peak area against analyte concentrations and was expressed by the linear regression coefficient (R^2). Generally, an R^2 value of >0.998 is considered as evidence of an acceptable fit of data to the regression line (Singh, 2013). In this study linear regression range was from 0.991-0.999 (Appendix 3).

3.3.8 Determination of selectivity of the method

The ability of the method to detect and separate OPs and OCs in the mixture without interferences from other components in the mixture was determined. The selectivity of the method was determined when chromatographic peaks obtained from the mixture of the pesticide standard analysis showed the absence of interfering peaks (Appendix 3).

3.3.9 Determination of limit of Detection (LOD) and limit of Quantification (LOQ)

The lowest concentration at which OCs and Ops could be detected and also quantified was determined using the formula $S_{LOD}=S_{RB}+3\sigma_{RB}$, $SLOQ=S_{RB}+10\sigma_{RB}$, where S_{LOD} is the signal at the limit of detection LOD, SLOQ signal at the limit of quantification LOQ, S_{RB} signal of the reagent blank, σ_{RB}standard deviation of the reagent blank, according to the directives of IUPAC and the American Chemical Society's Committee on Environmental Analytical Chemistry. LOD for OCs range from 0.1-0.3 ng/mL. LOD for OPs range from 0.02-0.3 ng/mL. Relative standard deviation (RSD) was less than 3% (Table A4 for OCs, Table A5 for OPs).

3.4 Data analysis

The laboratory data on milk samples was entered in Microsoft Excel for detailed data analysis and later exported to SPSS software for descriptive statistics.

Variations in the pesticide levels in milk collected from the different counties was evaluated using Analysis of variance and Tukey's post hoc test. Differences in the pesticides levels in milk samples collected in the dry and wet seasons were evaluated using the independent sample student's t-test. $p< 0.05$ was considered significant in all cases.

3.5 Laboratory analysis of Veterinary drug residues in milk

3.5.1 Chemicals and Reagents

Acetonitrile, water and methanol were of the highest purity available (LC-MS grade) were purchased from Merck. The standards for sulphonamides (sulfadimethoxine, sulfamethazine, sulfadoxine, sulfachloropyradazine, sulfamethoxypyradine, sulfamerazine, sulfathiazole, sulfamethoxazole, sulfagunadine, sulfadiazine, sulfapyridine) were purchased from Kobian Sigma-Aldrich, Germany. QuEChERS extraction tubes (50 mL) and pre-weighed salts (4 g sodium sulphate, 1 g sodium chloride) and clean up by dispersive solid phase extraction(d-SPE)15 mL PTFE centrifuge tubes with pre-weighed salts (50 mg PSA, 150 mg C18EC, 900 mg of anhydrous sodium sulphate) were all purchased from Agilent Technologies through Nesvax Innovations Ltd Nairobi, Kenya.

3.5.2 Preparation of Standard Solution

To make a stock solution of 1 mg/ml,10 mg of each of the standard powder (sulphonamides) was weighed into a separate 10 mL glass volumetric flask, dissolved and filled up to the mark with methanol.

An aliquot of 20 μL of each of the above solution was weighed into a 20 mL volumetric flask and topped up with methanol to make a mix of sulphonamides standard with concentration of 1 μg/mL which was used to prepare various working concentrations through serial dilutions. The standard stock solution and other working solutions were stored in a refrigerator at 4 °C.

The standard for spiking experiment was obtained by transferring predefined volumes of family mixes from 100 -1000 μL.

3.6 Extraction of Veterinary drug Residues in milk

Milk samples were extracted using QuEChERS technique by weighing 10 mL of whole milk into a 50 mL polypropylene centrifuge tube. An appropriate amount of the sulphonamides mixed with spiking standard was added to the fortified blank sample and vortexed for 60 seconds, allowing for 30 minutes for the interaction of the matrix and the standard before extraction.

An aliquot of 10 mL of 1% acetic acid in acetonitrile was added to each milk sample, capped and vortexed for 1-minute, pre-weighed salts (4 g sodium sulphate,1 g sodium chloride) were added and vigorously shaken by hand or vortexed for 1 minute to break up salt agglomerates then centrifuged at 5000 rpm for 10 min at 4 °C.

An aliquot of 6 mL of the supernatant layer was transferred into a 15 mL PTFE centrifuge tube containing 50 mg PSA, 150 mg C18EC, 900 mg anhydrous sodium sulphate, capped and vortexed for 1 minute then centrifuged at 5000 rpm for 10 minutes. An aliquot of 1 mL of extract was transferred into another tube and dried by nitrogen flow at 40 °C and reconstituted with 1 mL of acetonitrile/water (1/9 v/v) filtered with disposable filters 0.45 μm membrane into LC-MS/MS autosampler vails for UHPLC-MS/MS analysis.

3.6.1 Instrumentation

UHPLC Agilent Technologies 1290 Infinity II was coupled with Agilent Technologies 6460 triple quad mass spectrometer (UHPLC-MS/MS) operating in electrospray (ESI) source in positive mode. The LC analyses were run on a UHPLC Zobrax SB-Aq,RRHD 1.8 μm particle size (2.1 x 150 mm). The column was equilibrated at 35 °C. Data acquisition and quantification was performed using Mass hunter software Version B.07.00SP2 (Agilent).

Centrifugation were performed in a high-volume centrifuge Thermoscientific Heraeus Multifige XIR.A Vortex mixer from Digi system and sartorius analytical balance were used.

UHPLC-MS/MS analysis

The mobile phase consisted of 0.1% formic acid aqueous solution (A) and Acetonitrile (B) at a flow rate of 0.2 mL/min. Gradient elution employed with the ratio of A:B varied as follows:0.0 min, 98:2,1.50 min; 98:2,1.60 min, 98:2,15.0 min, 5:95,20.0min, 5:95. Instrument control, data acquisition and evaluation were done with Mass hunter software Version B.07.00SP2 (Agilent). MS applied parameters were: Ion source was AJS ESI mode, scan type MRM and daughters scan were used, precursor ion 2 product ions, dwell time 10 minutes, ion source gas temperature 325 °C, gas flow 11l/min, nebulizer 45psi, sheath gas temperature 250 °C, sheath gas flow 11 l/min, Capillary positive voltage 3500 V, Capillary negative voltage 3500 V.

3.6.2 Validation of a modified UHPLC-MS/MS Method.

To obtain reliable data and determine the quality of the method it was important to validate a modified UHPLC-MS/MS method. The following parameters were used to validate the method before it was used for analysis of milk; accuracy, linearity, selectivity, limit of detection (LOD), limit of quantification (LOQ).

3.6.3 Determination of accuracy of the method.

To determine the accuracy of the method recovery studies were carried out using milk blank samples spiked at 10 ppb, 50 ppb and 100 ppb with sulphonamides standard. The blank milk sample was analyzed before spiking to confirm the absence of sulphonamides residues. The amount of analyte recovered after complete sample extraction and processing step was determined. The average recoveries of spiked samples with sulphonamides standard ranged from 92% to 99%. All samples were treated and analyzed using UHPLC-MS/MS-MRM procedure described above (Appendix 5).

3.6.4 Determination of linearity of the method

To determine the linearity of the UHPLC-MS/MS method, a standard curve was constructed. The sulphonamides calibration standard mixture was prepared using concentrations of 10 ppb, 20 ppb, 30 ppb, 50 ppb and 100 ppb. The calibration standards were prepared from the mixed sulphonamides stock solution of 1ppm through serial dilution. Linearity was then determined by regression analysis of the peak area against analyte concentrations and was expressed by the linear regression coefficient (R^2). R^2 value of >0.998 is generally considered as proof of an acceptable fit of data to the regression line (Singh, 2013). In this study linear regression range was from 0.97-0.998 (Appendix6).

3.6.5 Determination of selectivity of the method

The ability of the method to detect and separate sulphonamides in the mixture without interferences from other components in the mixture was determined. The selectivity of the method was determined when chromatographic peaks obtained from the mixture of the sulphonamides standard analysis showed the absence of interfering peaks (Appendix 7).

3.6.6 Determination of limit of Detection (LOD) and limit of Quantification (LOQ)

The lowest concentration at which sulphonamides could be detected and also quantified was determined was using the formula $S_{LOD}=S_{RB}+3\sigma_{RB}$, $SLOQ=S_{RB}+10\sigma_{RB}$, where S_{LOD} is the signal at the limit of detection LOD, S_{LOQ} signal at the limit of quantification LOQ, S_{RB} signal of the reagent blank, σ_{RB} standard deviation of the reagent blank, according to the directives of IUPAC and the American Chemical Society's Committee on Environmental Analytical Chemistry. LOD for veterinary drugs range from 0.002-0.04 ng/mL, LOQ range from 0.02-0.05 ng/mL. Relative standard deviation (RSD) was less than 3% (Table A7).

3.7 Data analysis

The laboratory data on milk samples were entered in Microsoft Excel for detailed data analysis and later exported to SPSS software for descriptive statistics. Variations in the Veterinary drug residue in milk collected from the different counties was evaluated using Analysis of variance and Tukey's post hoc test. Differences in the veterinary drug residues levels in milk samples collected in the dry and wet seasons were evaluated using the independent sample student's t-test. $p< 0.05$ was considered significant in all cases.

CHAPTER FOUR

4. RESULTS AND DISCUSSION

4.1 Organophosphate pesticides in cow and camel milk

4.1.1 Organophosphate pesticides in cow milk from Kiambu County

A total of 90 cow milk samples from Kiambu County were analyzed for organophosphates pesticide residues. The mean levels of the organophosphates tested in Cow milk were all below the maximum residue limits set by the Codex Alimentarius, European Union Pesticide database and the United States Department of Agriculture (USDA) guidelines as shown in Table 4.1

The following organophosphates were identified; Chlorthiphos-1, coumaphos, disulfoton, fenamiphos, methacrifos, tetrachlorvinphos and malathion. (Figure 4.1)

Chlorthiophos-1 had the highest mean concentration(ng/mL) of 46.07±0.88 ranging between not detectable (limit of detection=0.2 ng/mL) to 387.47 ng/mL. Out of 90 samples 30(34.66%) samples had chlorthiophos-1. The Maximum Residue Limit (MRL) for chlorthiophos is not set by the Codex Alimentarius, European Union Pesticide database and the United States Department of Agriculture (USDA).

Coumaphos was detected in 72 (82.39%) samples with mean levels ranging between not detectable to 5.80 ng/mL and a mean concentration (ng/mL) of 4.53±2.10. This level is below the maximum residue limit of 1,250 ng/mL set by United State Department of Agriculture.

Disulfoton was detected in 40 (46.02%) samples and ranged between not detectable to 5.08 ng/mL with a mean concentration (ng/mL) of 5.80 ±0.38. This level is below the maximum residue level of 10 ng/mL set by Codex Alimentarius and European Union Pesticide database.

Fenamiphos was detected in 71 (80.68%) samples and ranged between not detectable to 1.65 ng/mL with a mean concentration (ng/mL) of 1.24 $\pm$0.61. The mean level is below the maximum residue level of 5 ng/mL set by Codex Alimentarius and European Union Pesticide database.

Methacrifos was detected in 64 (72.73%) samples and ranged between not detectable to 1.30 ng/mL with a mean concentration (ng/mL) of 0.88 $\pm$0.55. The mean level is below the maximum residue level of 10 ng/mL set by European Union Pesticide database.

Tetrachlorvinphos was detected in 48 (55.31%) samples and ranged between not detectable to 1.42 ng/mL with a mean concentration (ng/mL) of 0.77 $\pm$0.68. The mean level is below the maximum residue level of 5 ng/mL set by Codex Alimentarius and European Union Pesticide database.

Malathion was detected in 13(15.34%) samples with mean levels ranging between not detectable to 3.95 ng/mL and a mean concentration(ng/mL) of 0.15$\pm$0.08. This level is below the maximum residue limit set by European Union Pesticide database as 20 ng/mL and United States Department of Agriculture (USDA) as 500 ng/mL.

The means, minimum and maximum values of 14 organophosphates residues analyzed in milk are summarized in Table 4.1 below. Eight organophosphates were set by some regulatory bodies. However, four organophosphates maximum residue limits were not available.

Table 4.1: Mean, Minimum and Maximum values (ng/mL) of Organophosphates in cow and camel milk collected from Kiambu, Isiolo and Laikipia Counties

	Cow milk		**Camel milk**				**Implication**		
	Kiambu		**Isiolo**		**Laikipia**		**MRL (ng/mL)**		
Organophosphate	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**	**Codex Alimentarius**	**EU pesticides database**	**USDA**
Chlorthiophos-1	46.07	0.2-387.47	10.77	0.2-91.76	14.30	0.2-118.94	Not set	Not set	Not set
Chlorthiophos-2	0.02	0.02	0.02	0.02	0.02	0.02	Not set	Not set	Not set
Chlorthiophos-3	0.2	0.2	0.2	0.2	0.2	0.2	Not set	Not set	Not set
Coumaphos	4.53	0.2-5.80	5.74	0.2-6.23	3.34	0.2-5.78	Not set	Not set	1250
Disulfoton	2.19	0.2-5.08	0.2	0.2	2.67	0.2-5.92	10	10	Not set
Fenamiphos	1.24	0.2-1.65	0.16	0.2-0.35	1.29	0.2-1.93	5	5	Not set
Fenchlorphous	0.3	0.3	0.3	0.3	0.3	0.3	Not set	5	Not set
Leptophos	0.2	0.2	0.2	0.2	0.2	0.2	Not set	Not set	Not set
Malathion	0.15	0.2-3.95	1.34	0.2-2.80	0.17	0.2-0.46	Not set	20	500
Methacrifos	0.88	0.3-1.30	0.3	0.3	0.3	0.3	Not set	10	Not set
Profenofos	0.1	0.1	0.1	0.1	0.1	0.1	10	10	10
Terbufos	0.2	0.2	0.2	0.2	1.04	0.2-3.94	10	10	Not set
Tetrachlorvinphos	0.77	0.3-1.42	9.93	0.3-11.89	0.47	0.3-1.39	Not set	Not set	10
Tolclofos-methyl	0.2	0.2	0.2	0.2	0.2	0.2	Not set	10	Not set

MRL: Maximum residue limit; ng/mL: nano grams per milliliter; EU: European Union; USDA: United States Department of Agriculture.

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4.1.2 Organophosphate's pesticides in camel milk from Isiolo and Laikipia County

A total of 82 camel milk samples from Isiolo and Laikipia Counties were analyzed for organophosphates pesticide residues. The mean levels of the organophosphates tested in Camel milk were all below the maximum residue limits set by the Codex Alimentarius, European Union Pesticide database and the United States Department of Agriculture (USDA) guidelines as shown in Table 4.1

The following organophosphates were identified: chlorthiophos-1, disulfototon, fenamiphos, malathion, terbufos and tetrachlorvinphos. (Figure 4.1) Chlorthiophos-1 was detected in 25(39.84%) samples with mean levels ranging between not detectable to 91.76 ng/mL and a mean concentration (ng/mL) of 10.77 ± 0.94 in Isiolo county, while in Laikipia county one sample was detected with mean concentration(ng/mL) of 14.30 ± 0.12, ranging between not detectable to 118.94 ng/mL.

Disulfoton was detected in 6 (75%) samples in Laikipia with mean levels ranging between not detectable to 5.92 ng/mL and a mean concentration(ng/mL) of 2.67 ± 2.47 while Isiolo did not have any positive samples with disulfoton.

Fenamiphos was detected in 49 (77.34%) samples from Isiolo county with mean levels ranging between not detectable to 0.35 ng/mL and a mean concentration (ng/mL) of 0.16 ± 0.09. In Laikipia 6 (81.25%) were detected with mean levels ranging from not detectable to 1.93 ng/mL and mean concentration (ng/mL) of 1.29 ± 0.65. This level is below the maximum residue limit of 5 ng/mL set by Codex Alimentarius and European Union Pesticide database.

Malathion was detected in 45 (70.31%) samples from Isiolo county with mean levels ranging between not detectable to 2.80 ng/mL and a mean concentration (ng/mL) of 1.34 ± 0.76. In Laikipia 3 (43.75%) were detected with mean levels ranging from not detectable to 0.46 ng/mL and mean concentration (ng/mL) of 0.17 ± 0.65. This level is below the maximum residue limit set by European Union Pesticide database as 20 ng/mL and United States Department of Agriculture (USDA) as 500 ng/mL.

Terbufos was detected in 2 (31.25%) samples from Laikipia county with mean levels ranging between not detectable to 3.94 ng/mL and a mean concentration (ng/mL) of 1.04 ± 0.61. This level is below the maximum residue limit set as 10 ng/mL set by Codex Alimentarius and European Union Pesticide database. Isiolo did not have any positive samples for terbufos.

Tetrachlorvinphos was detected in 57 (89.06%) samples from Isiolo county with mean levels ranging between not detectable to 11.89 ng/mL and a mean concentration (ng/mL) of 9.93±3.50. In Laikipia county 3 samples (37.50%) were detected with mean levels ranging between not detectable to 1.39 ng/mL and a mean concentration (ng/mL) of 0.47±0.14. This level is below the maximum residue limit set as 10 ng/mL set by United States Department of Agriculture (USDA) as shown in Table 4.1

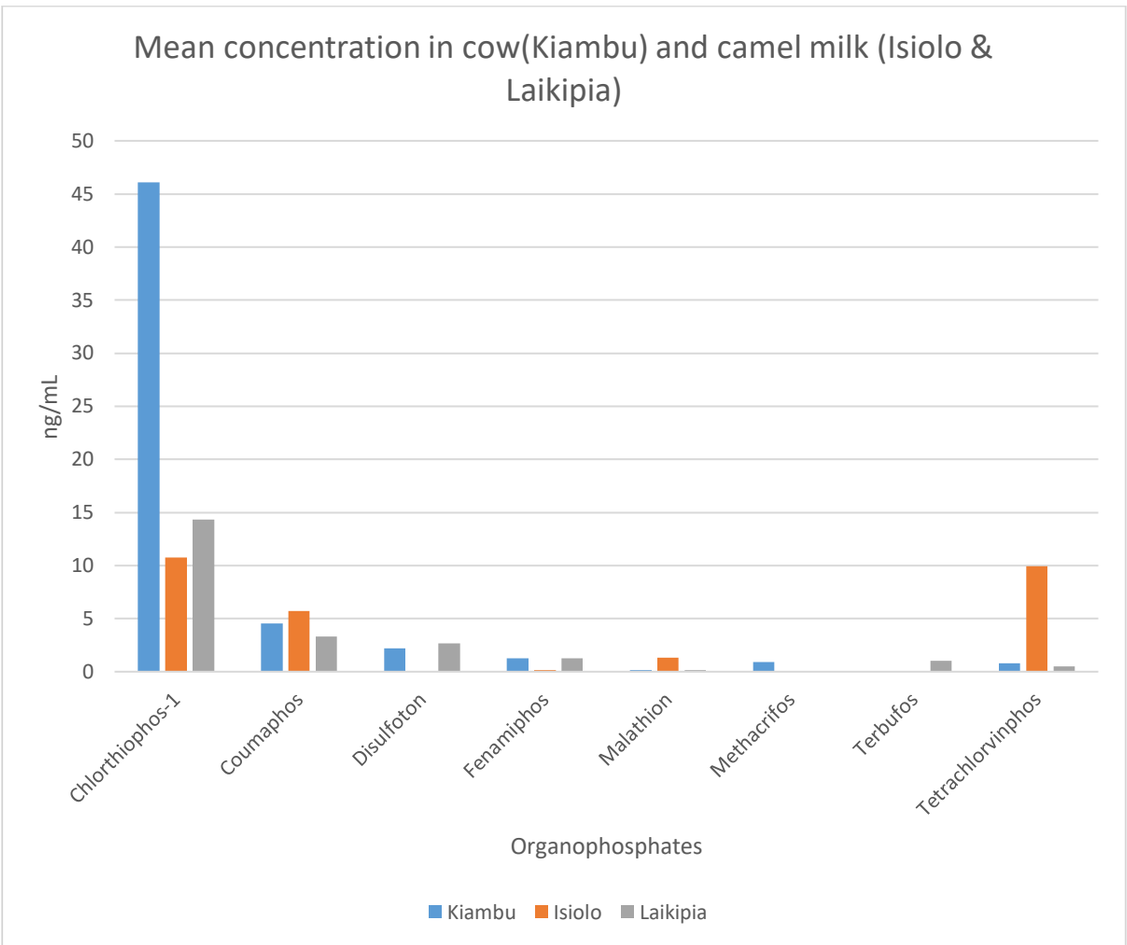


Figure 4.1: Mean concentration (ng/mL) of Organophosphates evaluated in cow and camel milk collected from Kiambu, Isiolo and Laikipia

Table 4.2 below is the summary of the comparison of the mean values of organophosphates evaluated in cow and camel milk collected from Kiambu, Isiolo and Laikipia Counties. The mean levels of coumaphos, malathion, and tetrachlorvinphos was significantly higher in Isiolo relative to Kiambu and Laikipia.

Table 4.2: Comparison of the mean values (ng/mL) of Organophosphates evaluated in Cow and Camel milk collected from Kiambu, Isiolo and Laikipia

	Cow milk	**Camel milk**	
Organophosphates	**Kiambu**	**Isiolo**	**Laikipia**
Chlorthiophos-1	46.07 (0.88)[b]	10.77 (0.94)[a]	14.30 (0.12)[b]
Chlorthiophos-2	0.02	0.02	0.02
Chlorthiophos-3	0.2	0.2	0.2
Coumaphos	4.53 (2.10) [b]	5.74 (1.04)[c]	3.34 (2.68)[a]
Disulfoton	2.19 (0.38)[b]	0.2	2.67 (2.47)[b]
Fenamiphos	1.24 (0.61)[b]	0.16(0.09)[a]	1.29 (0.65)[b]
Fenchlorphous	0.3	0.3	0.3
Leptophos	0.2	0.2	0.2
Malathion	0.15 (0.08)[a]	1.34 (0.76)[b]	0.17 (0.10)[a]
Methacrifos	0.88 (0.55)[b]	0.3	0.3
Profenofos	0.1	0.1	0.1
Terbufos	0.2	0.2	1.04 (0.61)[b]
Tetrachlorvinphos	0.77 (0.68)[a]	9.93 (3.50)[a]	0.47 (0.14)[a]
Tolclofos-methyl	0.2	0.2	0.2

Means (standard deviation) with the same superscripts along the columns are not significantly different at $p \leq 0.05$ (ANOVA and Tukey's post hoc test).

4.1.3 Comparison of mean values of Organophosphates evaluated in Cow and Camel milk collected from different wards within Kiambu, Isiolo and Laikipia Counties

The mean level of methacrifos and disulfoton in camel milk collected from individual wards in Laikipia County was significantly higher than the mean levels in Camel milk collected from individual wards in Isiolo County. Table 4.3 shows the mean levels of terbufos in camel milk collected in individual wards in Laikipia County was significantly higher than the mean levels in camel milk collected from individual wards in Isiolo County.

Table 4.3: Comparison of the mean values (ng/mL) of Organophosphates evaluated in Cow and Camel milk collected from different wards within Kiambu, Isiolo and Laikipia Counties

County	Ward	Methacrifos	Disulfoton	Terbufos	Tolclofos-methyl	Fenchlorphos	Malathion	Tetrachlorvinphos	Fenamiphos	Chlorthiophos-2	Profenofos	Chlorthiophos-1	Chlorthiophos-3	Leptophos	Coumaphos
Kiambu	Githunguri	0.95 (0.52)^{b}	2.62 (2.40)^{b}	0.98 (1.52)$^{\mathbf{bc}}$	0.2	0.3	0.23 (0.86)^{a}	0.78 (0.68)^{a}	1.23 (0.63)^{b}	0.02	0.1	45.51 (74.31)^{a}	0.3	0.2	4.26 (2.33)^{a}
	Kiamathae	0.79 (0.59)^{b}	2.05 (2.39)^{b}	0.53 (1.22)^{ab}	0.2	0.3	0.23 (0.69)^{a}	0.76 (0.69)^{a}	1.23 (0.61)^{b}	0.02	0.1	46.02 (95.05)^{a}	0.3	0.2	4.81 (1.84)^{ab}
	Komothai	0.95 (0.52)^{b}	2.37 (2.40)^{b}	0.66 (1.33)^{abc}	0.2	0.3	0.08 (0.22)^{a}	0.65 (0.69)^{a}	1.23 (0.62)^{b}	0.02	0.1	46.88 (96.22)^{a}	0.3	0.2	4.67 (1.99)^{ab}
	Ngewa	0.88 (0.55)^{b}	1.79 (2.33)^{b}	1.23 (1.61)^{c}	0.2	0.3	0.04 (0.12)^{a}	0.89 (0.66)^{a}	1.27 (0.59)^{b}	0.02	0.1	45.87 (84.44)^{a}	0.3	0.2	4.26 (2.32)^{a}
Isiolo	Kashuru	0.3	0.2	0.2	0.2	0.3	1.38 (0.72)^{b}	9.97 (3.47)^{bc}	0.12 (0.11)^{a}	0.02	0.1	7.49 (18.15)^{a}	0.3	0.2	5.61 (1.35)^{bc}
	Kina	0.3	0.2	0.2	0.2	0.3	1.36 (0.78)^{b}	9.02 (4.45)^{b}	0.18 (0.07)^{a}	0.02	0.1	12.49 (21.11)^{a}	0.3	0.2	5.94 (0.08)^{c}
	Mulango	0.3	0.2	0.2	0.2	0.3	1.27 (0.77)^{b}	11.15 (0.17)^{c}	0.15 (0.09)^{a}	0.02	0.1	11.69 (16.47)^{a}	0.3	0.2	5.61 (1.34)^{bc}
Laikipia	DolDol	1.07 (0.56)^{b}	2.67 (2.47)^{b}	1.04 (1.61)^{bc}	0.2	0.3	0.17 (0.20)^{a}	0.47 (0.64)^{a}	1.29 (0.65)^{b}	0.02	0.1	14.30 (39.12)^{a}	0.3	0.2	3.34 (2.68)^{a}

Means (standard deviation) with the same superscripts along the columns are not significantly different at $p \leq 0.05$ (ANOVA and Tukey's post hoc test).

Table 4.3 shows the mean levels of malathion in camel milk collected from individual wards in Isiolo County was significantly higher relative to the mean levels in cow milk collected from individual wards in Kiambu and camel milk collected from individual wards in Laikipia County. The mean levels of tetrachlorvinphos in camel milk collected from individual wards in Laikipia County were significantly lower than the mean levels in camel milk collected from individual wards in Isiolo County. Fenamiphos in camel milk collected from individual wards in Isiolo County were significantly lower than the mean levels in cow milk collected from Kiambu County. The mean levels of coumaphos in camel milk collected from Kashuru, Kina, and Mulango wards in Isiolo County was significantly higher than the mean levels in cow milk collected from Githunguri and Ngewa wards in Kiambu County.

Coumaphos levels in camel milk collected from Doldol in Laikipia County was significantly lower than the mean levels in camel milk collected from Kashuru, Kina, and Mulango wards in Isiolo County. However, there was no difference in the mean levels of chlorthiophos 2 & 3, fenchlorphous, leptophos, profenofos and toclofos-methyl in Cow and Camel milk collected from individual wards in Kiambu, Isiolo, and Laikipia Counties.

4.2.0 Organochlorines Pesticides in Cow milk and Camel milk from Counties

4.2.1 Organochlorines Pesticides in Cow milk from Kiambu County

A total of 90 cow milk samples from Kiambu County were analyzed for organochlorines pesticides residues. The mean level of heptachlor in cow milk collected from Kiambu was above the maximum residue limits set by the Codex Alimentarius and the European Union Pesticide Database (4.3). The following organochlorines were identified; α+β endosulphan, chlorfenson, dieldrin, mirex, cis +trans nonachlor, *o,p'*-DDD, DDT and heptachlor. Table 4.4 shows organochlorines pesticides

maximum residue limits were set by some regulatory bodies although 13 organochlorines have no set MRLs (Table 4.4).

α+β endosulphan was detected in 84 (93.02%) samples and ranged between the limit of detection (0.3 ng/mL) to 7.14 ng/mL with a mean concentration (ng/mL) of 5.10 $\pm$1.15. This level is below the maximum residue level of 10 ng/mL set by Codex Alimentarius as 10 ng/mL and European Union Pesticide database as 50 ng/mL.

Chlorfenson was detected in 70 (78.77%) samples and ranged between not detectable <0.20 -1.36 ng/mL with a mean concentration (ng/mL) of 1.03 $\pm$0.53.This level is below the maximum residue level of 50 ng/mL set by European Union Pesticide database.

Dieldrin was detected in 76 (85.47%) samples and ranged between not detectable to 2.90 ng/mL with a mean concentration (ng/mL) of 1.72 $\pm$0.73.This level is below the maximum residue level set as 6 ng/mL by Codex Alimentarius and European Union Pesticide database and as 300 ng/mL by the United States Department of Agriculture (USDA).

Heptachlor was detected in 78 (87.71%) samples and ranged between not detectable to 22.50 ng/mL. Heptachlor had the highest mean concentration (ng/mL) of 12.38 $\pm$5.07. This level is above the maximum residue level of 6 ng/mL set by Codex Alimentarius and 4 ng/mL set by European Union Pesticide database.

Mirex was detected in 77 (86.59%) samples and ranged between not detectable to 0.85 ng/mL with a mean concentration (ng/mL) of 0.72 $\pm$0.29. The maximum residue limit of mirex is not yet set by either Codex Alimentarius, European Union Pesticide database or United States Department of Agriculture (USDA).

Table 3.4: Mean, minimum and maximum values (ng/mL) of Organochlorines evaluated in Cow and Camel milk collected from Kiambu, Isiolo and Laikipia Counties

	Cow milk		Camel milk				MRL (ng/mL)		
	Kiambu		Isiolo		Laikipia				
Organochlorine	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**	**Codex Alimentarius**	**EU pesticides database**	**USDA**
α-BHC	0.2	0.2	0.2	0.2	0.2	0.2	Not set	Not set	Not set
β-BHC	0.2	0.2	0.2	0.2	0.2	0.2	Not set	Not set	Not set
γ-BHC	0.3	0.3	0.3	0.3	0.3	0.3	1	10	300
δ-BHC	0.2	0.2	2.35	0.2-2.94	0.2	0.2-0.15	Not set	Not set	Not set
α-endosulphan	2.41	0.2-3.41	0.36	0.2-1.43	1.57	0.2-2.67	Not set	Not set	Not set
β-endosulphan	2.69	0.1-3.91	2.74	0.1-4.66	1.89	0.1-3.21	Not set	Not set	Not set
α+β endosulphan	5.10	0.2-7.14	3.11	0.2-5.57	3.46	0.2-5.52	10	50	Not set
cis-Chlordane	0.2	0.2-0.96	0.2	0.2	0.2	0.2	Not set	Not set	Not set
trans-Chlordane	0.2	0.2	0.2	0.2	0.2	0.2	Not set	Not set	Not set
cis+trans Chlordane	0.2	0.2-0.96	0.2	0.2	0.2	0.2	2	2	Not set
Chlorfenson	1.03	0.2-1.36	0.2	0.2	0.81	0.2-1.34	Not set	50	Not set
Dieldrin	1.72	0.1-2.90	0.1	0.1	1.51	0.1-2.26	6	6	300
Endrin	0.2	0.2	0.2	0.2	0.2	0.2	Not set	0.8	Not set
Heptachlor	12.38	0.2-22.50	4.62	0.2-5.89	13.42	0.2-19.68	6	4	Not set
Hexachlorbenzene	0.2	0.2	0.2	0.2	0.2	0.2	Not set	Not set	Not set
Mirex	0.72	0.2-0.85	0.2	0.2	0.80	0.2-1.01	Not set	Not set	Not set
cis-Nonachlor	1.42	0.1-4.78	4.09	0.1-9.93	1.86	0.1-3.64	Not set	Not set	Not set
trans-Nonachlor	1.30	0.3-2.84	1.26	0.3-2.90	1.08	0.3-1.90	Not set	Not set	Not set
cis+transnonachlor	2.72	0.3-6.33	5.35	0.3-11.53	2.94	0.3-4.94	Not set	Not set	Not set
o,p'-DDD	8.97	0.2-16.87	0.2	0.2-1.61	8.91	0.2-10.51	Not set	Not set	Not set
DDT	3.27	0.2-4.12	12.95	0.2-13.78	2.98	0.2-3.51	20	40	1250

MRL: Maximum residue limit; ng/mL: nano grams per milliliter; USDA: United States Department of Agriculture; DDT: *o,p'*-DDT+*p,p'*-DDD+*p,p'*-DDE+*p,p'*-DDD

Cis + transnonachlor was detected in 68 (61.17%) samples and ranged between not detectable to 6.33 ng/mL with a mean concentration (ng/mL) of 2.72 $\pm$1.88. The maximum residue limit of Cis +transnonachlor is not yet set by either Codex Alimentarius, European Union Pesticide database or United States Department of Agriculture (USDA).

The *o,p'*-DDD was detected in 82 (91.62%) samples and ranged between not detectable to 0.16.87 ng/mL with a mean concentration (ng/mL) of 8.97 $\pm$2.84. The maximum residue limit of *o,p'*-DDD is not yet set by either Codex Alimentarius, European Union Pesticide database or United States Department of Agriculture (USDA).

DDT was detected in 84 (47.35%) samples and ranged between not detectable to 4.12 ng/mL with a mean concentration (ng/mL) of 3.27 $\pm$0.86. This level is below the maximum residue level set as 20 ng/mL by Codex Alimentarius and 40 ng/mL by European Union Pesticide database and as 1250 ng/mL by the United States Department of Agriculture (USDA).

4.2.2 Comparison of organochlorines Pesticides in Camel milk from Isiolo and Laikipia County.

A total of 82 camel milk samples from Isiolo and Laikipia County were analyzed for organochlorines pesticides residues. The following organochlorines were identified; α+β endosulphan, chlorfenson, dieldrin, mirex, cis +trans nonachlor, *o,p'*-DDD, DDT and heptachlor. The mean level of heptachlor in Camel milk collected from Laikipia was above the maximum residue limits set by the Codex Alimentarius and the European Union Pesticide Database. Table 4.5 above.

α+β endosulphan was detected in 70 (87.69%) samples and ranged between not detectable (Limit of detection=0.3 ng/mL) to 5.57 ng/mL with a mean concentration (ng/mL) of 3.11 $\pm$0.84 from Isiolo county while Laikipia county had α+β endosulphan in 5 (68.75%) with a mean concentration

(ng/mL) of 3.46 $\pm$1.78 This level is below the maximum residue level of 10 ng/mL set by Codex Alimentarius as 10 ng/mL and European Union Pesticide database as 50 ng/mL.

Chlorfenson, diedrin and mirex was detected in camel milk from Laikipia county with mean concentration ranging between not detectable to 2.26 ng/mL.

Heptachlor was detected in 66 (89.19%) samples from Isiolo ranging between not detectable to 5.89 ng/mL with mean concentration (ng/mL) of 4.62 $\pm$1.55. This level is within the maximum residue level of 6 ng/mL set by European Union Pesticide database. Laikipia county had high mean concentration of 13.42 ng/mL above the maximum residue level of 6 ng/mL set by Codex Alimentarius and 4 ng/mL set by European Union Pesticide database.

Cis + transnonachlor was detected in 60 (64.23%) samples from Isiolo ranging between not detectable to 11.53 ng/mL with a mean concentration (ng/mL) of 5.35 $\pm$4.40. Laikipia county had a lower mean concentration of 2.94 ng/mL. The maximum residue limit of Cis +transnonachlor is not yet set by either Codex Alimentarius, European Union Pesticide database or United States Department of Agriculture (USDA).

o,p'-DDD was detected in camel milk samples from Laikipia with a mean concentration of 8.91 ng/mL ranging between not detectable to 10.51 ng/mL. The maximum residue limit of *o,p'*-DDD is not yet set by either Codex Alimentarius, European Union Pesticide database or United States Department of Agriculture (USDA).

DDT was detected in 31 (30%) samples from Isiolo and ranged between not detectable to 13.78 ng/mL with a mean concentration (ng/mL) of 12.95 $\pm$2.32. In Laikipia county seven samples were detected with DDT with a mean concentration of 2.98 ng/mL. These levels were below the maximum residue level set as 20 ng/mL by Codex Alimentarius and 40 ng/mL by European Union

Pesticide database and as 1,250 ng/mL by the United States Department of Agriculture (USDA) (Table 4.5).

Table 4.5: Comparison of the mean values (ng/ml) of Organochlorines evaluated in Cow and Camel milk collected from Isiolo, Laikipia& Kiambu Counties

	Cow milk	**Camel milk**	
Organochlorine	**Kiambu**	**Isiolo**	**Laikipia**
α-BHC	0.2	0.2	0.2
β-BHC	0.2	0.2	0.2
γ-BHC	0.3	0.3	0.3
δ-BHC	0.2	2.35 (0.82)^{b}	0.2
cis-Chlordane	0.2	0.2	0.2
trans-Chlordane	0.2	0.2	0.2
cis+trans Chlordane	0.2	0.2	0.2
Chlorfenson	1.03 (0.53)^{b}	0.2	0.81 (0.65)^{a}
Dieldrin	1.72 (0.73)^{b}	0.1	1.51 (0.91)^{b}
α-endosulphan	2.41 (0.67)^{c}	0.36 (0.00)^{a}	1.57 (1.10)^{b}
β-endosulphan	2.69 (0.83)^{b}	2.74 (0.71)^{b}	1.89 (1.33)^{a}
α+β endosulphan	5.10(1.15) ^{b}	3.11(0.84)^{a}	3.46(1.78) ^{a}
Endrin	0.2	0.2	0.2
Heptachlor	12.38 (5.07)^{b}	4.62 (1.55)^{a}	13.42 (5.90)^{b}
Hexachlorbenzene	0.2	0.2	0.2
Mirex	0.72 (0.29)^{b}	0.2	0.80 (0.22)^{b}
cis-Nonachlor	1.42 (0.71)^{a}	4.09 (0.24)^{b}	1.86 (1.70)^{a}
trans-Nonachlor	1.30 (0.72)^{a}	1.26 (0.70)^{a}	1.08 (0.76)^{a}
cis+transnonachlor	2.72(1.88) ^{a}	5.35(4.40) ^{b}	2.94(1.97) ^{a}
o'p-DDD	8.97 (2.84)^{b}	0.2	8.91 (2.52)^{b}
DDT	3.27(0.86) ^{a}	12.95(2.32) ^{b}	2.98(1.17) ^{a}

Means (standard deviation) with the same superscripts across the rows are not significantly different at $p \leq 0.05$ (ANOVA and Tukey's post hoc test). DDT: o'p-DDT+p'p-DDD+p'p-DDE+p'p-DDT. ND: Not detected/below detection limit.

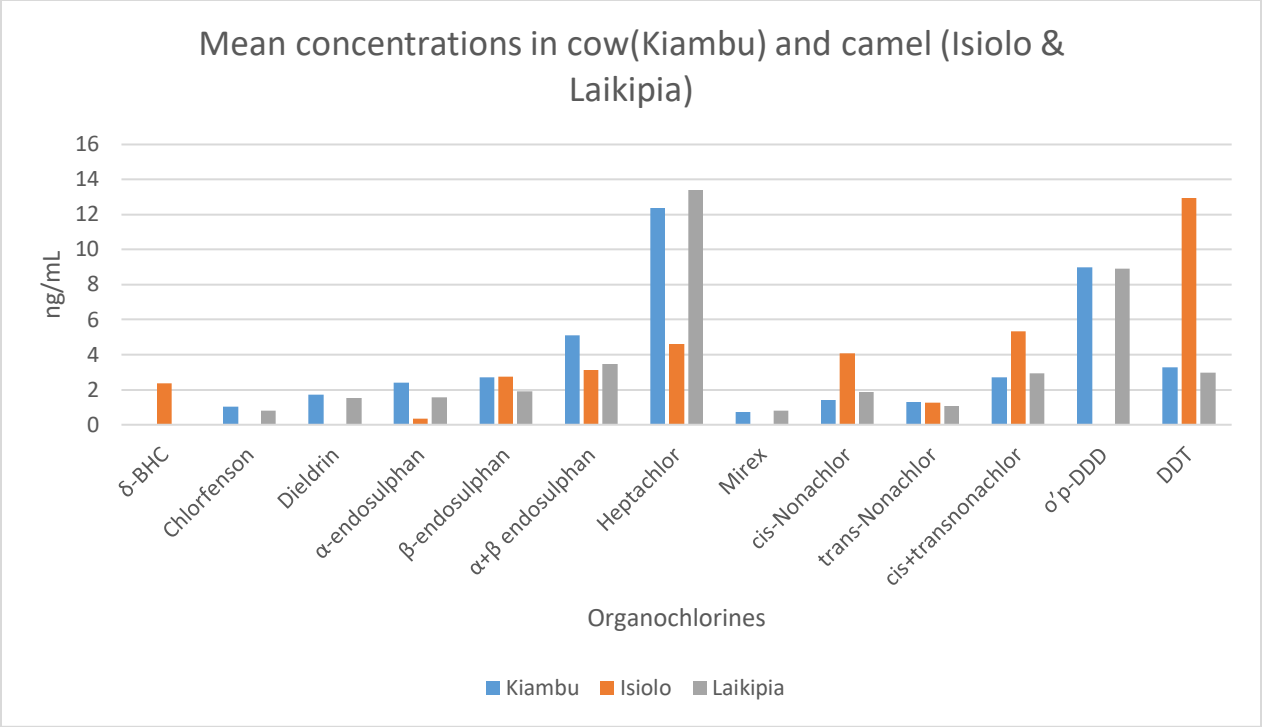


Figure 4.5: Comparison of the mean values (ng/ml) of Organochlorines evaluated in Cow and Camel milk collected from Isiolo, Laikipia& Kiambu Counties

Table 4.5 shows the mean levels of δ-BHC in milk collected from Isiolo was significantly higher than the mean levels of δ-BHC in milk in milk collected from Kiambu or Laikipia. The mean levels of α-endosulphan in milk collected from Kiambu is significantly higher than the mean levels of -endosulphan in milk collected from Isiolo and Laikipia. The mean levels of *β*-endosulphan in milk collected from Laikipia is significantly lower than the mean levels of *β*-endosulphan in milk collected from Kiambu or Isiolo. The mean levels of heptachlor in milk collected from Isiolo are significantly lower than the mean levels of Heptachlor in Kiambu or Laikipia. The mean levels of cis-Nonachlor in milk collected from Isiolo are significantly higher than the mean levels of cis-Nonachlor in milk collected from Kiambu or Laikipia. The mean levels of *o,p'*-DDD in milk collected from Isiolo is significantly lower than the mean levels of *o,p'*-DDD in milk collected from

Kiambu or Laikipia. The mean levels of DDT in milk collected from Isiolo were significantly higher than the mean levels of DDT in milk collected from Kiambu or Laikipia.

4.3 Veterinary drug residues

Table 8 is a summary of the mean, minimum, and maximum levels of veterinary drug residues in Cow and Camel milk collected from Kiambu, Isiolo, and Laikipia Counties in Kenya. The mean levels of veterinary drug residues in cow and camel milk collected from Kiambu, Isiolo, and Laikipia Counties were all within the maximum residue limit of 100 ng/mL.

4.3.1 Veterinary drug residues in Cow milk from Kiambu county

A total of 90 cow milk samples from Kiambu County were analyzed for veterinary drug residues. The following veterinary drugs; Sulfadiazine, sulfapyridine, sulfamerazine, sulfamethoxazole, sulfamethoxypyradine and were detected in 68 (75.56%) samples, 74 (82.22%) samples, 60 (66.67%) samples, 61 (67.78%) samples and 89 (98.88%) samples respectively. sulfachloropyrazine, sulfamethazine, sulfadimethoxine, sulfathiazole and sulfaguanidine had mean concentration ranging between not detected (Limit of detection= 0.02 ng/mL) to 25.98 ng/mL. Sulfadoxine was not detected (Table 4.6).

Table 4.6: The mean, minimum and maximum levels (ng/ml) of Veterinary drug residues in Cow and Camel milk collected from Kiambu, Isiolo and Laikipia Counties in Kenya.

County	Range	Sulfadiazine	Sulfachloropyradazine	Sulfathiazole	Sulfapyridine	Sulfamerazine	Sulfaguanadine	Sulfamethoxazole	Sulfamethoxypyradine	Sulfamethazine	Sulfadimethoxine	Sulfadoxine
Isiolo	Minimum	0.02	0.02	0.03	0.02	0.02	0.04	4.43	0.04	0.04	10.65	2.15
	Mean	0.78	0.38	2.31	0.55	0.02	4.30	4.49	0.06	0.62	10.68	4.86
	Maximum	1.23	1.21	2.96	2.98	0.02	4.99	4.80	4.56	10.87	12.06	5.82
Laikipia	Minimum	0.02	0.02	2.56	0.00	0.02	4.44	4.43	0.04	0.04	10.65	4.89
	Mean	0.77	0.42	2.58	0.52	0.02	4.73	4.45	0.04	0.04	10.66	4.89
	Maximum	1.21	1.20	2.79	2.77	0.02	4.79	4.75	0.04	0.04	10.67	4.90
Kiambu	Minimum	0.02	0.02	6.42	0.02	0.02	0.82	0.00	0.04	0.04	0.03	0.03
	Mean	4.68	6.36	6.45	4.35	4.46	3.38	0.88	8.37	6.26	5.37	6.45
	Maximum	7.88	11.99	6.80	6.70	10.87	25.98	9.56	11.99	11.98	9.78	10.65

Sulfadimethoxine had the highest mean concentration of 10.68 ng/mL in camel milk samples from Isiolo. sulfadiazine, sulfachloropyrazine, sulfathiazole, sulfapyridine, sulfamerazine, sulfaguanadine, sulfamethoxazole, sulfamethoxypyradine, sulfamethazine and sulfadoxine had mean concentration ranging from not detected (limit of detection=0.02 ng/mL) to 12.06 ng/mL.

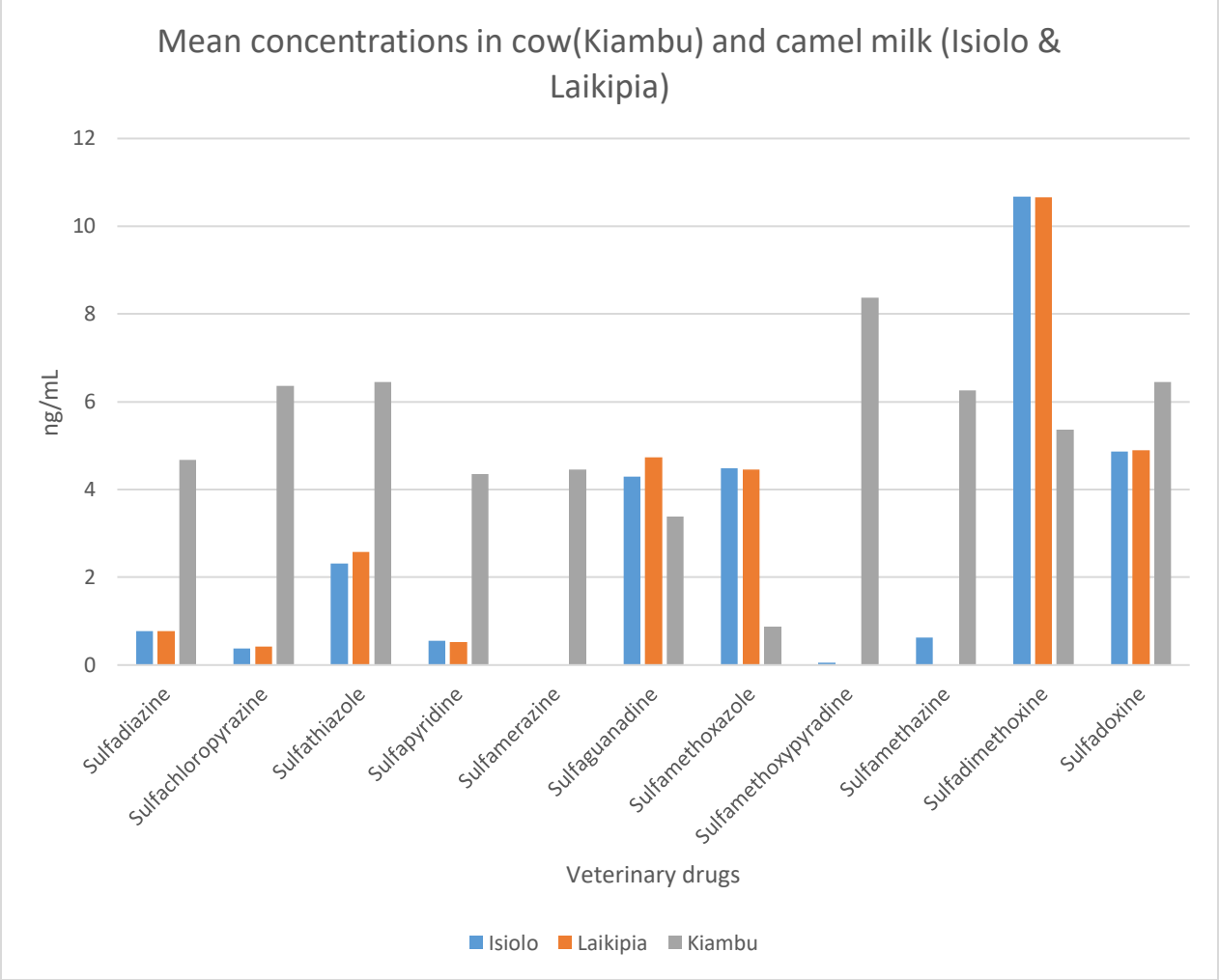


Figure 4.6: Mean concentrations of veterinary drugs residues in cow and camel milk collected in Isiolo, Laikipia and Kiambu counties in Kenya

4.3.2 Comparison of veterinary drug residues in Camel milk from Isiolo and Laikipia county

Table 4.7 shows a summary of the comparison of the mean levels of veterinary drug residues in Cow and Camel milk collected from Kiambu, Isiolo, and Laikipia Counties. The mean levels of Sulfadiazine, sulfachloropyridazine, sulfathiazole, sulfapyridine, sulfamerazine, sulfamethoxypyradine, sulfamethazine and sulfadoxine in cow milk collected from Kiambu County was significantly higher than the mean levels in camel milk collected from Isiolo, and Laikipia Counties. The mean levels of Sulfamethoxazole and sulfadimethoxine in cow milk collected from Kiambu County was significantly lower than the mean levels in camel milk collected from Isiolo and Laikipia Counties. However, the mean levels of sulfamethoxypyradine in cow milk collected from Kiambu County was significantly lower than the mean levels in camel milk collected from Isiolo and Laikipia Counties.

Table 4.7: Comparison of the mean levels(ng/ml) of Veterinary drug residues in Cow and Camel milk collected from Kiambu, Isiolo and Laikipia Counties

County	Sulfa Diazine	Sulfachloro Pyradazine	Sulfa Thiazole	Sulfa Pyridine	Sulfa merazine	Sulfa Guanidine	Sulfa methoxazol	Sulfa methoxypy	Sulfa methazine	Sulfa dimethoxin	Sulfa doxine
Isiolo	0.78 (0.57)[a]	0.38 (0.26)[a]	2.31 (0.77)[a]	0.55 (0.07)[a]	0.02 (0.01)[a]	4.30 (1.38)[b]	4.49 (0.11)[b]	0.06 (0.04)[a]	0.62 0.08[a]	10.68 (0.13)[a]	4.86 (0.33)[a]
Laikipia	0.77 (0.58)[a]	0.42 (0.28)[a]	2.58 (0.06)[a]	0.52 (0.1)[a]	0.02 (0.01)[a]	4.73 (0.07)[b]	4.45 (0.07)[b]	0.04 (0.02)[a]	0.04 (0.00)[a]	10.66 (0.01)[b]	4.89 (0.0)[a]
Kiambu	4.68 (3.83)[b]	6.36 (5.00)[b]	6.45 (0.06)[b]	4.35 (2.79)[b]	4.46 (3.37)[b]	3.38 (0.65)[a]	0.88 (0.37)[a]	8.37 (3.12)[b]	6.26 (5.21)[b]	5.37 (4.56)[a]	6.45 (5.15)[b]

Means (standard deviation) with the same superscript letters along the column for respective drugs are not significantly different at the $p \leq 0.05$ level (Tukey's test).

Table 4.8 shows a comparison of the mean levels of veterinary drug residues in Cow and Camel milk collected from different wards in Kiambu, Isiolo and Laikipia Counties. The mean levels of sulfamethoxypyradine in cow milk collected from individual wards in Kiambu County were significantly higher than the mean levels in camel milk collected from individual wards in Isiolo and Laikipia Counties. The mean levels of Sulfamethazine in camel milk collected from individual wards in Laikipia County were significantly higher than the mean levels in cow milk collected from individual wards in Kiambu County. The mean levels of Sulfamethazine in cow milk collected from individual wards in Kiambu County were significantly higher than the mean levels in camel milk collected from individual wards in Isiolo and Laikipia. The mean levels of sulfadimethoxime in camel milk collected from individual wards in Laikipia County were significantly higher than the mean levels in cow milk collected from individual wards in Kiambu County. However, the mean levels of sulfadimethoxine in cow milk collected from Kiamathae ward in Kiambu County was significantly lower than the mean levels in cow milk collected from Githunguri and Ngewa wards in Kiambu County. The mean levels of sulfadoxine in camel milk collected from Doldol ward in Laikipia County was significantly lower than the mean levels in cow milk collected from Ngewa ward in Kiambu County. The mean levels of sulfadoxine in cow milk collected from Githunguri ward in Kiambu County was significantly higher than the mean level in camel milk collected from Mulango ward in Isiolo County. However, the mean level of sulfadoxine in cow milk collected from Githunguri ward in Kiambu County was significantly higher than the mean level in camel milk collected from Mulango ward in Isiolo County. The mean level of sulfadoxine in cow milk collected from Kiamathae ward in Kiambu County was significantly lower than the mean level in cow milk collected from Githunguri and Ngewa wards in the same county.

Table 4.8: Comparison of the mean levels (ng/mL) of Veterinary drug residues in Cow and Camel milk collected from different wards in Kiambu, Isiolo and Laikipia counties

County	Wards	Sulfadiazine	Sulfachloropyridazine	Sulfathiazole	Sulfapyridine	Sulfamerazine	Sulfaguanadine	Sulfamethoxazole	Sulfamethoxypyradine	Sulfamethazine	Sulfadimethoxine
Isiolo	Kashuru	0.80 (0.06)^{a}	0.39 (0.06)^{a}	2.56 (0.01)^{b}	0.14 (0.01)^{a}	0.02 (0.01)^{a}	4.62 (0.75)^{abc}	4.45 (0.07)^{c}	0.04 (0.02)^{a}	0.04 (0.02)^{a}	10.66 (0.01)^{e}
	Kina	0.80 (0.07)^{a}	0.39 (0.06)^{a}	2.51 (0.41)^{b}	0.73 (0.21)^{a}	0.02 (0.01)^{a}	4.75 (0.06)^{ac}	4.47 (0.09)^{c}	0.04 (0.02)^{a}	1.07 (0.26)^{a}	10.67 (0.04)^{e}
	Mulango	0.92 (0.20)^{a}	0.27 (0.05)^{a}	2.43 (0.57)^{b}	0.40 (0.06)^{a}	0.02 (0.01)^{a}	4.49 (1.05)^{abc}	4.48 (0.11)^{c}	0.04 (0.00)^{a}	0.04 (0.02)^{a}	10.70 (0.22)^{e}
Nanyuki	Doldol	0.59 (0.20)^{a}	0.50 (0.09)^{a}	1.87 (1.17)^{a}	0.90 (0.24)^{ab}	0.02 (0.01)^{a}	3.55 (2.08)^{abc}	4.53 (0.14)^{c}	0.23 (0.09)^{a}	1.11 (0.95)^{a}	10.68 (0.08)^{e}
Kiambu	Githunguri	5.46 (3.62)^{cd}	7.98 (4.82)^{d}	6.47 (0.09)^{c}	4.83 (2.68)^{de}	5.33 (3.46)^{cd}	2.76 (0.74)^{ab}	0.58 (0.08)^{a}	9.20 (3.03)^{de}	6.77 (4.82)^{c}	6.49 (4.30)^{bcd}
	Gitwe	3.90 (2.0)^{bc}	5.00 (0.89)^{cd}	6.44 (0.01)^{c}	3.70 (2.97)^{cd}	3.63 (0.38)^{bc}	3.94 (0.95)^{abc}	0.48 (0.09)^{a}	7.33 (2.98)^{bc}	5.11 (0.36)^{bc}	4.65 (0.77)^{ab}
	Kiamathae	2.34 (0.67)^{ab}	2.80 (0.56)^{ab}	6.44 (0.02)^{c}	2.60 (0.72)^{bc}	2.32 (0.13)^{b}	4.21 (2.27)^{abc}	0.72 (0.03)^{a}	6.44 (2.74)^{b}	2.95 (0.81)^{ab}	1.93 (0.76)^{a}
	Komothai	4.16 (3.93)^{bc}	5.90 (0.04)^{cd}	6.46 (0.05)^{c}	4.08 (2.85)^{cd}	4.10 (0.37)^{bc}	3.80 (0.78)^{abc}	0.99 (0.34)^{a}	8.07 (3.21)^{bcd}	5.78 (0.28)^{bc}	4.68 (0.62)^{bc}
	Ngewa	6.24 (3.20)^{cd}	8.08 (4.50)^{cd}	6.43 (0.01)^{c}	5.50 (2.20)^{de}	5.52 (2.83)^{cd}	2.41± 0.82^{a}	0.83 (0.07)^{a}	9.23 (2.78)^{cde}	8.63 (4.58)^{cd}	7.40 (0.80)^{bd}

Means (standard deviation) with the same superscript letters along the column are not significantly different at $p \leq 0.05$ level (Tukey's test)

4.4 Effect of seasonal variations on the mean levels (ng/mL) of Organophosphates in Cow and Camel milk from Kiambu, Isiolo and Laikipia

Table 4.9 is a summary of the effect of seasonal variation on the mean levels of organophosphates in Cow and Camel milk collected from Kiambu, Isiolo, and Laikipia Counties. There was no difference in the mean level of methacrifos, disulfoton, terbufos, tolclofos-methyl, fenchlorphos, malathion, fenamiphos, chlorthiophos-2, profenofos, chlorthiophos-1, chlorthiophos-3, leptophos, and coumaphos in camel milk collected during the dry season of January relative to camel milk collected during the wet season (December) in the study area (Table 4.9). However, the mean level of tetrachlorvinphos in camel milk collected during the dry season was significantly higher than the mean level in camel milk collected during the wet season. There was no difference in the mean level of methacrifos, disulfoton, terbufos, tolclofos-methyl, fenchlorphos, tetrachlorvinphos, malathion, fenamiphos, chlorthiophos-2, profenofos, chlorthiophos-1, chlorthiophos-3, and leptophos in cow milk collected during the dry season of February relative to cow milk collected during the wet season of December in the study area. However, the mean level of coumaphos in cow milk collected during the wet season of December was significantly higher than the mean level in cow milk collected during the dry season of February.

Table 4.9: The effect of seasonal variation on the mean levels (ng/mL) of Organophosphates in Cow and Camel milk collected from Kiambu, Isiolo and Laikipia Counties

COUNTY	Sample	Season	Methacrifos	Disulfoton	Terbufos	Tolclofos-methyl	Fenchlorphos	Malathion	Tetrachlorvinphos	Fenamiphos	Chlorthiophos-2	Profenofos	Chlorthiophos-1	Chlorthiophos-3	Leptophos	Coumaphos
Isiolo & Laikipia	Camel milk	Dry January	0.11 (0.07)^{a}	0.11 (0.01)^{a}	0.2 (0.01)^{a}	0.2	0.3	1.21 (0.85)^{b}	9.69 (3.80)^{c}	0.23 (0.02)^{a}	0.02	0.1	16.32 (0.54)^{a}	0.2	0.2	5.73 (0.97)^{c}
		Wet December	0.13 (0.01)^{a}	0.50 (0.20)^{a}	0.24 (0.08)^{a}	0.2	0.3	1.21 (0.77)^{b}	7.97 (4.95)^{b}	0.34 (0.02)^{a}	0.02	0.1	5.39 (0.60)^{a}	0.2	0.2	5.19 (1.92)^{bc}
Kiambu	Cow milk	Dry February	0.93 (0.52)^{b}	2.06 (0.37)^{b}	0.82 (0.43)^{b}	0.2	0.3	0.07 (0.01)^{a}	0.75 (0.08)^{a}	1.23 (0.62)^{b}	0.02	0.1	50.34 (0.48)^{b}	0.2	0.2	4.05 (2.43)^{a}
		Wet December	0.84 (0.57)^{b}	2.29 (0.40)^{b}	0.82 (0.23)^{b}	0.2	0.3	0.22 (0.05)^{a}	0.78 (0.28)^{a}	1.24 (0.61)^{b}	0.02	0.1	42.82 (0.47)^{b}	0.2	0.2	4.90 (1.73)^{b}

Means (standard deviations) with the same superscript letters along the column for respective drugs and animals are not significantly different at the $p \leq 0.05$ level (Tukeys's test) during the wet and dry seasons.

Figure 4.8 shows the comparison of organophosphate pesticides in cow milk samples collected during the wet and dry seasons. Chlorthiophos-1 registered the highest concentration in cow milk samples for both wet and dry seasons, followed by coumaphos.

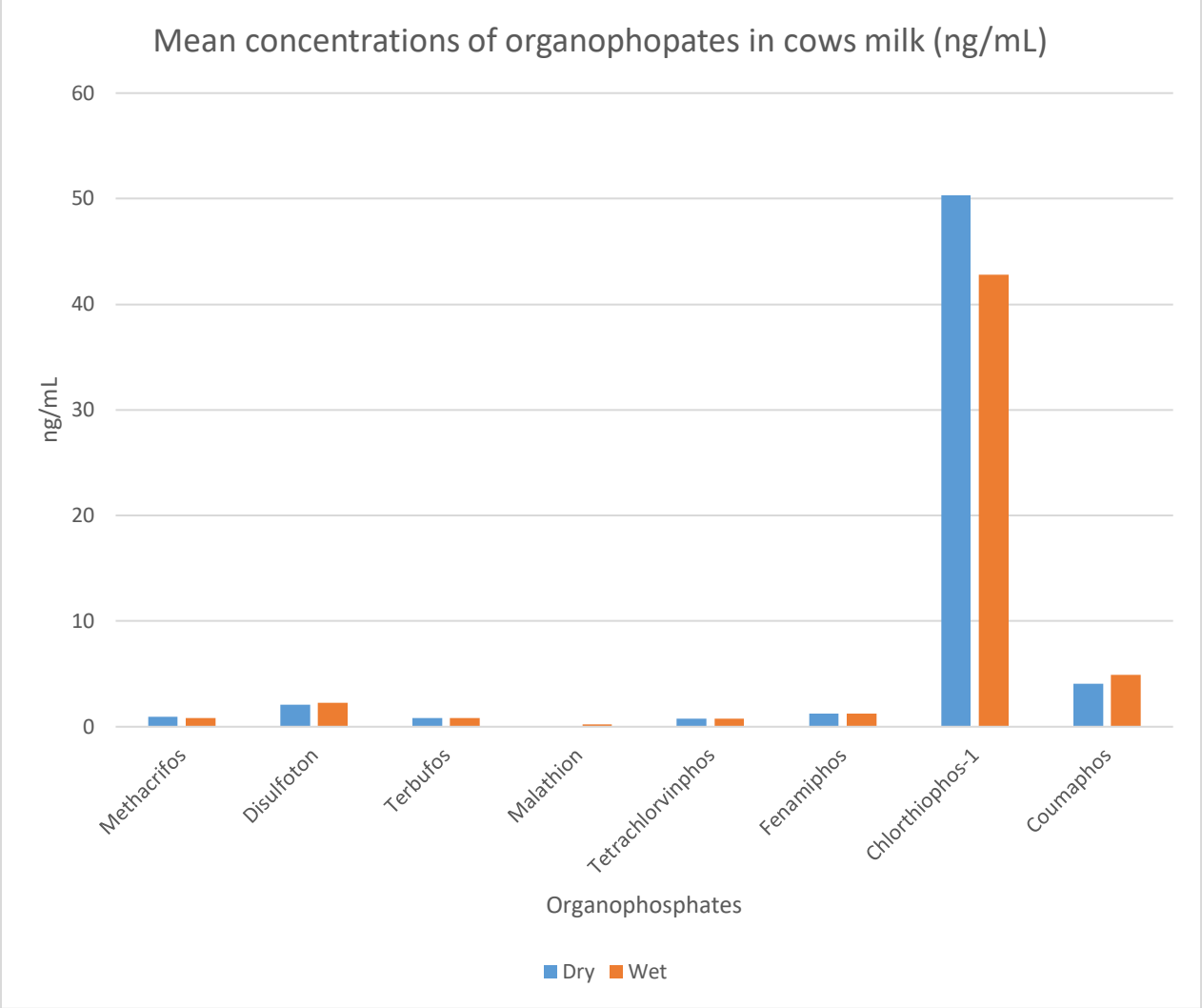


Figure 4.8: Seasonal variation on the mean levels (ng/mL) of Organophosphates in cow milk collected from Kiambu.

Figure 4.9 shows the comparison of organophosphate pesticides in camel milk samples collected during the wet and dry seasons. Tetrachlorvinphos, Chlorthiophos-1 and coumaphos registered the highest concentration in amel milk samples for both wet and dry seasons.

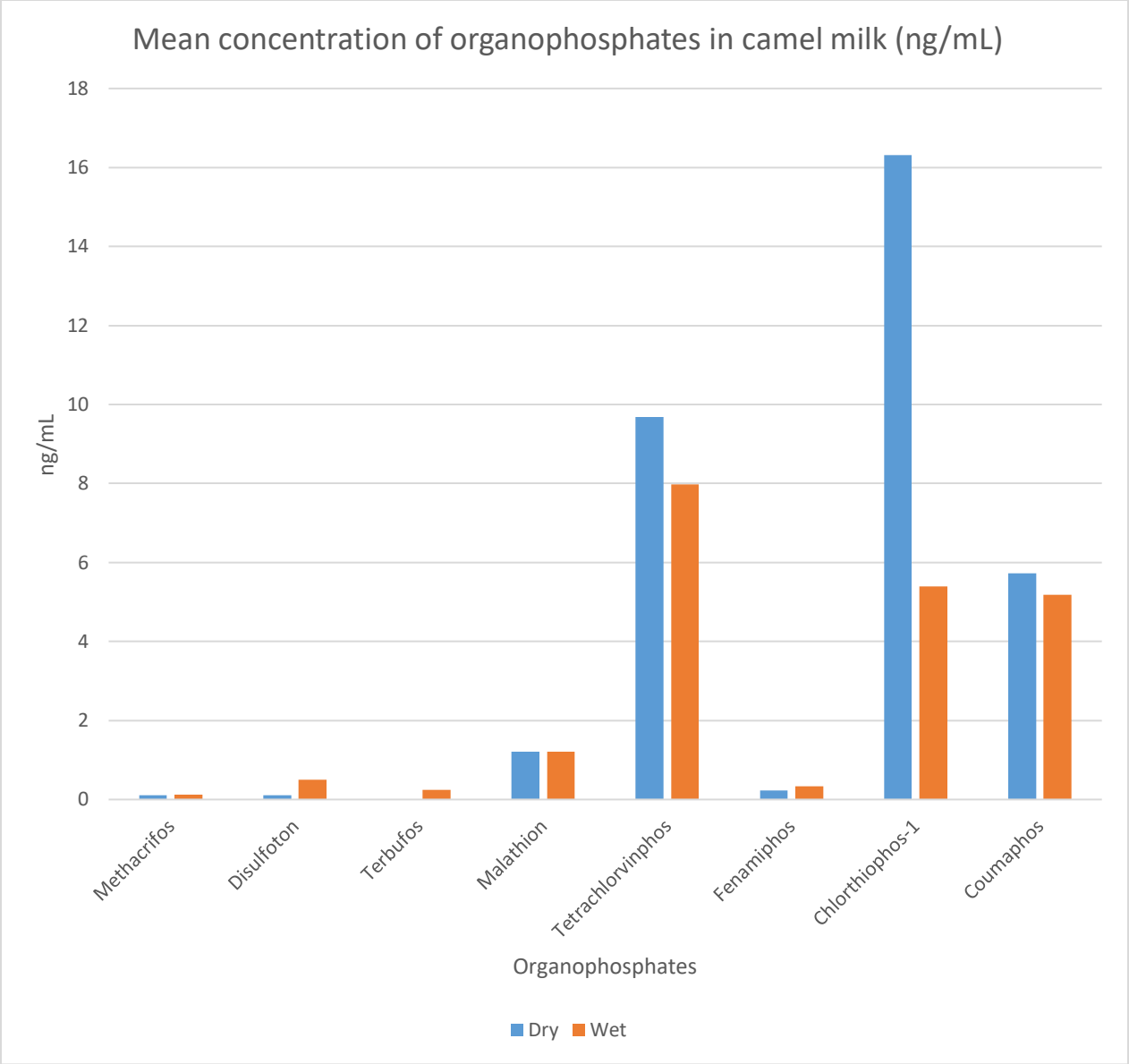


Figure 4.9: Seasonal variation on the mean levels (ng/mL) of Organophosphates in camel milk collected from Isiolo and Laikipia counties

4.5 Effect of seasonal variations on the mean levels (ng/mL) of Organochlorines in Cow and Camel milk from Kiambu, Isiolo and Laikipia

Table 4.10 shows a summary of the effect of seasonal variation on the mean level of organochlorines in cow and camel milk collected from Kiambu, Isiolo, and Laikipia Counties. There was no difference in the mean level of α-BHC, β-BHC, hexachlorobenzene, δ-BHC, heptachlor, cis-chlordane, trans-chlordane, *p,p'*-DDE, α-endosulphan, dieldrin, trans-nonachlor, chlorfenson, *p,p'*-DDD, endrin, β-endosulphan, *p,p'*-DDT, *o,p'*-DDT and mirex in camel milk collected during the dry season of January relative to camel milk collected during the wet season of December in the study area. However, the mean level of γ-BHC, o'p-DDD and cis-nonachlorin camel milk collected during the wet season was significantly higher than the mean level in camel milk collected during the dry season. Table 12. There was no difference in the mean level of α-BHC, β-BHC, hexachlorobenzene, γ-BHC, δ-BHC, heptachlor, cis-chlordane, trans-chlordane, *p,p'*-DDE, α-endosulphan, chlorphenson, *p,p'*-DDD, endrin, *p,p'*-DDT, *o,p'*-DDT and mirex in cow milk collected during the dry season of February relative to cow milk collected during the wet season of December in the study area. However, the mean level of dieldrin, *o,p'*-DDD, trans-nonachlor, and *β*-endosulphan in cow milk collected during the wet season was significantly higher than the mean level in cow milk collected during the dry season.

Table 4.10 The effect of seasonal variation on the mean levels (ng/mL) of Organochlorines in Cow and Camel milk collected from Kiambu, Isiolo and Laikipia Counties.

County	Animal	Season	alpha-BHC	beta-BHC	Hexachlorobenzene	gamma-BHC (Lindane)	delta-BHC	Heptachlor	cis-Chlordane	trans-Chlordane	p,p'-DDE	alpha-Endosulfan	Dieldrin	o,p'-DDD	trans-Nonachlor	Chlorfenson	p,p'-DDD	Endrin	beta-Endosulfan	p,p'-DDT	cis-Nonachlor	o,p'-DDT	Mirex
Isiolo & Laikipia	Camels	Dry January	0.2 (0.01)^{a}	0.2 (0.01)^{a}	0.2 (0.01)^{a}	0.06 (0.05)^{b}	2.03 (1.10)^{b}	5.12 (3.28)^{b}	0.2 (0.01)^{a}	0.2 (0.01)^{a}	0.2 (0.01)^{a}	0.41 (0.03)^{a}	0.10 (0.02)^{a}	0.71 (0.32)^{a}	1.28 (0.76)^{ab}	0.10 (0.05)^{a}	0.3 (0.01)^{a}	0.2 (0.01)^{a}	2.67 (0.84)^{ab}	0.01 (0.01)^{a}	3.06 (0.96)^{b}	11.89 (3.89)^{b}	0.07 (0.04)^{a}
		Wet December	0.2 (0.01)^{a}	0.2 (0.01)^{a}	0.2 (0.01)^{a}	0.08 (0.05)^{c}	2.19 (0.98)^{b}	6.11 (4.00)^{b}	0.2 (0.01)^{a}	0.2 (0.01)^{a}	0.2 (0.01)^{a}	0.59 (0.09)^{a}	0.24 (0.08)^{a}	1.35 (0.28)^{b}	1.20 (0.65)^{ab}	0.08 (0.01)^{a}	0.3 (0.01)^{a}	0.2 (0.01)^{a}	2.63 (0.84)^{ab}	0.02 (0.01)^{a}	4.74 (0.09)^{c}	11.79 (3.85)^{b}	0.11 (0.09)^{a}
Kiambu	Cows	Dry February	0.2 (0.01)^{a}	0.2 (0.01)^{a}	0.2 (0.01)^{a}	0.3 (0.01)^{a}	0.13 (0.04)^{a}	10.66 (5.90)^{a}	0.2 (0.01)^{a}	0.2 (0.01)^{a}	0.2 (0.01)^{a}	2.29 (0.81)^{b}	1.44 (0.88)^{b}	8.16 (3.49)^{b}	1.08 (0.81)^{a}	1.04 (0.53)^{b}	0.3 (0.01)^{a}	0.2 (0.01)^{a}	2.47 (1.08)^{a}	0.13 (0.03)^{b}	0.76 (0.41)^{a}	2.85 (1.21)^{a}	0.75 (0.25)^{b}
		Wet December	0.2 (0.01)^{a}	0.2 (0.01)^{a}	0.2 (0.01)^{a}	0.3 (0.01)^{a}	0.11 (0.05)^{a}	13.74 (3.82)^{a}	0.2 (0.01)^{a}	0.2 (0.00)^{a}	0.2 (0.01)^{a}	2.50 (0.51)^{b}	1.93 (0.49)^{c}	9.61 (1.99)^{c}	1.47 (0.58)^{b}	1.02 (0.54)^{b}	0.3 (0.01)^{a}	0.2 (0.01)^{a}	2.87 (0.50)^{b}	0.13 (0.02)^{b}	1.95 (0.75)^{b}	3.38 (0.10)^{a}	0.70 (0.31)^{b}

Means (standard deviations) with the same superscript letters along the column for respective drugs and animals are not significantly different at the $p \leq 0.05$ level (Tukeys's test) during the wet and dry seasons.

ND: Not detected/below detection limit.

Figure 4.10 shows the comparison of organochlorine pesticide residues in camel milk. The highest levels of OCPs registered were *o,p*-DDT, heptachlor, *cis*-Nanochlor, *beta*-endosulphan and *delta*-BHC.

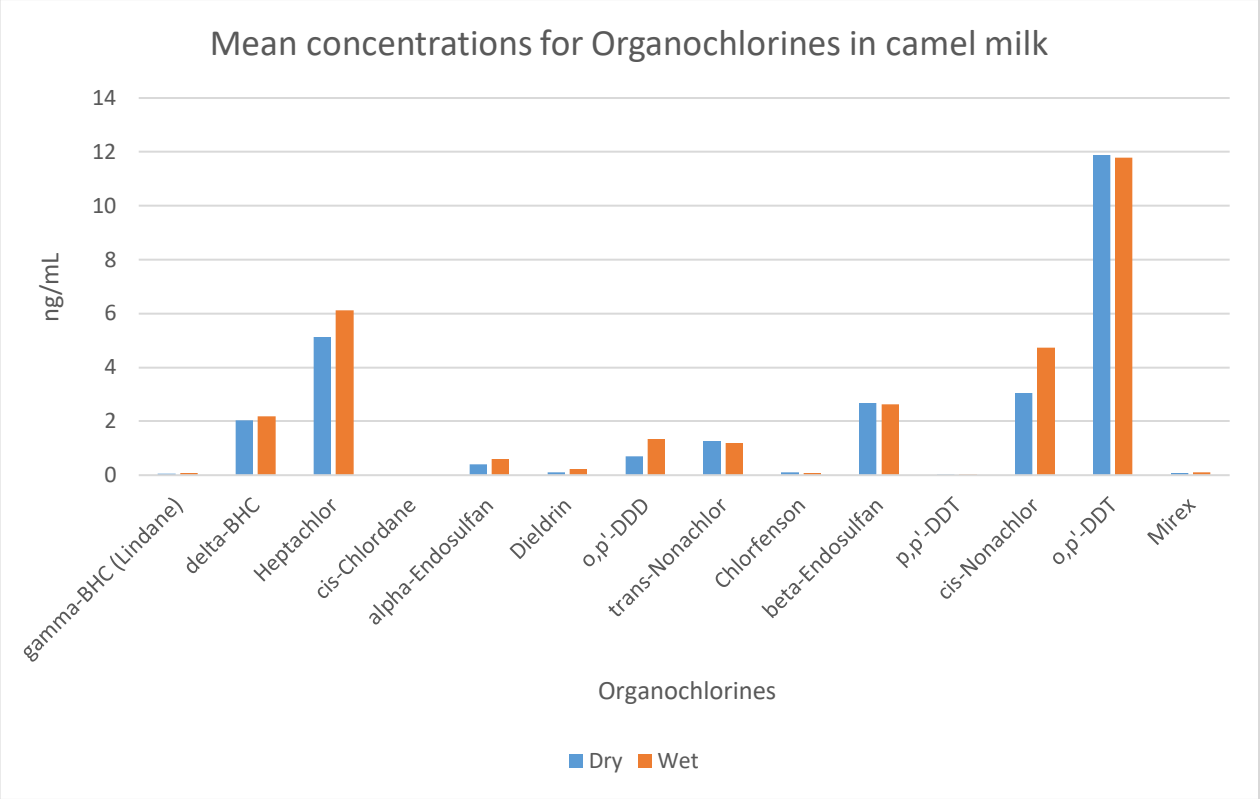


Figure 4.10: Seasonal variation on the mean levels (ng/mL) of Organochlorines in Camel milk collected from Isiolo and Laikipia Counties

Figure 4.11 shows the comparison of organochlorine pesticide residues in cow milk. The highest levels of OCPs registered were heptachlor and *o,p*-DDD.

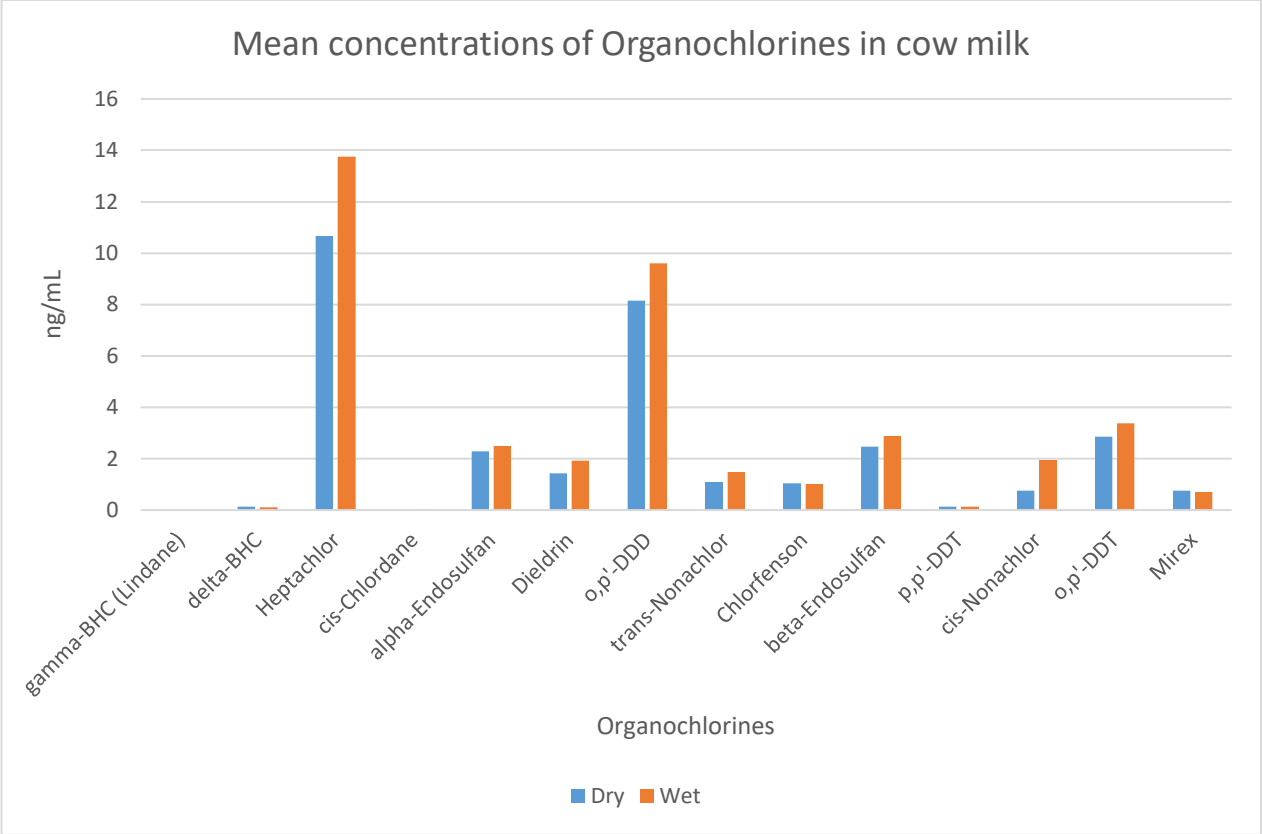


Figure 4.11: Seasonal variation on the mean levels (ng/mL) of Organochlorines in Cow milk collected from Kiambu County

4.6 The effect of seasonal variation on the mean levels (ng/mL) of Veterinary drug residues in cow and camel milk collected from Kiambu, Isiolo and Laikipia Counties.

Table 4.11 shows a summary of the effect of seasonal variation on the mean level of veterinary drug residues in cow and camel milk collected from Kiambu, Isiolo and Laikipia Counties. There was no difference in the mean level of sulfadiazine, sulfachloropyradazine, sulfapyridine, sulfamerazine, sulfaguanadine, sulfamethoxazole, sulfathiazole, sulfamethoxypyradine, sulfamethazine, sulfadimethoxine and sulfadoxine in camel milk collected during the dry season of January relative to camel milk collected during the wet season of December.

Table 4.11: The effect of seasonal variation on the mean level (ng/mL) of Veterinary drug residues in Cow and Camel milk collected from kiambu, Isiolo and Laikipia Counties

County	Animal	Season	SulfaDiazine	Sulfachloro pyradazine	Sulfahiazole	SulfaPyridine	Sulfamerazine	Sulfaguanadine	Sulfamethoxazole	Sulfametho xypyradine	Sulfamethazine	Sulfadimethoxine	Sulfadoxine
Isiolo	Camels	Dry January	0.74 (0.08)^{a}	0.42 (0.07)^{a}	2.21 (0.89)^{a}	0.62 (0.12)^{a}	0.02 (0.01)^{a}	4.08 (1.64)^{ab}	4.50 (0.12)^{c}	0.04 (0.0)^{a}	0.82 (0.17)^{a}	10.67 (0.05)^{c}	4.82 (0.42)^{a}
		Wet December	0.82 (0.05)^{a}	0.36 (0.05)^{a}	2.48 (0.49)^{b}	0.47 (0.02)^{a}	0.02 (0.01)^{a}	4.62 (0.75)^{b}	4.47 (0.09)^{c}	0.11 (0.01)^{a}	0.27 (0.09)^{a}	10.69 (0.16)^{c}	4.91 (0.11)^{a}
Kiambu	Cows	Dry February	5.80 (3.43)^{c}	7.75 (4.52)^{c}	6.46 (0.07)^{c}	5.15 (2.42)^{c}	5.28 (3.12)^{c}	3.22 (0.20)^{a}	1.16 (0.74)^{b}	9.26 (2.86)^{c}	8.01 4.88^{c}	6.65 4.19^{b}	7.83 4.64^{b}
		Wet December	3.90 (0.92)^{b}	5.39 (0.11)^{b}	6.45 (0.04)^{c}	3.79 (2.91)^{b}	3.88 (0.44)^{b}	3.50 (0.94)^{a}	0.67 (0.01)^{a}	7.74 (3.15)^{b}	5.04 (5.00)^{b}	4.47 (0.62)^{a}	5.48 (0.30)^{a}

Means (standard deviations) with the same superscript letters along the column for respective drugs and animals are not significantly different at the $p \leq 0.05$ level (Tukeys's test) during the wet and dry seasons. ND: Not detected/below detection limit.

However, the mean level of sulfathiazole in camel milk collected during the wet season was significantly higher than the mean level in camel milk collected during the dry season. On the other hand, the mean level of sulfadiazine, sulfachloropyradazine, sulfapyridine, sulfamerazine, sulfamethozaxole, sulfamethoxypyradine, sulfamethazine, sulfadimethoxine and sulfadoxine in cow milk collected during the wet season of December was significantly lower than the mean levels in cow milk collected during the dry season of February.

Figure 4.12 shows the comparison of veterinary drug residues in camel milk. The highest levels of registered were sulfamethoxine, sulfadoxine, sulfamethoxazole and sulfaguanadine in wet and dry season.

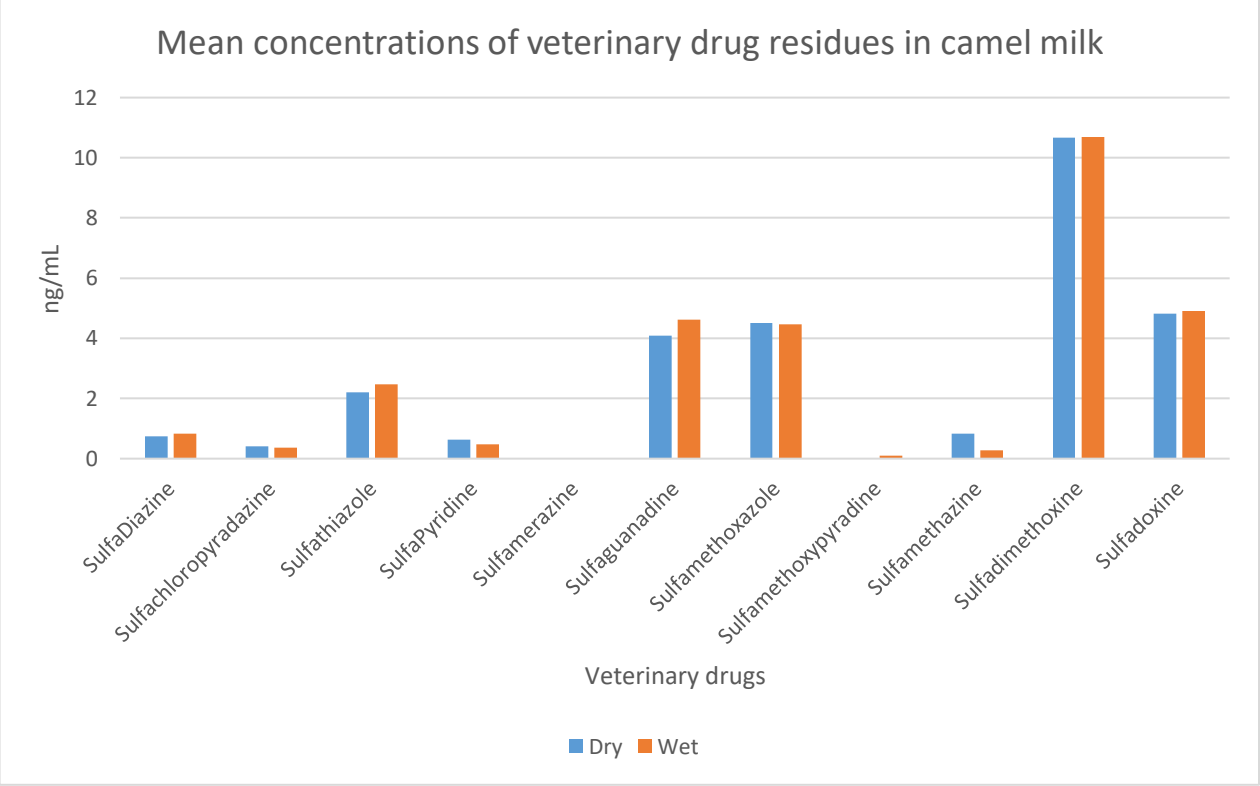


Figure 4.12: seasonal variation on the mean level (ng/mL) of Veterinary drug residues in Camel milk collected from, Isiolo and Laikipia Counties

Figure 4.13 shows the comparison of veterinary drug residues in cow milk. High frequence of contamination was registered in the cow milk with the highest levels of registered were sulfamethoxypyradine, sulfamethazine, sulfadoxine and sulfachloropyradazine.

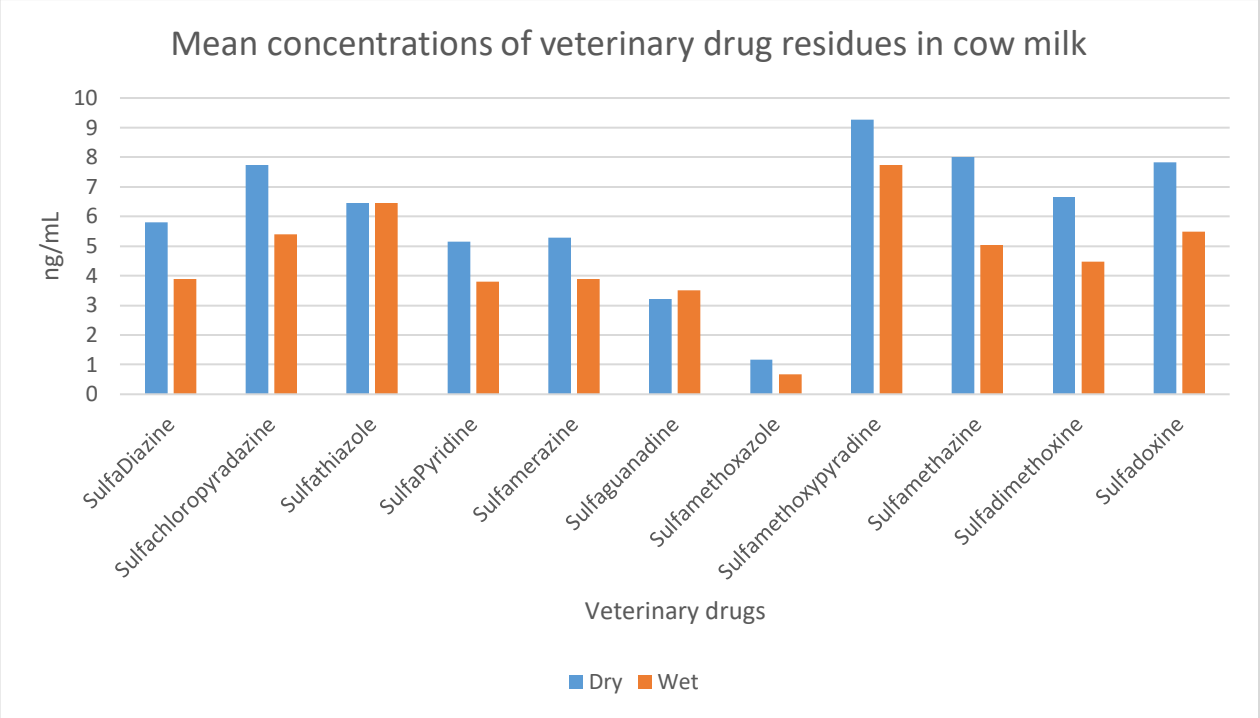


Figure 4.13: seasonal variation on the mean level (ng/mL) of Veterinary drug residues in Camel milk collected from, Isiolo and Laikipia Counties

4.7 Discussion of results

4.7.1 Pesticide residues in cow and camel milk collected from Kiambu, Isiolo and Laikipia County

The high toxicity of pesticides towards human, animal and environmental health has resulted in the total ban or restriction of their use. However, some of the harmful pesticides are still available for use in Kenya and continue to threaten the animal, environment and the health of Kenyans (Kaigwara *et al.*, 2002). Knowledge on the levels of pesticides in general in food products such as milk may

be important in evaluating dietary risk exposure in children and adults. Previous studies on pesticides residues in Kenya have mainly focused on levels in water, fish, beef, sediments, tomatoes and camel meat (Werimo *et al.,* 2009). It was established that the mean levels of all the 14 organophosphates tested were below the maximum residue limit set by the EU pesticides database, Codex Alimentarius and the USDA. Organophosphates are less persistent, unstable and degrade rapidly by hydrolysis on exposure to air, soil and sunlight unlike organochlorines (Simo *et al.,* 2010). This may be a reason they were below the maximum residue levels. In addition, farmers in the study area may be adequately educated on the use of these pesticides, the use of these pesticides or good agricultural practices while handling milk. However, the observation that cow milk registered higher mean levels of Chlorthiphos-1 (46.7 ng/mL) in Kiambu county relative to camel milk may have something to do with the fact that Kiambu county where the cow milk was collected is an agriculture intensive area and there may be a possibility of prior use of these pesticides relative to Isiolo and Laikipia counties where camel milk was collected which are mainly inhabited by pastoralists who may not be practicing large scale agriculture. Leaching and surface run-off could be another potential reason for the high levels of these organophosphates (Srivastava *et al.,* 2008). It was observed that the mean levels of heptachlor evaluated in cow and camel milk collected in the study area were above the maximum residue limits set by the Codex Alimentarius, EU pesticides database. These findings are in agreement with those of Omwenga and colleagues who reported high levels of heptachlor in farmed fish collected in Kiambu and Machakos Counties (Omwenga *et al.,* 2016). Heptachlor is a persistent organic pollutant (POP) which has been reported to be present in soil for up to 14 years after its initial application (ToxFAQs 2020). Like other POP's, it is lipophilic and poorly soluble in water and tends to accumulate in the body fat of humans and animals (ToxFAQs, 2020). This organochlorine was banned in the US in the 1980's and is

considered a potential carcinogen by the International Agency for Research on Cancer and the Environmental Protection Agency (EPA). Drinking water and breast milk have been reported as possible routes of exposure. Animals exposed to heptachlor during gestation and infancy have been found to have changes in their nervous system and immune function (Fan *et al.,* 2020). Exposure to higher doses in newborn animals have led to decreased body weight and death (Fan *et al.,* 2020). BHC isomer and DDT were detected in some of the milk samples in the study area. The use of these pesticides was banned in Kenya on account of their persistence in the environment and toxicity to untargeted organisms (Omwenga *et al.,* 2016). Some of the isomers of BHC were initially used for seed dressing to protect crops against termites and for use in cattle dips and spraying against ticks. Could farmers in the study area be using these pesticides illegally? Or could their detection be related to previous application? These are pertinent queries that require further inquiry. Kiambu had the highest OC mean levels in cow milk, chlorfenson 1.03 ng/mL, dieldrin 1.72 ng/mL, α+β endosulphan 5.10 ng/mL, trans-nonachlor 1.30 ng/mL, *o,p'*-DDT 8.97 ng/mL. These mean levels were below the set MRL levels of Codex Alimentarius, EU Pesticides database and USDA. The levels of dieldrin were lower than those detected in raw fresh milk sampled in Kampala markets in Uganda with concentration of 7 ng/mL (Kampire *et al.,* 2011). Furthermore, a study carried out in Giza, Egypt reported higher mean concentration of dieldrin in raw milk 2.966± 0.135 ng/mL (Ahmed and Zaki, 2009). However, Salem et al. (2009) did not detect any dieldrin residues in milk sampled from Jordan. A study carried out in Assiut agro-industrial areas, Egypt detected several OP and OC pesticides residues in raw buffalo milk samples. Lindane and malathion exceeded EC MRLs (2008) together with chlorpyrifos, methoxychlor and HCB (Shaker *et al.,* 2015). A study of raw milk samples collected in nine Italian dairy plants detected traces of pesticides residues in 4.4% of the samples analyzed. The main pesticide was chlorpyrifos (Gazzotti

et al., 2009). Mean concentrations of 13 OP pesticides residues from Mexican milk samples were below established MRLs and ranged between 0.0051 and 0.0203 ppm (Salas *et al.,* 2003).

Among the 14 organophosphates tested, only the MRL of Profenofos was captured by all 3 MRL database such as the Codex Alimentarius, EU Pesticides database, and the USDA. Moreover, of all the 18 organochlorines tested only γ-BHC, Dieldrin and DDT were captured by all the three MRL databases. It is worth noting that there was hardly a consensus on the MRL values among the 3 databases. Taken together, these observations underscore the need to harmonize the MRL's database such that there is a standard/common criteria/database for the general evaluation of MRL's.

4.7.2 Veterinary drug residues in cow and camel milk collected from Kiambu, Isiolo and Laikipia County

Eleven veterinary drugs tested had mean concentrations ranging from <0.02 ng/mL to 25.98 ng/mL. All the multiple veterinary drug residues detected were under the maximum residue levels as regulated by the United States, European Union and Codex Alimentarius Commission in all the raw milk samples from Kiambu, Isiolo and Laikipia. This result was consistent with a study done by Aboge (2002) who detected no anti-microbial residues in Nairobi, Kiambu and Nakuru districts from milk samples. However, this finding differs from that of Brown (2020) who found Beta-lactam 7% and tetracycline 3% of samples of milk from Kibera.

The observed lack of anti-microbial residues in milk in this study may be attributed to control by some dairy cooperatives societies' management who often contact area veterinarians to get reports on which farmers animals have been treated and expected periods of milk withdrawal (Aboge, 2002).

A study carried out in seven districts of Hebei province China did not detect veterinary drugs residues above the maximum residue limit, however four veterinary drugs lincomycin, trimethoprim, sulfacetamide, penicillin G were detected at 20.7 ppb, 9.3, 1.08 and 2.86, respectively (Han *et al.*, 2017).

Seventeen fresh milk samples were tested in Taiwan and no veterinary drug residue was detected apart from lincomycin with concentrations ranging from 6.9 to 92.3 ng/mL (Tang *et al.*, 2011).

4.7.3 Effects of seasonal variation on the mean levels of organophosphates in cow and camel milk collected from Kiambu, Isiolo and Laikipia counties

There was no difference in the mean levels of organophosphates tested both in wet and dry season in camel raw milk. However, the mean levels of tetrachlorvinphos in camel milk was higher in dry season therefore these residues probably resulted from water, soil, air or contaminated feeds.

There were no differences in the mean levels of organophosphates from cow milk both in wet and dry season. However, the mean levels of coumaphos in cow milk collected during wet season was higher than in dry season mainly because Kiambu is an agricultural area and during the rainy season there are many agricultural activities such as spraying pesticides to protect plants and animals from insects and pests.

4.7.4 Effects of seasonal variation on the mean levels of Organochlorines in cow and camel milk collected from Kiambu, Isiolo and Laikipia counties

There were no differences in the mean levels of all the Organochlorines tested in camel milk during the wet and dry season. However mean levels of gamma-BHC (lindane), *o,p'*-DDD and cis-nonachlor in camel milk was higher in wet season relative to dry season which could be attributed to agricultural activities during wet season such as spraying pesticides to plants and animals to protect them against insects and pests.

There were no differences in the mean levels of all the Organochlorines tested in cow milk during wet and dry season. However, the mean levels of dieldrin, *o,p'*-DDD, transnonachlor and β-endosulphan in cow milk was higher in wet season relative to dry season. This is consistent with other researchers in the country who detected high mean levels of methoxychlor and *p,p'*-DDD in milk samples during wet season (Mwenda *et al.*, 2017).

4.7.5 Effects of seasonal variation on the mean levels of Veterinary drugs in cow and camel milk collected from Kiambu, Isiolo and Laikipia counties

There were no differences in the mean levels of all the veterinary drugs tested in camel milk during wet and dry season. However, the mean levels of sulfathiazole in camel milk during wet season was higher than in dry season. The mean levels of veterinary drugs tested in cow milk were higher in dry season relative to wet season. However, the mean levels of sulfaguanadine and sulfamethoxazole were high in wet season.

CHAPTER FIVE

5. CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

Organophosphates pesticides comprising of Chlorthiophos-1,2,3, coumaphos, disulfoton, fenamiphos, fenchlorphous, leptophos, malathion, methacrifos, profenofos, terbufos, tetrachlorvinphos, tolclofos-methyl were detected in cow and camel milk from Kiambu, Isiolo and Laikipia counties. The mean levels of the organophosphates detected were all below the maximum residue limit set by the Codex Alimentarius, European Union Pesticide database and the United States Department of Agriculture guidelines. This may have attributed to organophosphates being less persistent, unstable and degrade rapidly by hydrolysis on exposure to air, soil and sunlight unlike organochlorines which are very persistent and stable in the environment. Currently most of the Organochlorines have been banned replacing them with organophosphates (Simo *et al.*, 2010). In addition, farmers in the study area may be adequately educated on the use of these pesticides or the use of these pesticides may be generally low in the study area. Farmers may be practicing good agricultural practices while handling milk.

Organochlorines pesticide residues comprising of alpha-BHC, beta-BHC, gamma-BHC (lindane), delta-BHC, alpha-endosulphan, beta-endosulphan, alpha+beta endosulphan, cis-chlordane, transchlordane, chlorfenson, dieldrin, endrin, heptachlor, hexachlorbenzene, mirex, cis-nonachlor, trans-nonachlor, cis+ trans nonachlor, *o,p'*-DDD, DDT were within the acceptable limits. However, both cow and camel milk collected from the study area had mean levels of heptachlor above the maximum residue limit set by the Codex Alimentarius, European Union Pesticide database and the United States Department of Agriculture.

Organochlorine pesticide residues were generally higher in Kiambu and Laikipia counties compared to Isiolo county. This may be attributed to the fact that Kiambu and Laikipia counties are high potential areas; there is a high chance of organochlorines usage in the past. Another reason may be due to agricultural activities practiced in these areas such as spraying pesticides in plants and animals for diseases and vector control. In addition, milk has high lipid content and Organochlorine pesticides are highly lipophilic and tend to accumulate in matrix with high fat content such as milk. Another possible source of might have been from air, soil or surface run-off from agricultural fields.

Veterinary drug residues comprising sulfadimethoxine, sulfadoxine, sulfachloropyradazine, sulfamethoxypyradine, sulfamethazine, sulfamerazine, sulfathiazole, sulfamethoxazole, sulfagunadine, sulfadiazine and sulfapyridine mean levels for cow and camel milk were all below the maximum residue limit set by the Codex Alimentarius, European Union database and the United States Department of Agriculture.

Raw cow and camel milk from Kiambu, Isiolo and Nanyuki is considered relatively safe for human consumption because of the low prevalence of veterinary drug residues. However, strict control measurements for veterinary drug residues in raw cow and camel milk are required because some veterinary drugs were detected in milk from some areas.

6.2 RECOMMENDATIONS

1. Strict national regulations, policies, guidelines and legislation should be passed on livestock sector to avoid unnecessary use of pesticides and veterinary drugs.
2. More studies should be done mostly in the rural areas where high mean levels of organochlorines were previously detected in milk.

3. Usage of veterinary drugs without a veterinarian prescription should be discouraged, educating and training farmers on drug withdrawal period of treated animals.
4. National residue monitoring plans and chemical residue control should be designed and implemented to set standards on the usage of chemicals.
5. Training farmers on good agricultural practices and monitoring are useful to reduce pesticides and veterinary drug residues in milk.
6. Further research on the effect of chemical residues to human and control systems should be put in place.

REFERENCES

Abdelkhalek, A., Elsherbini, M., & Gunbaej, E. E. (2015). Assessment of heavy metal residues in milk powder and infant milk formula sold in Mansoura City, Egypt. *Alexandria Journal of Veterinary Sciences*, *47*(1), 71-77.

Abdulkhaliq, A., Swaileh, K., Hussein, R. M., & Matani, M. (2012). Levels of metals (Cd, Pb, Cu and Fe) in cow's milk, dairy products and hen's eggs from the West Bank, Palestine. *International Food Research Journal 19(3):1089-1094.*

Aboge, G. O. (2002). *Antimicrobial residues detected in marketed milk in urban and rural areas in Kenya* (Doctoral dissertation, University of Nairobi).

Abong'o D. A, SO Wandiga, and IO Jumba, (2018). Occurrence and Distribution of Organochlorine Pesticide Residue Levels in Water, Sediment and Aquatic Weeds in the Nyando River Catchment, Lake Victoria, Kenya," *African Journal of Aquatic Science* 43,(3) : 255–70, https://doi.org/10.2989/16085914.2018.1490244.

Ahlberg, S. H., Korhonen, H., Lindfors, E., & Kang'ethe, E. K. (2016). Analysis of antibiotic residues in milk from smallholder farms in Kenya. *African Journal of Dairy Farming and Milk Production*.3(4):152-158

Ahmed, N. S., & Zaki, E. M. S. (2009). Detection of some organochlorine pesticides in raw milk in giza governorate. *J. Appl. Sci. Res*, *5*(12), 2520-2523.

Alexsandro Dallegrave et al., "Methodology for Trace Analysis of 17 Pyrethroids and Chlorpyrifos in Foodstuff by Gas Chromatography–Tandem Mass Spectrometry," *Analytical and Bioanalytical Chemistry* 408, no. 27 (November 2016): 7689–97, https://doi.org/10.1007/s00216-016-9865-5.

Anderson, E. S. (1968). The ecology of transferable drug resistance in the enterobacteria. *Annual Reviews in Microbiology*, *22*(1), 131-180.

Armentano, A., Summa, S., Magro, S. L., Palermo, C., Nardiello, D., Centonze, D., & Muscarella, M. (2018). Rapid method for the quantification of 13 sulphonamides in milk by conventional high-performance liquid chromatography with diode array ultraviolet detection using a column packed with core-shell particles. *Journal of Chromatography A*, *1531*, 46-52.

Ashbolt, N. J., Amézquita, A., Backhaus, T., Borriello, P., Brandt, K. K., Collignon, P., ... & Topp, E. (2013). Human health risk assessment (HHRA) for environmental development and transfer of antibiotic resistance. *Environmental health perspectives*, *121*(9), 993-1001.

Aytenfsu, S., Mamo, G., Kabede, B. (2016). Review on Chemical Residues in Milk and Their Public Health Concern in Ethiopia.*Journal of Nutrition & Food Science*.6:4

Azegele, A. E. (2010). *Quantitative Assessment of Sulfadimidine Residues levels in Eggs sold in Nairobi, Kenya* (Doctoral dissertation, University of Nairobi, Kenya).

Benford D, (2000). Acceptable Daily Intake- A tool for ensuring food safety. *International Life Science Institute*. ILSI Press, 1126 Sixteenth Street, N.W Washington, D.C 20036-4810 U.S A p 3-6.

Boeckel, T. P. V., Brower, C., & Gilbert, M. (2015). Grenfel BTl, Levin SA, Robinson TP, et al. *Global trends in antimicrobial use in food animals. PNAS*, *112*(18), 5649-5654.

Brown, K., Mugoh, M., Call, D. R., & Omulo, S. (2020). Antibiotic residues and antibiotic-resistant bacteria detected in milk marketed for human consumption in Kibera, Nairobi. *Plos one*, *15*(5), e0233413.

Cheong, C. K., Hajeb, P., Jinap, S., & Ismail-Fitry, M. R. (2010). Sulfonamides determination in chicken meat products from Malaysia. *Int. Food Res. J*, *17*, 885-892.

Chopra, I., Hawkey, P. M., & Hinton, M. (1992). Tetracyclines, molecular and clinical aspects. *Journal of Antimicrobial Chemotherapy*, *29*(3), 245-277.Chromatography Coupled with Tandem Mass Spectrometry. Food Chemistry. 197, 730–736.Chromatography–Negative Chemical Ionisation Tandem Mass Spectrometric Determination

Dallegrave, A., Pizzolato, T. M., Barreto, F., Eljarrat, E., & Barceló, D. (2016). Methodology

Darwish, W. S., Eldaly, E. A., El-Abbasy, M. T., Ikenaka, Y., Nakayama, S., & Ishizuka, M. (2013). Antibiotic residues in food: the African scenario. *Japanese Journal of Veterinary Research*, *61*(Supplement), S13-S22.

Decision, C. (2002). 657/EC implementing Council Directive 96/23/EC concerning the performances of analytical methods and the interpretation of results. *Official Journal of the European Communities*, (L221),6-8.

Deme, P., Azmeera, T., Devi, B. L. A. P., Jonnalagadda, P. R., Prasad, R. B. N., & Sarathi, U.

Deti, H., Hymete, A., Bekhit, A. A., Mohamed, A. M. I., & Bekhit, A. E. D. A. (2014). Persistent organochlorine pesticides residues in cow and goat milks collected from different regions of Ethiopia. *Chemosphere*, *106*, 70-74.

Dixon-Holland D.E and Katz S.E, (1988). Competitive direct enzyme linked immunosorbent assay for detection of sulfamethazine residues in swine and muscle tissue. *Journal of the Association of Official Analytical Chemists* 71: 1137-1140.

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS). (2010). Statement on the divergence between the risk assessment of lycopene by EFSA and the Joint FAO/WHO Expert Committee on Food Additives (JECFA). *EFSA Journal*, *8*(7), 1676.

European Food Safety Authority (EFSA). (2007). Opinion of the Scientific Panel on contaminants in the food chain (CONTAM) related to hormone residues in bovine meat and meat products. *EFSA Journal*, *5*(7), 510,20-21.

FAO. 2019. *The future of livestock in Kenya. Opportunities and challenges in the face of uncertainty*. Rome. 56 pp. for Trace Analysis of 17 Pyrethroids and Chlorpyrifos in Foodstuff by Gas Chromatography–

Gazzotti, T., Sticca, P., Zironi, E., Lugoboni, B., Serraino, A., & Pagliuca, G. (2009). Determination of 15 organophosphorus pesticides in Italian raw milk. *Bulletin of environmental contamination and toxicology*, *82*(2), 251-254.

Getenga, Z. M., Keng'ara, F. O., & Wandiga, S. O. (2004). Determination of organochlorine pesticide residues in soil and water from River Nyando drainage system within Lake Victoria Basin, Kenya. *Bulletin of environmental contamination and toxicology*, *72*(2), 335-343.

GoK, (2020) Report, The Public Petition (NO. 70 of 2019) regarding withdrawal of harmful Chemical Pesticides in the Kenyan Market

Government of Kenya (GoK). 2017. Economic Survey. Kenya National Bureau of Statistics (KNBS). ISBN: 978-9966-102-00-3

Griggs, D. J., Nilsson, M., Stevance, A., & McCollum, D. (2017). *A guide to SDG interactions: from science to implementation*. International Council for Science, Paris.

Guadin V (2003). Inter-laboratory studies for evaluation of ELISA kits for detection of chloramphenicol residues in milk and muscles. *Food and Agricultural Immunology,* 15: 143-157.

Han, R. W., Yu, Z. N., Zhen, T. Y., & Wang, J. (2017). Survey of veterinary drug residues in raw milk in Hebei Province, China. *Journal of food protection*, *80*(11), 1890-1896.

Han, Y., Song, L., Zhao, P., et al., (2016a). Residue Determination of Glufosinate in Plant

Hock, W. K., Day, L. G., & Morley, A. W. (1991). Farm Chemicals Manual: A Guide to Safe Use and Handling, The Agric. and Vet. *Chem. Assoc*.

Jackman R, Brown A.J, Dell A.N and Everest S.J, (1993). Production of antisera to 12 sulfonamides and their use in enzyme immunoassays. *In Haagsma N, Ruiter A, Czedik-EyeenbergP.B , editors. Euroresidue II:* Conference of Residues of Veterinary Drugs in Foods. University of Utrecht, Utrecht, the Netherlands. Pp 391-394.

Kampire, E., Kiremire, B. T., Nyanzi, S. A., & Kishimba, M. (2011). Organochlorine pesticide in fresh and pasteurized cow's milk from Kampala markets. *Chemosphere*, *84*(7), 923-927.

Kandimalla, V. B., Kandimalla, N., Hruska, K., & Franek, M. (2007). Detection of sulfamethazine in water, milk and pig manure by dipstick immunoassay. *VETERINARNI MEDICINA-PRAHA-*, *52*(10), 445.

Kanja L.W., Kinyamu J.K, J.U Skaare, Maitho T.E (1999), Levels of Organochlorine Pesticides Residue in Milk of Urban Mothers, Kenya, 732-733. University of Nairobi Research Archive

Kanja, L. W. (1988). *Organochlorine pesticides in Kenyan mothers milk: levels and sources* (Doctoral dissertation,University of Nairobi).

Kanja, L. W., Skaare, J. U., Ojwang, S. B. O., & Maitai, C. K. (1992). A comparison of organochlorine pesticide residues in maternal adipose tissue, maternal blood, cord blood, and human milk from mother/infant pairs. *Archives of environmental contamination and toxicology, 22*(1), 21-24.

Kapusnik-Uner, J., Sande, M., & Chamber, H. (2001). Antimicrobial agents: tetracyclines, chloramphenicol, erythromycin, and others. In *Goodman and Gilman's the pharmacological basis of theraputics* (pp. 1123-1153). McGraw-Hill, New York.

Karanja, A. M. (2003). The dairy industry in Kenya: the post-liberalization agenda. *Tegemeo Institute of Agricultural Policy and Development, Egerton University, Kenya*, *60*.

Katarzyna Madej, Tatyana K. Kalenik, and Wojciech Piekoszewski, "Sample Preparation and Determination of Pesticides in Fat-Containing Foods," *Food Chemistry* 269 (December 2018): 527–41, https://doi.org/10.1016/j.foodchem.2018.07.007.

Keating, MI., (1983). Tick control by chemical ixocides in Kenya : A review from 1912 to 1981. *Tropical animal health production',* 15: 1-6.

Kennedy, D. G., Cannavan, A., & McCracken, R. J. (2000). Regulatory problems caused by contamination, a frequently overlooked cause of veterinary drug residues. *Journal of Chromatography A, 882*(1-2), 37-52.

Kituyi, E. N., Wandiga, S. O., & Jumba, I. O. (1997). Occurrence of chlorfenvinphos residues in cow's milk sampled at a range of sites in western Kenya. *Bulletin of environmental contamination and toxicology*, *58*(6), 969-975.

Klaassen, C. D. (1991). Absorption, distribution, and excretion of toxicants. *Casarett and Doull's toxicology: The basic science of poisons*, 50-87.

Kurjogi, M., Issa Mohammad, Y. H., Alghamdi, S., Abdelrahman, M., Satapute, P., & Jogaiah, S. (2019). Detection and determination of stability of the antibiotic residues in cow's milk. *PloS one*, *14*(10), e0223475.

Lake R and Kahler T (2012). Increased data quality for sulfonamide residue analysis by HPLC and UHPLC using unique Biphenyl column selectivity. *Innovative Chromatography solution:* RESTEK.

Lee, M. H., Lee, H. J., & Ryu, P. D. (2001). Public health risks: Chemical and antibiotic residues-review. *Asian-Australasian Journal of Animal Sciences*, *14*(3), 402-413.

Lupindu, A. M., Dalsgaard, A., Msoffe, P. L., Ngowi, H. A., Mtambo, M. M., & Olsen, J. E. (2015). Transmission of antibiotic-resistant Escherichia coli between cattle, humans and the environment in peri-urban livestock keeping communities in Morogoro, Tanzania. *Preventive Veterinary Medicine*, *118*(4), 477-482.

Madadi, V. O. (2005). *Chemodynamic studies and assessment of pesticide residues in lake Victoria catchment area for rivers Sio and Nzoia* (Doctoral dissertation,University of Nairobi).

Magalhães, C. G., De Paiva, C. R., Botelho, B. G., De Oliveira, A. M. G., De Souza, L. F., Nonaka, C. V., ... & Carvalho, M. A. R. (2012). In-house validation of Premi® Test, a microbiological screening test with solvent extraction, for the detection of antimicrobial residues in poultry muscles. *Food Additives & Contaminants: Part A*, *29*(4), 535-540.

Meng, F. (2016). Baker's Yeast Beta-Glucan Decreases Episodes of Common Childhood Illness In 1 to 4 Year Old Children during Cold Season in China. *Journal of Nutrition& Food Sciences*, *6*(518), 2.

Mitema, E. S., Kikuvi, G. M., Wegener, H. C., & Stohr, K. (2001). An assessment of antimicrobial consumption in food producing animals in Kenya. *Journal of veterinary pharmacology and therapeutics*, *24*(6), 385-390.Lee H.J, Lee M.H, and Ruy P.D, (2001). Public health risks: Chemical and antimicrobial residues. *Asian-Aust. J. Anim. Sci.,*14: 402-413.

Mwenda, N. J. (2011). *Assessment of organochlorine pesticide residues in cow milk, sediments and water from Nairobi West region* (Doctoral dissertation, University of Nairobi).

Nag, S. K., & Raikwar, M. K. (2008). Organochlorine pesticide residues in bovine milk. *Bulletin of Environmental Contamination and Toxicology*, *80*(1), 5-9.

Ndunda, E. N., Madadi, V. O., & Wandiga, S. O. (2018). Organochlorine pesticide residues in sediment and water from Nairobi River, Kenya: levels, distribution, and ecological risk assessment. *Environmental Science and Pollution Research*, *25*(34), 34510-34518.

NEAP, (1994) Report, The Kenya National Environmental Action Plan, Ministry of Environment and natural Resources, Nairobi, Kenya of Multiclass Pesticide Residues in Edible Oils. Food Chemistry. 142, 144–151.

Olatoye, I. O., & Ehinmowo, A. A. (2010). Oxytetracycline residues in edible tissues of cattle slaughtered in Akure, Nigeria. *Nigerian Veterinary Journal*, *31*(2).

Olson Jr, J. C., & Sanders, A. C. (1975). Penicillin in milk and milk products: Some regulatory and public health considerations. *Journal of milk and food technology*, *38*(10), 630-633.

Omwenga, I., Kanja, L., Nguta, J., Mbaria, J., & Irungu, P. (2016). Organochlorine pesticide residues in farmed fish in Machakos and Kiambu counties, Kenya. *Cogent Environmental Science*, *2*(1), 1153215.

Ong'aro, D. S. (2012). *Strategies to improve firm-farmer relationship in dairy value chains: an assessment study in Borabu and Kiambu Districts, Kenya* (Doctoral dissertation, Van Hall Larenstein).

Origin Foods Using Modified Quick Polar Pesticides (QuPPe) Method and Liquid

Ortelli, D., Sporri, A. S., & Edder, P. (2018). Veterinary drug residue in food of animal origin in Switzerland: a health concern? *CHIMIA International Journal for Chemistry*, *72*(10), 713-717.

Orwa, J. D., Matofari, J. W., Muliro, P. S., & Lamuka, P. (2017). Assessment of sulphonamides and tetracyclines antibiotic residue contaminants in rural and peri urban dairy value chains in Kenya. *International Journal of Food Contamination*, *4*(1), 1-11.

Pavlov, A., Lashev, L., Vachin, I., & Rusev, V. (2008). Residues of antimicrobial drugs in chicken meat and offals. *Trakia J Sci*, *6*(1), 23-25.

Peng, X-T., Jiang, L., Gong, Y., Hu, X-Z., Peng, L-J., & Feng, Y-Q. (2015). Preparation of Mesoporous ZrO2-Coated Magnetic Microsphere and its Application in the Multi-ResidueAnalysis of Pesticides and PCBs in Fish by GC–MS/MS. Talanta. 132, 118–125

Roberts, M. C. (1996). Tetracycline resistance determinants: mechanisms of action, regulation of expression, genetic mobility, and distribution. *FEMS microbiology reviews*, *19*(1), 1-24.

Roudaut, B., & Moretain, J. P. (1990). Sulphonamide residues in milk of dairy cows following intravenous injection. *Food Additives & Contaminants*, *7*(4), 527-533.

Salas, J. H., González, M. M., Noa, M., Pérez, N. A., Díaz, G., Gutiérrez, R., ... & Osuna, I. (2003). Organophosphorus pesticide residues in Mexican commercial pasteurized milk. *Journal of agricultural and food chemistry*, *51*(15), 4468-4471

Sasanya, J. J., Ikeng, J. W. O., Ejobi, F., & Muganwa, M. (2005). Use of sulfonamides in layers in Kampala district, Uganda and sulfonamide residues in commercial eggs. *African health sciences*, *5*(1), 33-39.

Sattar S, Hassan, M.M, Islam, S.K.M.A, Alam M, Faruk M.S.A, Chowdhury, S, and Saifuddin, A.K.M (2014). Antimicrobial residues in broiler and layer meat in Chittagong district of Bangladesh. *Veterinary World*, EISSN: 2231-0916.

Sattar, S., Hassan, M. M., Islam, S. K. M., Alam, M., Al Faruk, M. S., Chowdhury, S., & Saifuddin, A. K. M. (2014). Antibiotic residues in broiler and layer meat in Chittagong district of Bangladesh. *Veterinary World*, *7*(9).

Simo, O. P., Zhang, Q. (2002) The Degradation of Organophosphorus Pesticides in Natural Waters.A Critical Review, Critical Reviews in Environmental Science and Technology, 32(1),17-72.

Sirdar, M. M. (2011). *Antibiotic residues in commercial layer hens in Khartoum state, Sudan, 2007-2008* (Doctoral dissertation, University of Pretoria).

Schultz, J. C., Adamson Jr, J. S., Workman, W. W., & Norman, T. D. (1963). Fatal liver disease after intravenous administration of tetracycline in high dosage. *New England Journal of Medicine*, *269*(19), 999-1004.

Seri, H. I. (2013, May). Introduction to veterinary drug residues: hazards and risks. In *Proceedings of the Workshop: Veterinary Drug Residues in Food Derived from Animals (Our Goal of Protecting Consumers)*.

Shahid, M. A., Siddique, M., Rehman, U. S., Hameed, S., & Hussain, A. (2007). Evaluation of a microbiological growth inhibition assay as a screening test for the presence of antibiotic residues in poultry meat. *American Journal of Food Technology*, *2*(5), 457-461.

Shaker, E. M., & Elsharkawy, E. E. (2015). Organochlorine and organophosphorus pesticide residues in raw buffalo milk from agro-industrial areas in Assiut, Egypt. *Environmental toxicology and pharmacology*, *39*(1), 433-440.

Shallcross, L. J., Howard, S. J., Fowler, T., & Davies, S. C. (2015). Tackling the threat of antimicrobial resistance: from policy to sustainable action. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *370*(1670), 20140082.

Shankar,B.P, ManjunathaPrabhu, B.H, Chadan, S, Ranjith, D and Shivakumar,V, (2010). Rapid methods for detection of Veterinary Drug residues in meat. *Veterinary World Vol.*3(5): *241-246*

Shitandi, A., & Sternesjö, Å. S. E. (2001). Detection of antimicrobial drug residues in Kenyan milk. *Journal of Food Safety*, *21*(4), 205-214.

Singh, R. (2013). HPLC method development and validation-an overview. *Journal of Pharmaceutical Education & Research*, *4*(1),26-33

Srivastava, S., Narvi, S. S., & Prasad, S. C. (2008). Organochlorines and organophosphates in bovine milk samples in Allahabad region. *Scientific Information Database*, 2(2),165-168

Stolker A.A.M, Rutgers P, Oosterink E, Lasaroms J.J.P, Peters R.J.B, van Rhijn J.A and Nielen M.W.F, (2008). Comprehensive screening and quantification of veterinary drugs in milk using UPLC- ToF-MS. *Analytical and Bioanalytical Chemistry* 10:1007/s00216- 008-2168-8.

Tandem Mass Spectrometry. Analytical Bioanalytical Chemistry. 408, 7689–7697.

Tang, Y. Y., Lu, H. F., Lin, H. Y., Shih, Y. C., & Hwang, D. F. (2012). Multiclass analysis of 23 veterinary drugs in milk by ultraperformance liquid chromatography–electrospray tandem mass spectrometry. *Journal of Chromatography B*, *881*, 12-19.

V. R. V. (2014). An Improved Solid-phase Extraction Clean-up Method for the Gas

Van Boeckel, T. P., Brower, C., Gilbert, M., Grenfell, B. T., Levin, S. A., Robinson, T. P., ... & Laxminarayan, R. (2015). Global trends in antimicrobial use in food animals. *Proceedings of the National Academy of Sciences*, *112*(18), 5649-5654.

Vlieghe, E., Phoba, M. F., Tamfun, J. M., & Jacobs, J. (2009). Antibiotic resistance among bacterial pathogens in Central Africa: a review of the published literature between 1955 and 2008. *International journal of antimicrobial agents*, *34*(4), 295-303.

Wandiga, S. O., & Mutere, A. (1988). Determination of γ-BHC in breast milk of Kenyan women. *Bulletin of the Chemical Society of Ethiopia*, *2*(1),39-44.

Wandiga, S. O., Yugi, P. O., Barasa, M. W., Jumba, I. O., & Lalah, J. O. (2002). The distribution of organochlorine pesticides in marine samples along the Indian Ocean coast of Kenya. *Environmental technology*, *23*(11), 1235-1246.

Wandiga, S.O, Madadi, O.V., Kircmire B.T.,and M. Kishimba, (2006). Lake Victoria: Will it support Life tomorrow? A Case Study for Abatement of Pollution and Eutrophication of Fresh Waters, in Environment for Development an Ecosystem Assessment of Lake Victoria Basin Environment and Socio-Economic Status, Trend and Human Vulnerabilities, Pan Africa Secretariat and UNEP: 69-75.

Wang S, Zhang H.Y, Wang L, Duan Z.J and Kennedy I, (2006). Analysis of sulphonamide residues in edible animal products. A review. *Food Additives and Contaminants, April 2006;* 23(4): 362-384.

Werimo, K., Bergwerff, A. A., & Seinen, W. (2009). Residue levels of organochlorines and organophosphates in water, fish and sediments from Lake Victoria-Kenyan portion. *Aquatic Ecosystem Health & Management*, *12*(3), 337-341.

World Health Organization. (2002). Use of Antimicrobials Outside Human Medicine and Resultant Antimicrobial Resistance in Humans (Fact Sheet No. 268). *Geneva, Switzerland: Author*.

World Health Organization. (2012). Report of the 3rd meeting of the WHO advisory group on integrated surveillance of antimicrobial resistance, 14-17 June 2011, Oslo, Norway.

Zhang, H., & Wang, S. (2009). Review on enzyme-linked immunosorbent assays for sulfonamide residues in edible animal products. *Journal of immunological methods*, *350*(1-2), 1-13.

Zikankuba, V. L., Mwanyika, G., Ntwenya, J. E., & James, A. (2019). Pesticide regulations and their malpractice implications on food and environment safety. *Cogent Food & Agriculture*, *5*(1), 1601544.

APPENDICES

Table A1: Accuracy data for Organochlorine's analysis method at various spiked concentrations

% Recovery			
	Spiked concentration (ppb)		
OCs	**10**	**50**	**100**
Alpha-BHC	97.3±0.07	97.2±0.24	80.7±0.10
beta-BHC	95.0±0.07	97.5±0.09	82.5±0.45
Hexachlorobenzene	86.1±0.08	91.5±0.12	81.6±0.08
gamma-BHC (Lindane)	94.7±0.43	81.7±0.02	80.3±0.15
delta-BHC	97.8±2.82	81.6±0.04	85.2±0.28
Heptachlor	95.9±0.23	85.5±0.24	86.9±0.14
cis-Chlordane	87.6±0.001	81.3±0.01	75.6±0.02
trans-Chlordane	93.8±0.21	81.8±0.003	76.8±0.32
p,p'-DDE	87.1±0.06	87.5±0.017	75.8±0.27
alpha-Endosulfan	86.7±0.003	95.9±0.02	76.9±0.06
Dieldrin	97.1±0.06	81.7±0.14	78.9±0.19
o,p'-DDD	96.0±0.21	81.7±0.01	80.3±0.31
trans-Nonachlor	96.0±0.06	89.5±0.03	80.6±0.07
Chlorfenson	86.1±0.07	93.7±0.07	80.6±0.03
p,p'-DDD	88.9±0.001	95.3±0.11	70.8±0.01
Endrin	78.8±0.05	89.5±0.01	89.4±0.19
beta-Endosulfan	87.7±0.29	73.1±0.02	80.1±0.01
p,p'-DDT	96.7±0.30	77.5±0.01	80.0±0.01
cis-Nonachlor	98.7±0.01	87.6±0.01	76.8±0.02
o,p'-DDT	96.2±0.23	89.2±0.24	78.7±0.15
Mirex	88.2±0.21	81.1±0.02	80.4±0.18

***Table** A2: Accuracy data for Organophosphate's analysis method at various spiked concentrations*

% Recovery			
	Spiked concentration(ppb)		
Ops	**10**	**50**	**100**
Disulfoton	85.0±0.35	95.2±0.06	98.7±0.15
Terbufos	88.0±0.25	97.3±0.01	97.7±0.23
Tolclofos-methyl	96.5±0.02	95.5±0.15	97.8±0.01
Fenchlorphos	88.1±0.07	97.6±0.07	96.7±0.01
Malathion	97.2±0.08	95.3±0.15	98.6±0.23
Tetrachlorvinphos	78.8±0.02	97.8±0.06	80.5±0.01
Fenamiphos	88.2±0.22	95.7±0.14	89.5±0.29
Chlorfenson	89.7±0.01	99.5±0.29	108.5±0.17
Chlorthiophos-2	86.7±0.15	97.7±0.01	80.7±0.14
Profenofos	96.1±0.07	99.5±0.14	89.5±0.35
Chlorthiophos-1	97.8±0.01	99.6±0.07	79.5±0.06
Leptophos	78.3±0.07	97.7±0.14	80.7±0.03
Coumaphos	86.9±0.01	99.2±0.19	98.9±0.03
Methacrifos	106.2±0.22	91.7±0.12	98.4±0.27

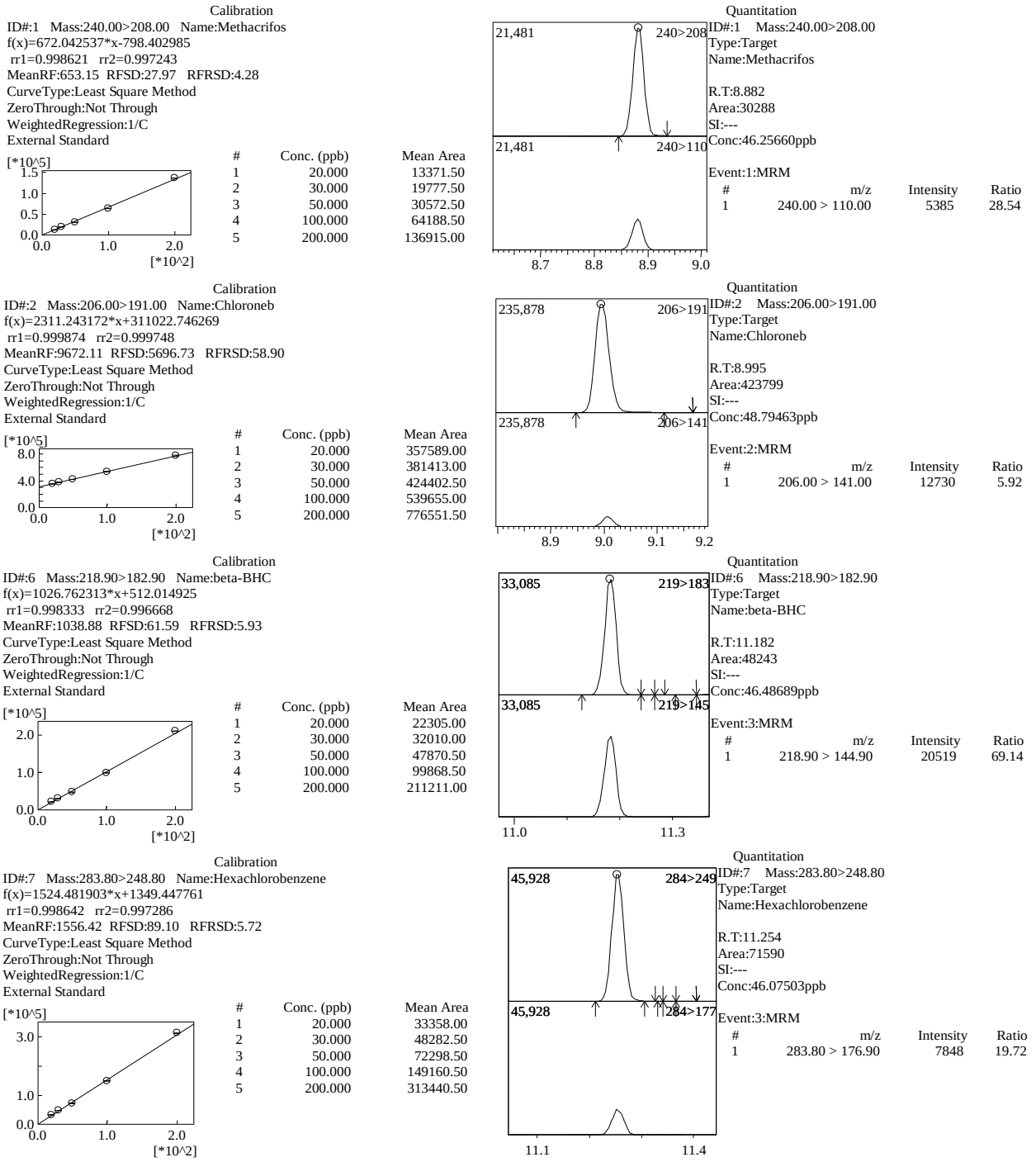


Figure A.1 MRM calibration & Chromatograms for methacrifos, chloroneb, beta-BHC & hexachlorbenzene

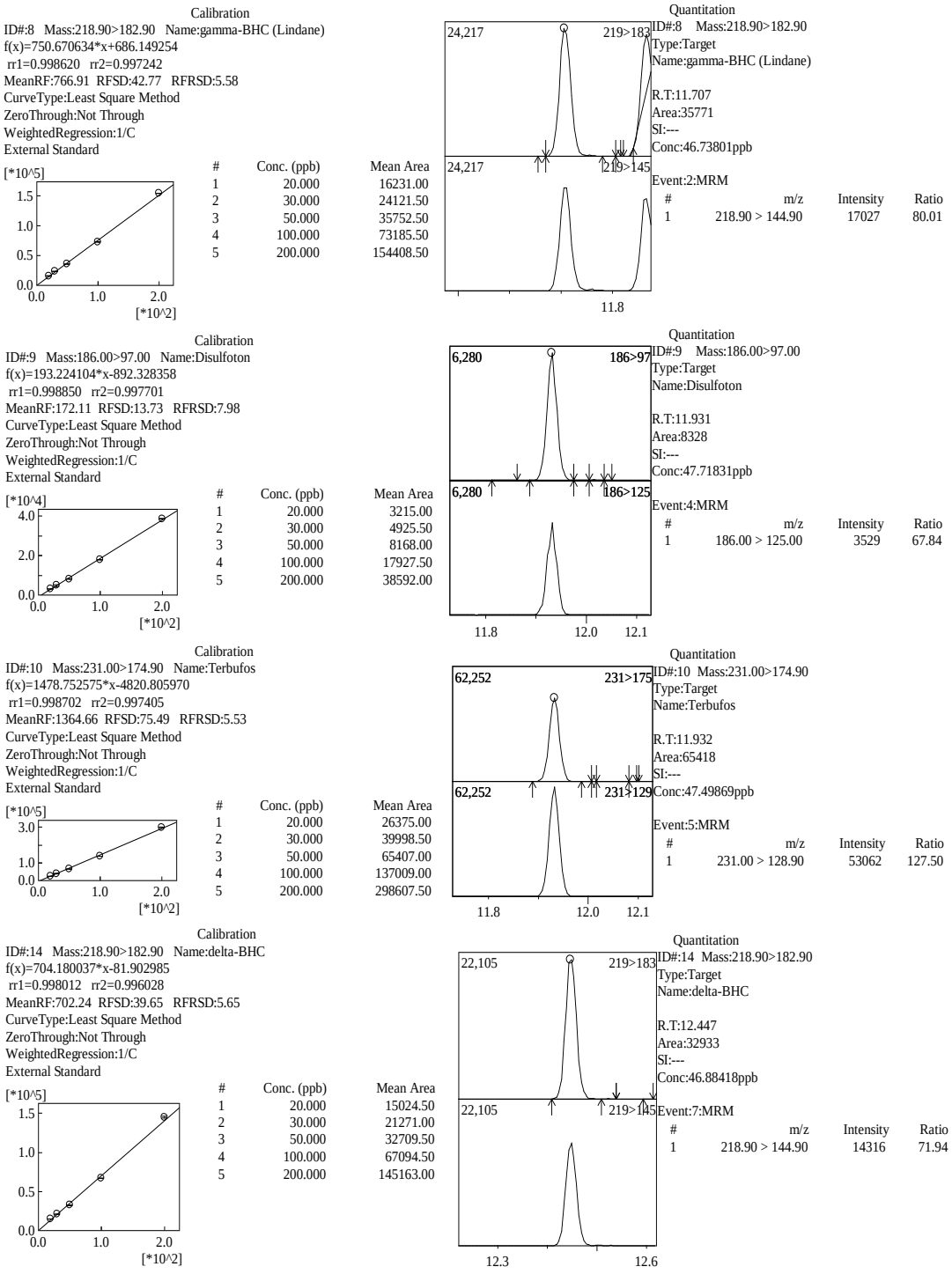
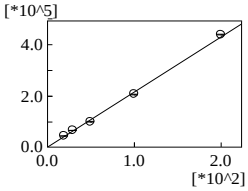


Figure A.2 MRM Calibration & Chromatograms for Lindane, disulfoton, terbufos & delta-BHC

Calibration
ID#:17 Mass:264.90>249.90 Name:Tolclofos-methyl
f(x)=2140.518060*x+47.955224
rr1=0.998600 rr2=0.997202
MeanRF:2141.65 RFSD:101.02 RFRSD:4.72
CurveType:Least Square Method
ZeroThrough:Not Through
WeightedRegression:1/C
External Standard



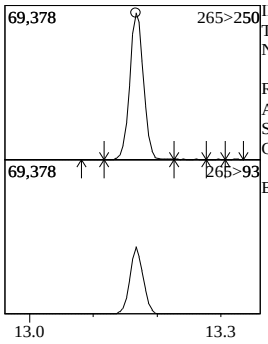
#	Conc. (ppb)	Mean Area
1	20.000	44411.00
2	30.000	66780.00
3	50.000	100270.00
4	100.000	206277.00
5	200.000	438709.00

Quantitation
ID#:17 Mass:264.90>249.90
Type:Target
Name:Tolclofos-methyl

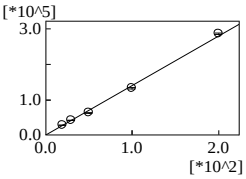
R.T:13.168
Area:99618
SI:---
Conc:46.51680ppb

Event:4:MRM

#	m/z	Intensity	Ratio
1	264.90 > 93.00	26785	43.70



Calibration
ID#:18 Mass:271.80>236.90 Name:Heptachlor
f(x)=1389.812985*x-618.738806
rr1=0.997970 rr2=0.995943
MeanRF:1375.17 RFSD:76.09 RFRSD:5.53
CurveType:Least Square Method
ZeroThrough:Not Through
WeightedRegression:1/C
External Standard



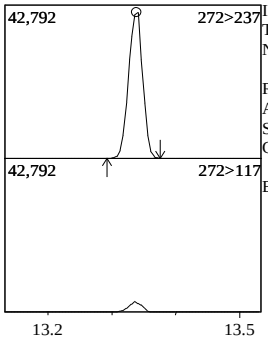
#	Conc. (ppb)	Mean Area
1	20.000	28833.50
2	30.000	42417.00
3	50.000	63236.50
4	100.000	132764.00
5	200.000	285580.50

Quantitation
ID#:18 Mass:271.80>236.90
Type:Target
Name:Heptachlor

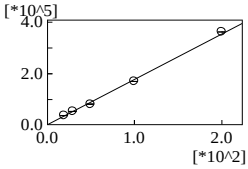
R.T:13.339
Area:62541
SI:---
Conc:45.44478ppb

Event:3:MRM

#	m/z	Intensity	Ratio
1	271.80 > 117.00	2449	6.78



Calibration
ID#:24 Mass:173.10>99.00 Name:Malathion
f(x)=1766.072612*x-643.708955
rr1=0.998213 rr2=0.996429
MeanRF:1750.84 RFSD:94.13 RFRSD:5.38
CurveType:Least Square Method
ZeroThrough:Not Through
WeightedRegression:1/C
External Standard



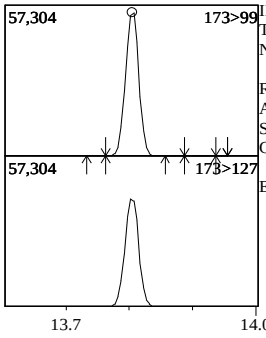
#	Conc. (ppb)	Mean Area
1	20.000	36935.00
2	30.000	53521.00
3	50.000	80688.00
4	100.000	169863.00
5	200.000	362203.50

Quantitation
ID#:24 Mass:173.10>99.00
Type:Target
Name:Malathion

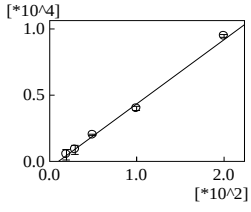
R.T:13.805
Area:81278
SI:---
Conc:46.38638ppb

Event:5:MRM

#	m/z	Intensity	Ratio
1	173.10 > 127.00	37876	75.33



Calibration
ID#:27 Mass:125.00>89.00 Name:Chlorbenside
f(x)=48.509216*x-489.037313
rr1=0.997688 rr2=0.995381
MeanRF:36.94 RFSD:8.44 RFRSD:22.85
CurveType:Least Square Method
ZeroThrough:Not Through
WeightedRegression:1/C
External Standard



#	Conc. (ppb)	Mean Area
1	20.000	537.50
2	30.000	894.50
3	50.000	2018.50
4	100.000	4015.00
5	200.000	9493.00

Quantitation
ID#:27 Mass:125.00>89.00
Type:Target
Name:Chlorbenside

R.T:14.816
Area:1970
SI:---
Conc:50.37655ppb

Event:1:MRM

#	m/z	Intensity	Ratio
1	125.00 > 99.00	381	34.54

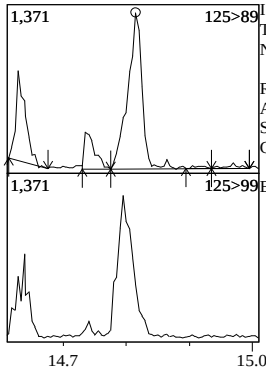


Figure A.3 MRM Calibration & Chromatograms for toclofos-methyl, heptachlor, malathion, chlorbenside

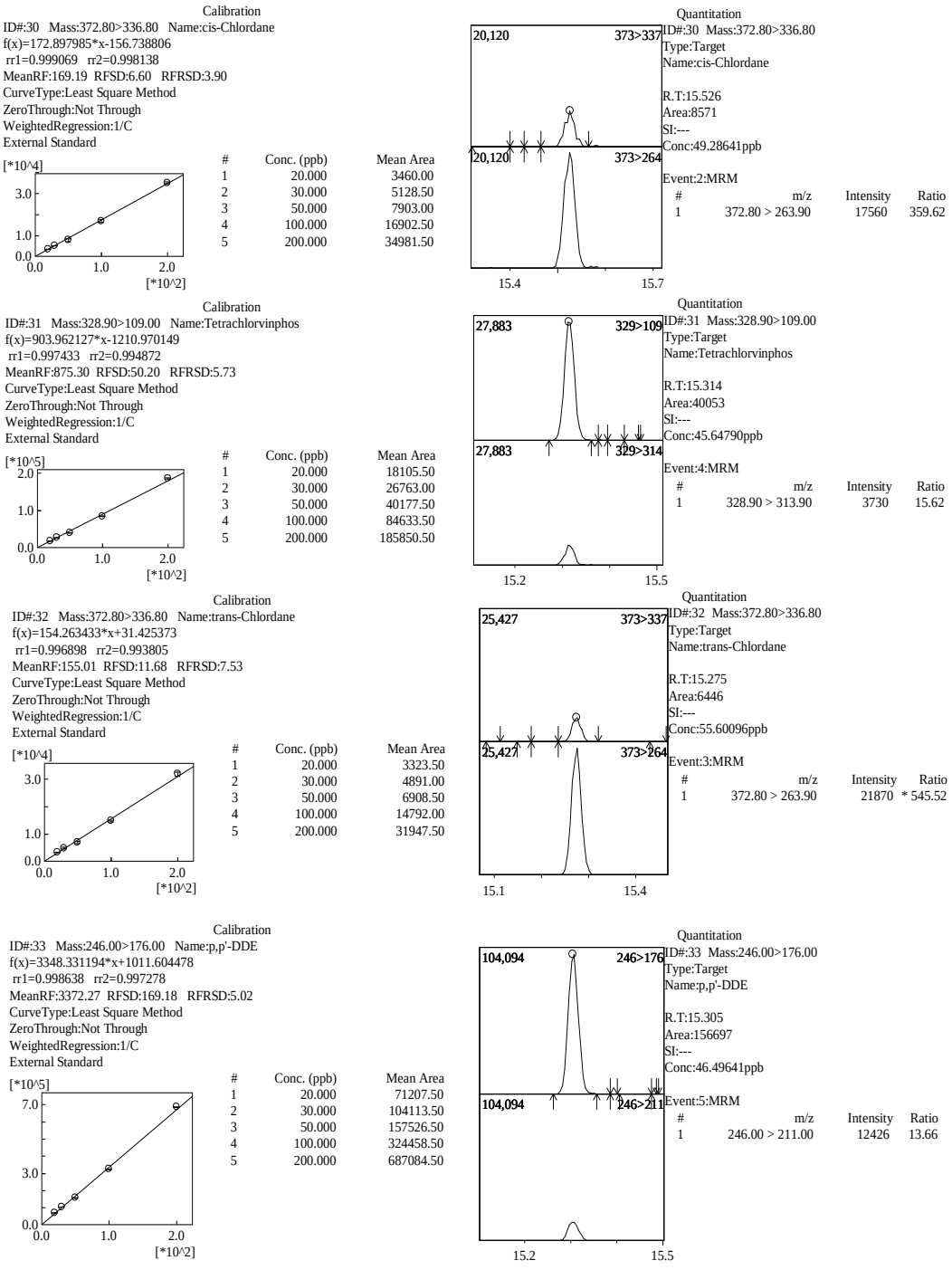


Figure A.4 MRM Calibration & Chromatograms for cis & trans chlordane, tetrachlorvinphos & p, p'-DDE

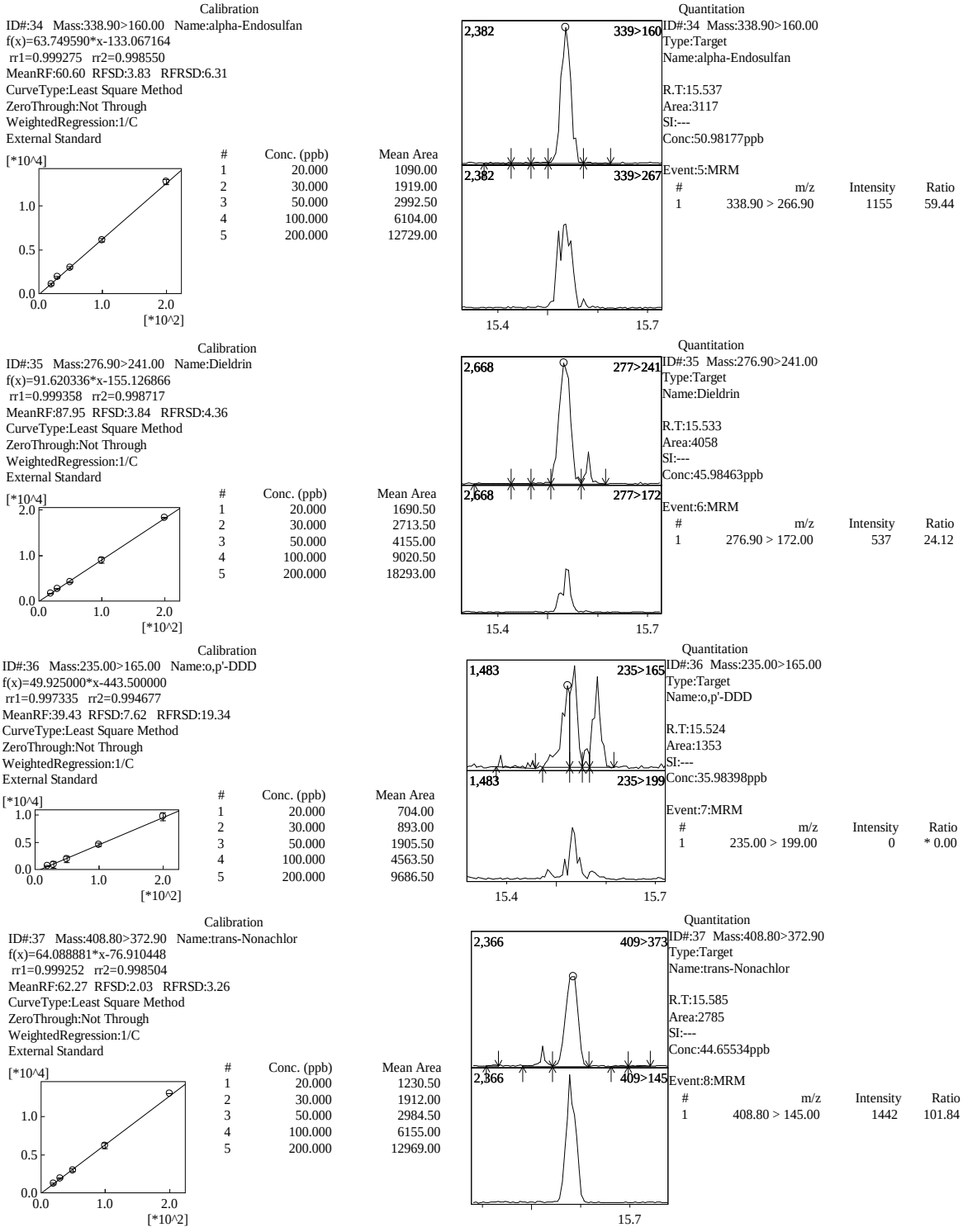


Figure A.5 MRM Calibration & Chromatograms for endulsulfan, dieldrin, p,p'-DDD & trans-nonachlor

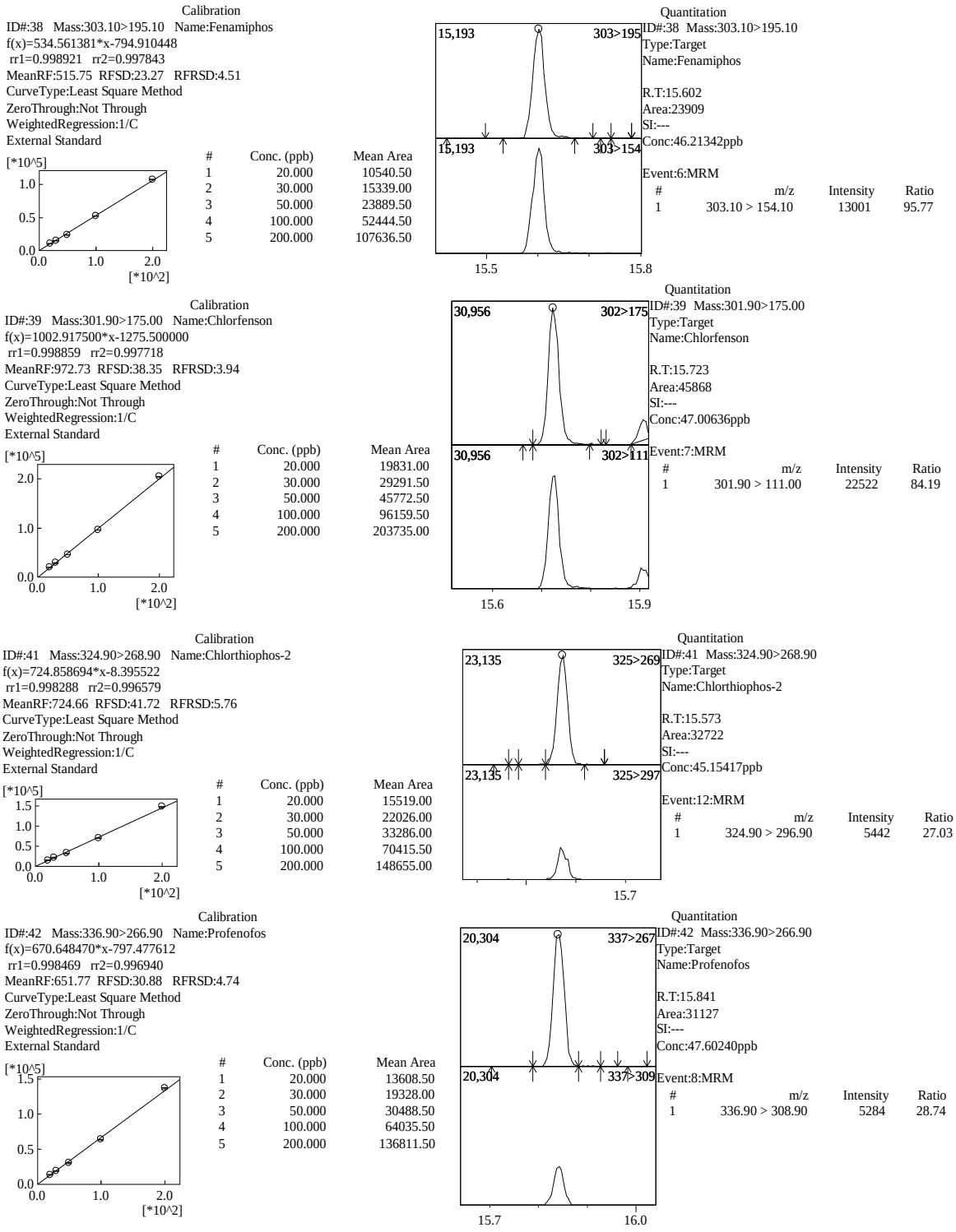
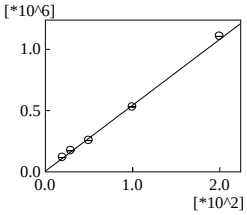


Figure A.6 MRM Calibration and Chromatograms for fenamiphos, chlorfenson, chlorthiophos-2, profenofos

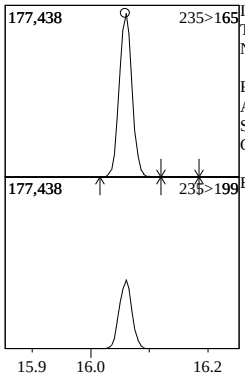
Calibration
ID#:44 Mass:235.00>165.00 Name:p,p'-DDD
f(x)=5402.101306*x+6035.395522
rr1=0.998917 rr2=0.997835
MeanRF:5544.94 RFSD:300.71 RFRSD:5.42
CurveType:Least Square Method
ZeroThrough:Not Through
WeightedRegression:1/C
External Standard



#	Conc. (ppb)	Mean Area
1	20.000	117641.00
2	30.000	173994.00
3	50.000	258896.50
4	100.000	532497.00
5	200.000	1107989.00

Quantitation
ID#:44 Mass:235.00>165.00
Type:Target
Name:p,p'-DDD

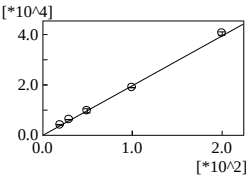
R.T:16.060
Area:257134
SI:---
Conc:46.48165ppb



Event:2:MRM

#	m/z	Intensity	Ratio
1	235.00 > 199.00	64812	41.65

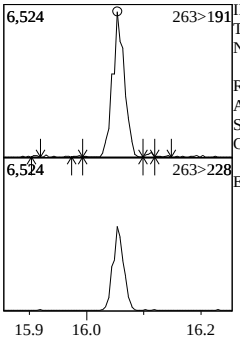
Calibration
ID#:45 Mass:262.90>191.00 Name:Endrin
f(x)=195.379254*x+263.559701
rr1=0.998934 rr2=0.997869
MeanRF:201.62 RFSD:10.07 RFRSD:4.99
CurveType:Least Square Method
ZeroThrough:Not Through
WeightedRegression:1/C
External Standard



#	Conc. (ppb)	Mean Area
1	20.000	4291.00
2	30.000	6231.50
3	50.000	9779.00
4	100.000	18879.50
5	200.000	40288.50

Quantitation
ID#:45 Mass:262.90>191.00
Type:Target
Name:Endrin

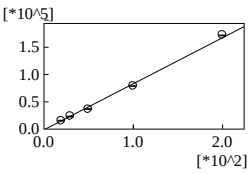
R.T:16.054
Area:9052
SI:---
Conc:44.98144ppb



Event:10:MRM

#	m/z	Intensity	Ratio
1	262.90 > 228.00	2946	60.36

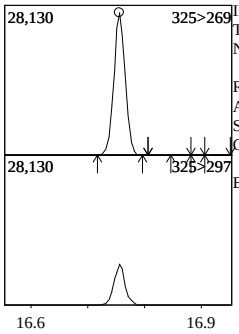
Calibration
ID#:46 Mass:324.90>268.90 Name:Chlorthiophos-3
f(x)=846.018284*x-1936.462687
rr1=0.997984 rr2=0.995973
MeanRF:800.19 RFSD:41.97 RFRSD:5.25
CurveType:Least Square Method
ZeroThrough:Not Through
WeightedRegression:1/C
External Standard



#	Conc. (ppb)	Mean Area
1	20.000	16002.00
2	30.000	24145.00
3	50.000	37277.50
4	100.000	78791.50
5	200.000	172509.00

Quantitation
ID#:46 Mass:324.90>268.90
Type:Target
Name:Chlorthiophos-3

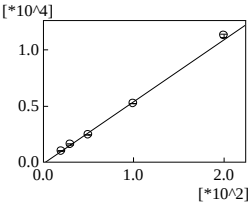
R.T:16.756
Area:37529
SI:---
Conc:46.64847ppb



Event:4:MRM

#	m/z	Intensity	Ratio
1	324.90 > 296.90	6803	28.00

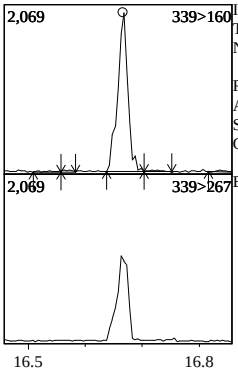
Calibration
ID#:47 Mass:338.90>160.00 Name:beta-Endosulfan
f(x)=55.513918*x-132.313433
rr1=0.998276 rr2=0.996555
MeanRF:52.38 RFSD:2.84 RFRSD:5.42
CurveType:Least Square Method
ZeroThrough:Not Through
WeightedRegression:1/C
External Standard



#	Conc. (ppb)	Mean Area
1	20.000	1027.00
2	30.000	1603.50
3	50.000	2428.00
4	100.000	5225.00
5	200.000	11260.50

Quantitation
ID#:47 Mass:338.90>160.00
Type:Target
Name:beta-Endosulfan

R.T:16.667
Area:2425
SI:---
Conc:46.06617ppb



Event:6:MRM

#	m/z	Intensity	Ratio
1	338.90 > 266.90	972	59.78

Figure A.7 MRM Calibration and Chromatograms for p, p'-DDD, endrin, endosulfan & chlorthiophos-3

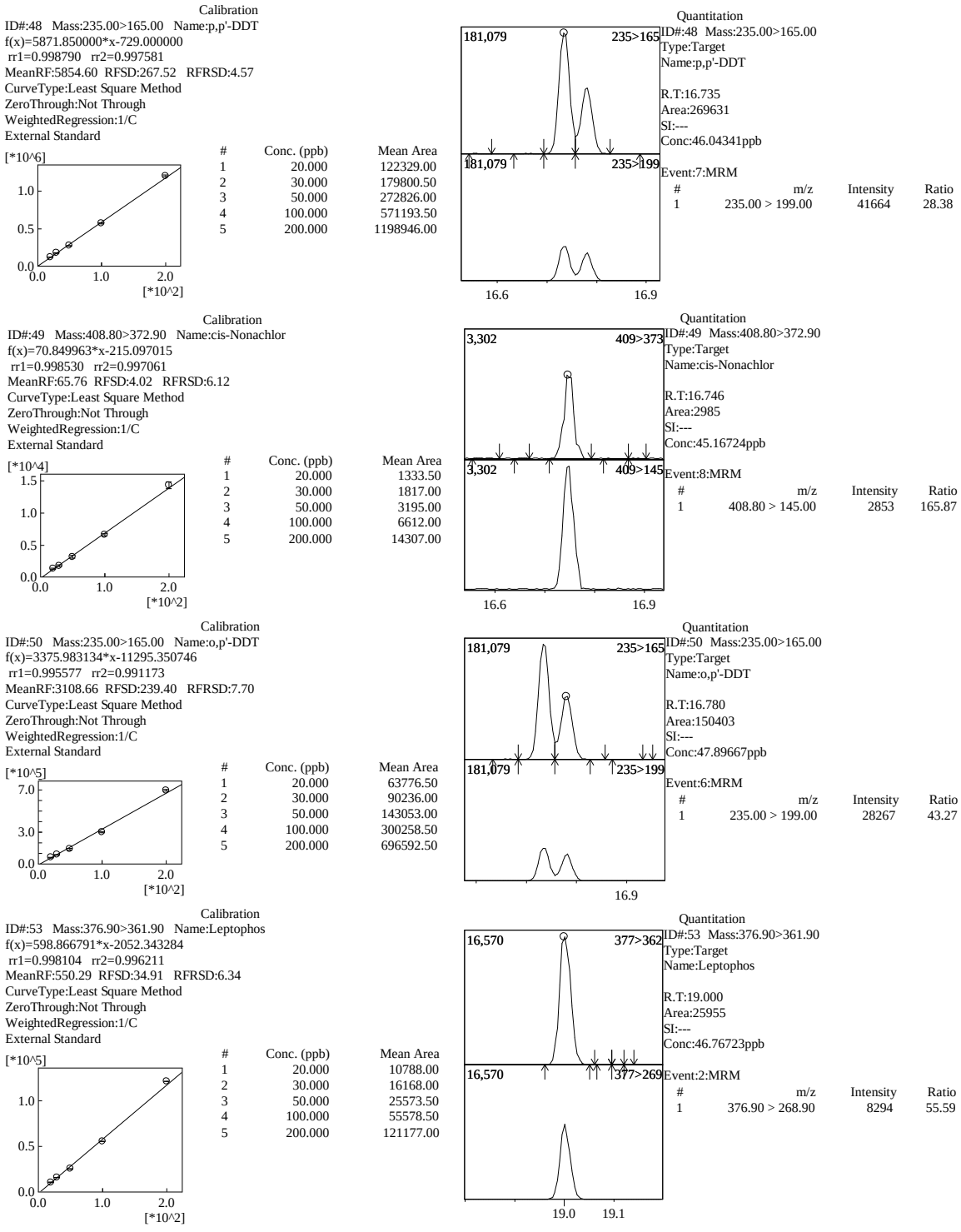


Figure A.8 MRM Calibration & Chromatograms for p.p'-DDT, cis-nonachlor, o,p'-DDT &leptophos

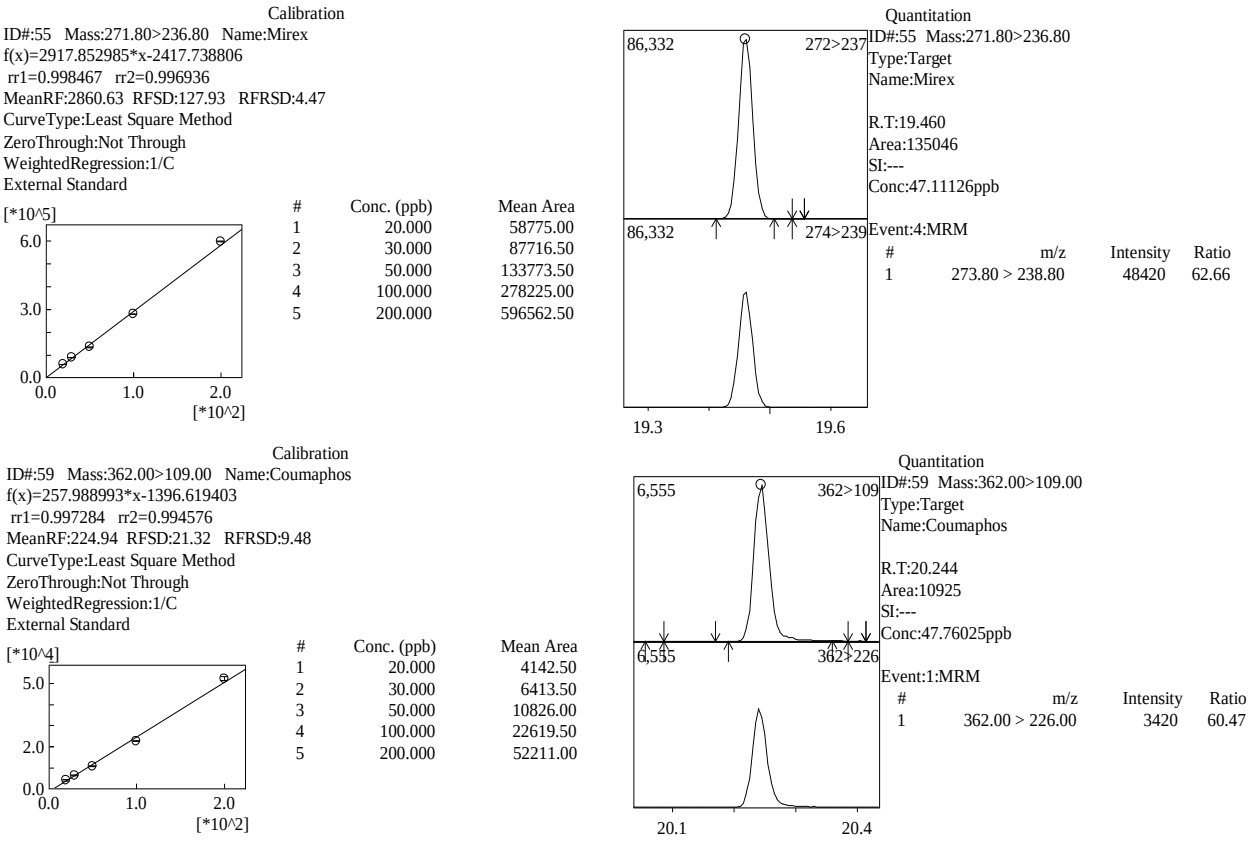


Figure A.9 MRM Calibration & Chromatogram for mirex & Coumaphos

Table A3: LOD, LOQ and RSD% for OCs

Serial No.	Compound Name	LOD (ng/ml)	LOQ (ng/ml)	RSD%
1.	alpha-BHC	0.2	0.3	2.1
2.	beta-BHC	0.2	0.2	0.7
3.	Hexachlorobenzene	0.2	0.3	2.9
4.	gamma-BHC (Lindane)	0.3	0.3	2.1
5.	delta-BHC	0.2	0.3	0.1
6.	Heptachlor-endo-epoxide	0.2	0.3	2.7
7.	cis-Chlordane	0.2	0.2	1.2
8.	trans-Chlordane	0.2	0.3	0.4
9.	p,p'-DDE	0.2	0.3	2.8
10.	alpha-Endosulfan	0.2	0.3	2.3
11.	Dieldrin	0.1	0.2	3.2
12.	o,p'-DDD	0.2	0.3	2.8
13.	trans-Nonachlor	0.3	0.4	3.9
14.	Chlorfenson	0.2	0.3	2.9
15.	p,p'-DDD	0.3	0.4	3.5
16.	Endrin	0.2	0.3	0.3
17.	beta-Endosulfan	0.1	0.2	2.0
18.	p,p'-DDT	0.1	0.1	0.7
19.	cis-Nonachlor	0.1	0.1	0.7
20.	o,p'-DDT	0.2	0.2	0.1
21.	Mirex	0.2	0.3	0.1

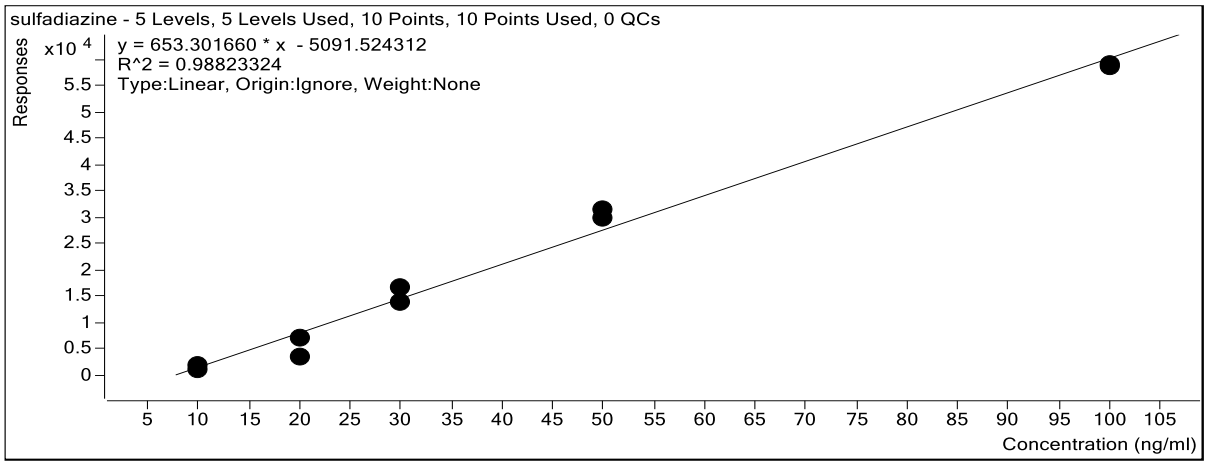
Table A4: LOD, LOQ and RSD% FOR OPs

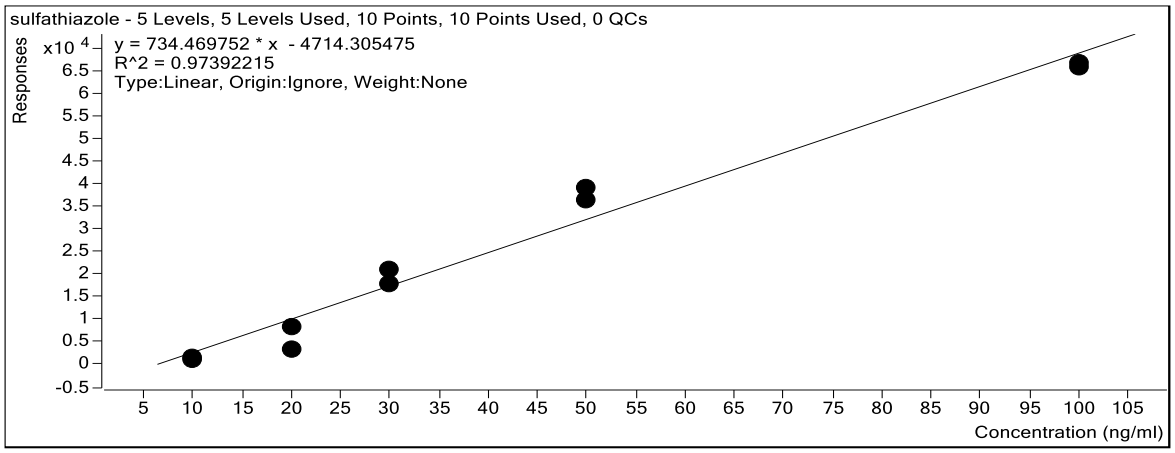
Serial No.	Compound name	LOD (ng/ml)	LOQ (ng/ml)	RSD%
1.	Methacrifos	0.3	0.4	2.7
2.	Disulfoton	0.2	0.3	1.3
3.	Terbufos	0.2	0.3	0.2
5.	Tolclofos-methyl	0.2	0.3	1.5
6.	Fenchlorphos	0.3	0.4	3.1
7.	Malathion	0.2	0.3	3.1
8.	Tetrachlorvinphos	0.3	0.4	5.8
9.	Fenamiphos	0.2	0.2	0.1
10.	Chlorthiophos-2	0.02	0.03	0.6
11.	Profenofos	0.1	0.2	1.3
12.	Chlorthiophos-1	0.2	0.3	2.4
13.	Chlorthiophos-3	0.2	0.3	0.9
14.	Leptophos	0.2	0.2	0.3
15.	Coumaphos	0.2	0.3	2.8

Table A5*: Accuracy data for Veterinary drug analysis method at various spiked concentrations*

% recovery		
	Spiked concentration (ppb)	
Veterinary drugs	**50**	**100**
Sulfadiazine	92.92±0.41	99.32±0.17
Sulfathiazole	98.91±0.15	98.68±0.01
Sulfapyridine	97.54±0.14	98.71±0.09
Sulfamerazine	99.44±0.09	98.87±0.15
Sulfamethazine	97.76±0.14	97.67±0.003
Sulfamethoxypyradine	99.11±0.019	98.73±0.08
Sulfamonomethoxine	96.92±0.14	98.87±0.13
Sulfaguanadine	99.46±0.07	98.69±0.14
Sulfamethoxazole	95.51±0.23	98.81±0.04
Sulfadimethoxine	95.92±0.01	98.67±0.002
Sulfadoxine	97.92±0.007	99.96±0.009
Sulphisoxazole	97.46±0.07	97.87±0.12
Sulfamethazole	97.34±0.15	97.86±0.006
Sulfanilamide	99.33±0.13	98.73±0.12
Sulfachloropyradazine	97.42±0.05	98.97±0.007
Sulfaguanidine	99.11±0.1	98.85±0.006

Appendix 4: ***Calibration Curves for Sulphonamides***





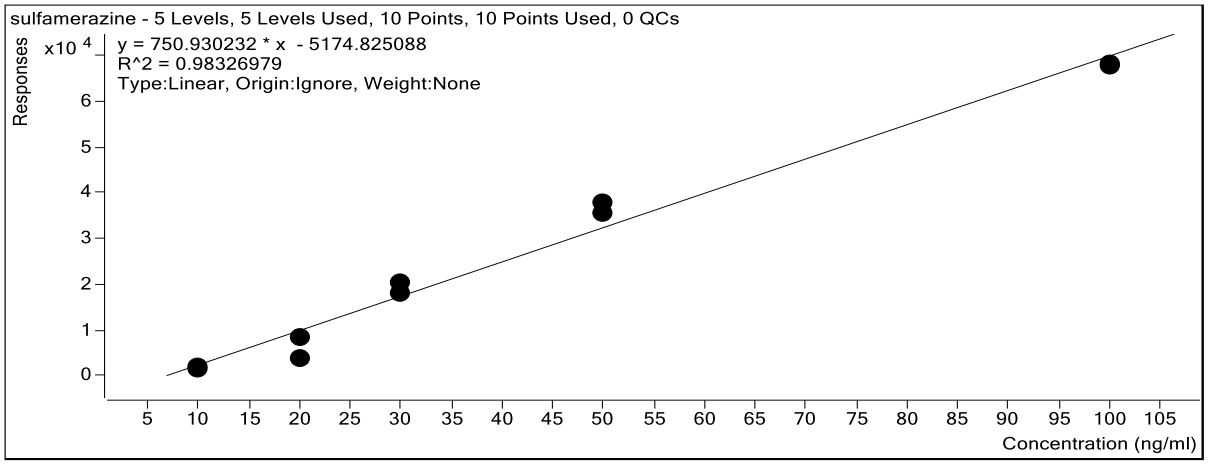
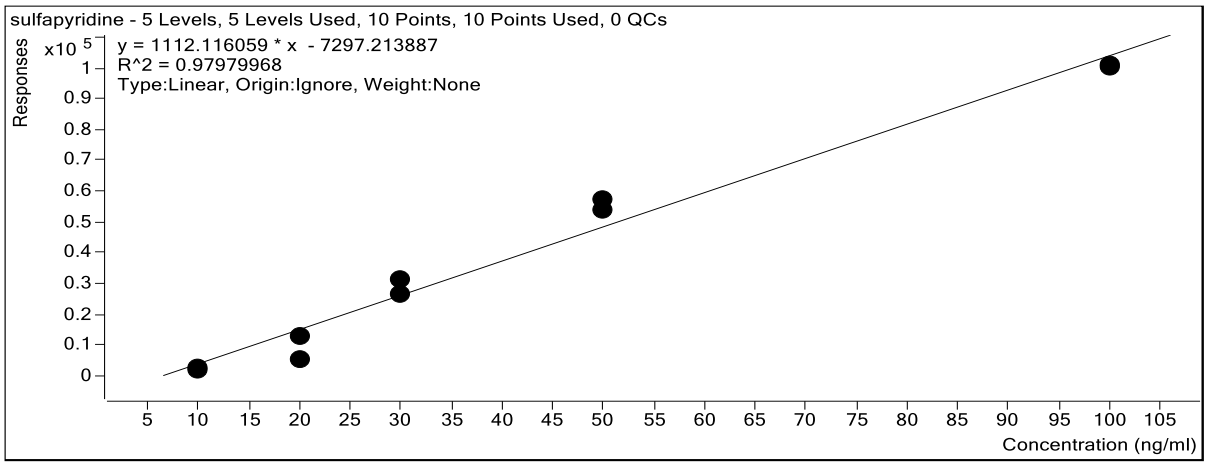
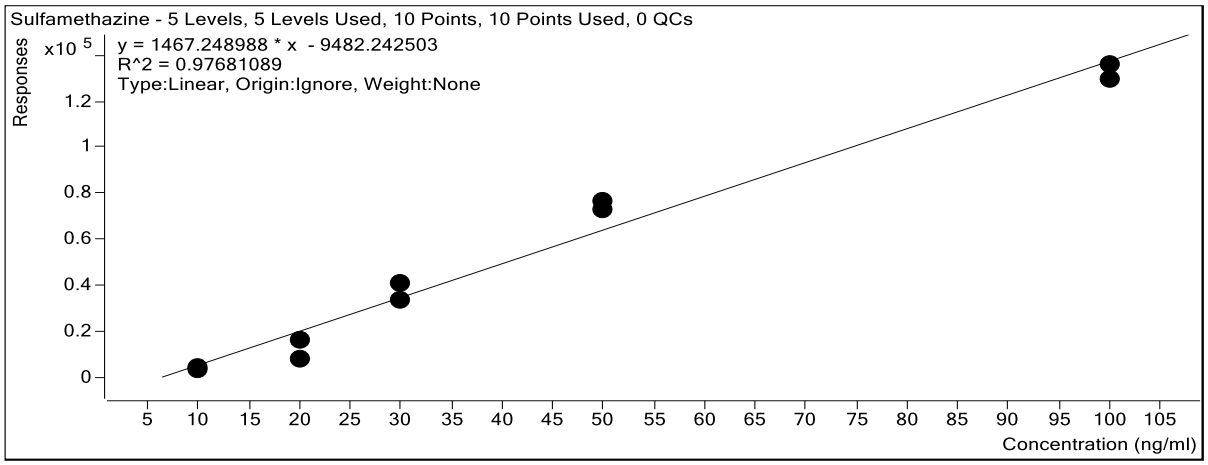


Figure A.10 Calibration curves for sulfadiazine,sulfathizole & sulfamerazine





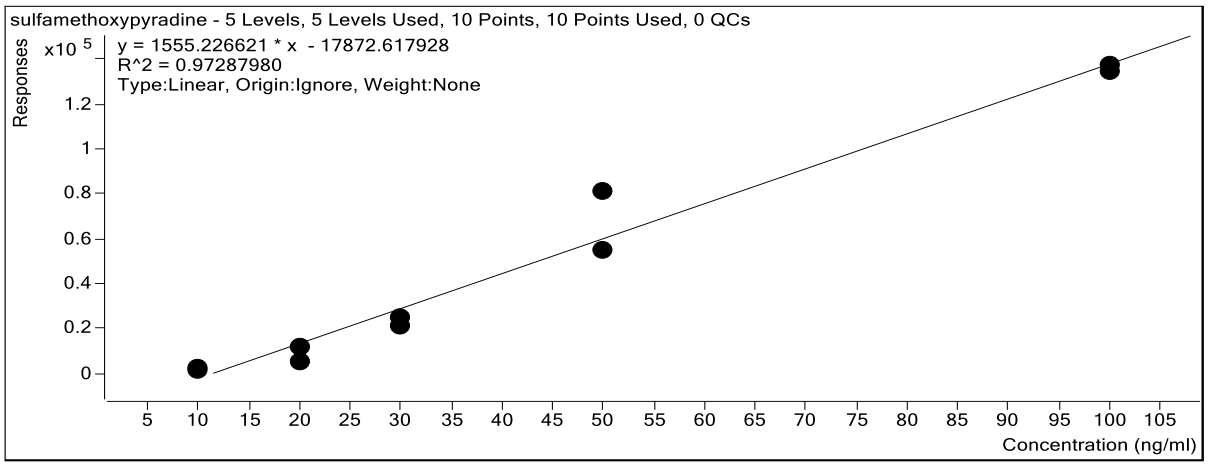
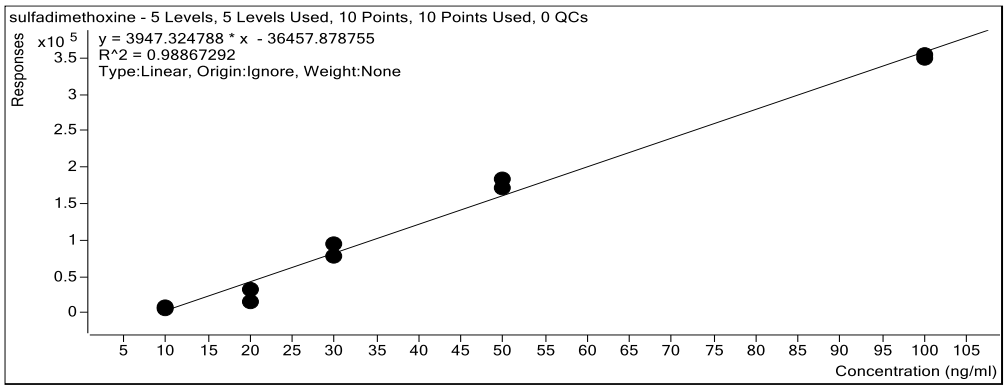


Figure A.11 Calibration curves for sulfapyridine, sulfamethazine & sulfamethoxypyradine



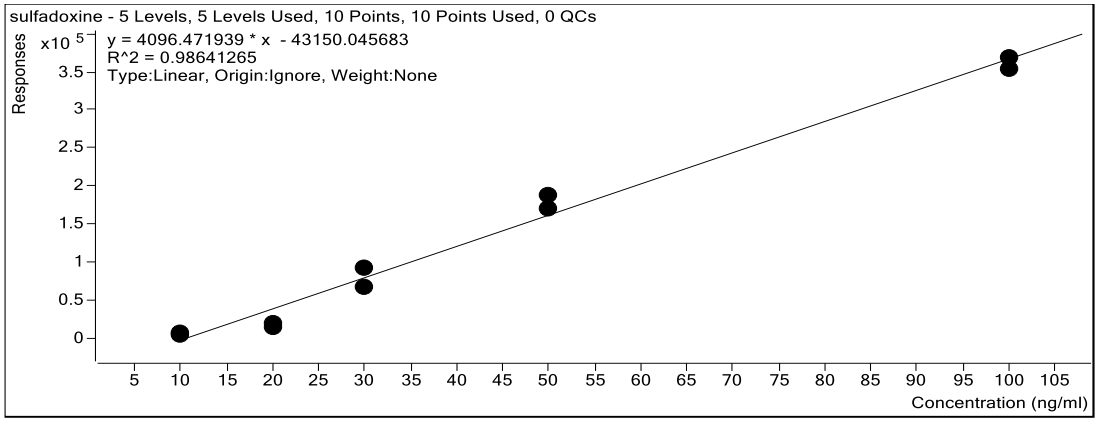


Figure A.12 Calibration curves for sulfadimethoxine & sulfadoxine

*Appendix 5**: Chromatograms for Sulphonamides***

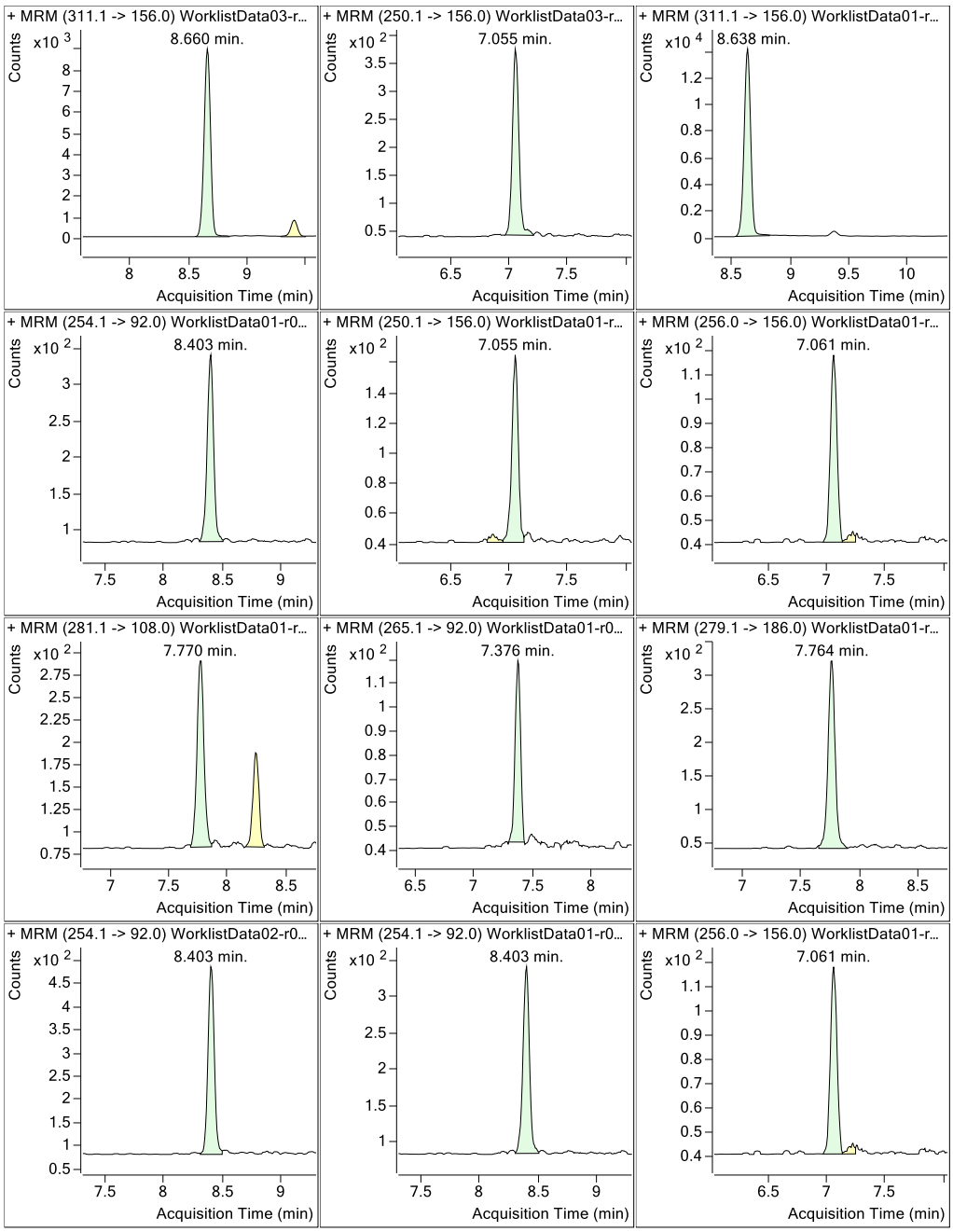
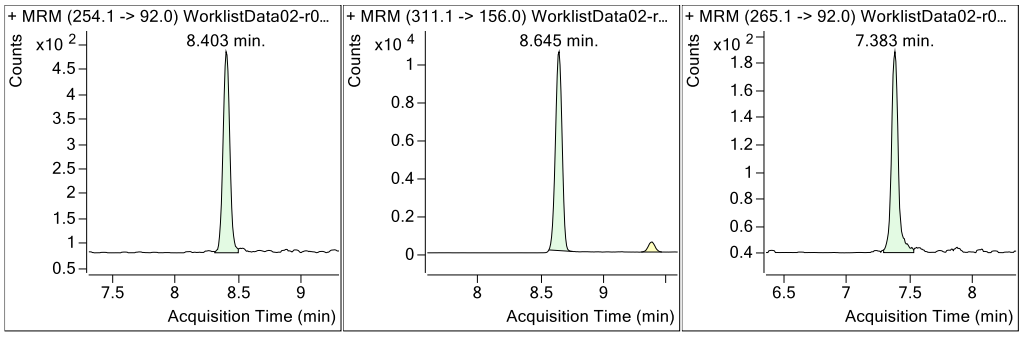
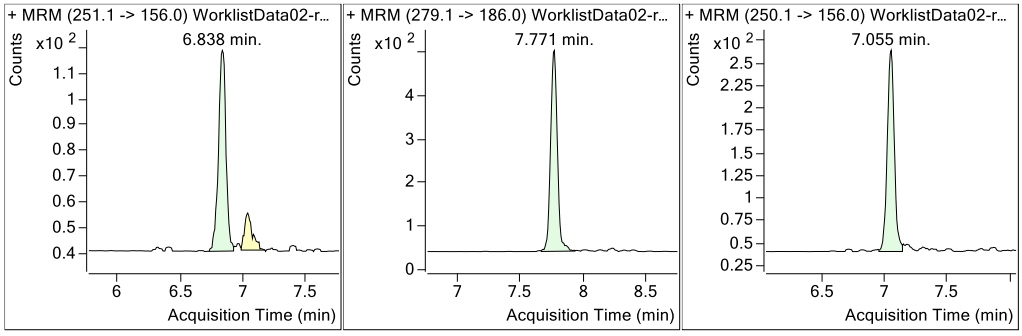
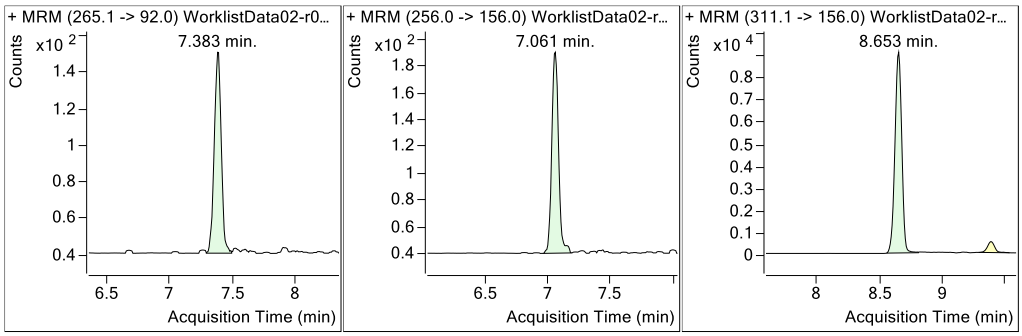
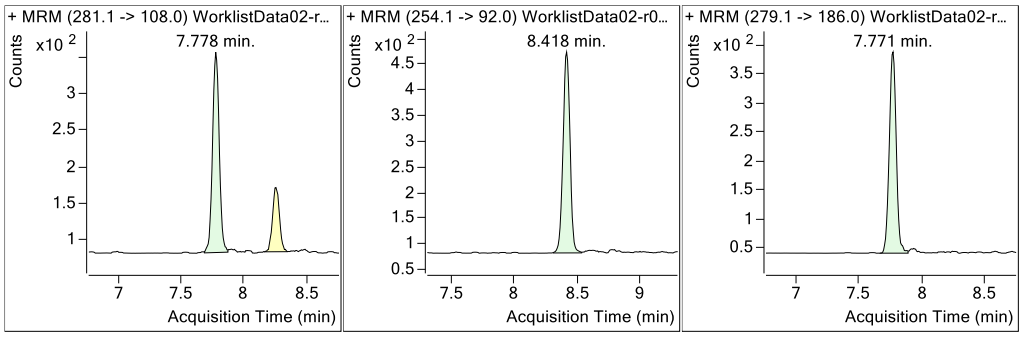


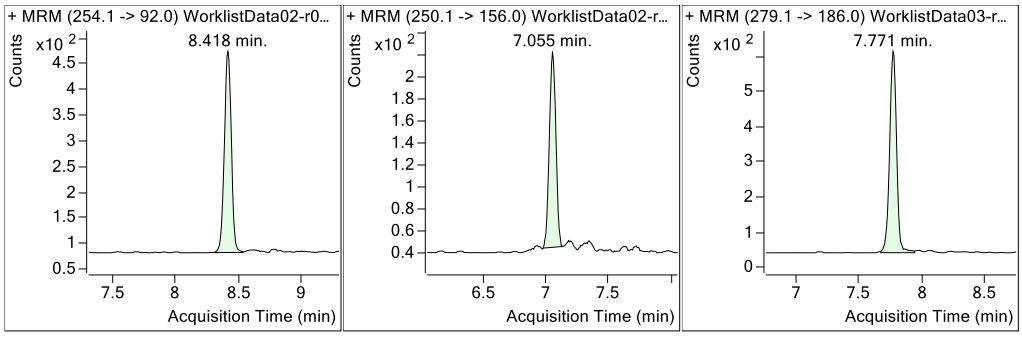
Figure A.13 Chromatograms for sulphonamides

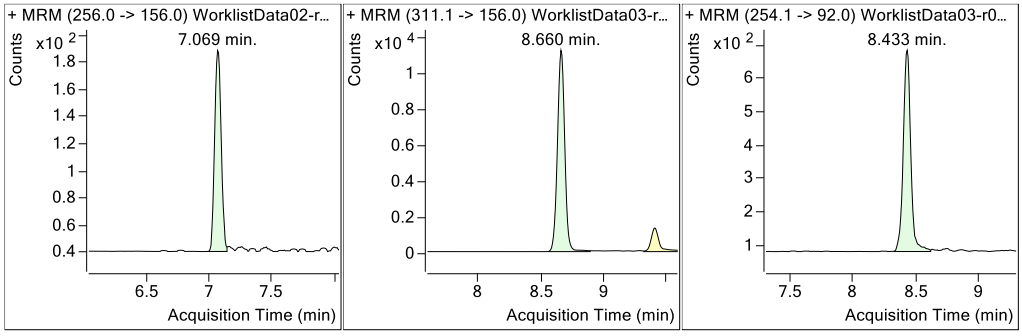

+ MRM (254.1 -> 92.0) WorklistData02-r0...
8.403 min.
+ MRM (311.1 -> 156.0) WorklistData02-r...
8.645 min.
+ MRM (265.1 -> 92.0) WorklistData02-r0...
7.383 min.
Counts
Acquisition Time (min)

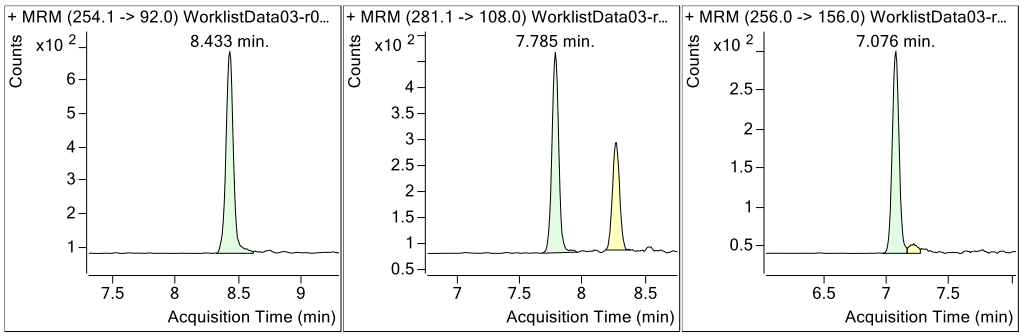

+ MRM (251.1 -> 156.0) WorklistData02-r...
6.838 min.
+ MRM (279.1 -> 186.0) WorklistData02-r...
7.771 min.
+ MRM (250.1 -> 156.0) WorklistData02-r...
7.055 min.
Counts
Acquisition Time (min)

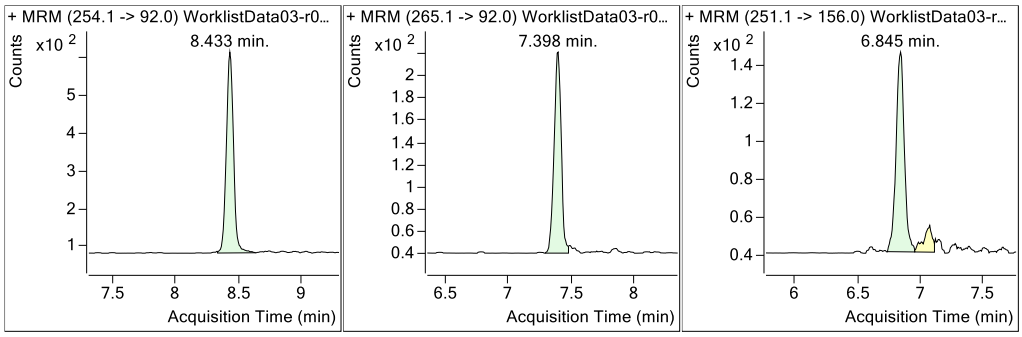

+ MRM (265.1 -> 92.0) WorklistData02-r0...
7.383 min.
+ MRM (256.0 -> 156.0) WorklistData02-r...
7.061 min.
+ MRM (311.1 -> 156.0) WorklistData02-r...
8.653 min.
Counts
Acquisition Time (min)


+ MRM (281.1 -> 108.0) WorklistData02-r...
7.778 min.
+ MRM (254.1 -> 92.0) WorklistData02-r0...
8.418 min.
+ MRM (279.1 -> 186.0) WorklistData02-r...
7.771 min.
Counts
Acquisition Time (min)


+ MRM (254.1 -> 92.0) WorklistData02-r0...
8.418 min.
+ MRM (250.1 -> 156.0) WorklistData02-r...
7.055 min.
+ MRM (279.1 -> 186.0) WorklistData03-r...
7.771 min.
Counts
Acquisition Time (min)


+ MRM (256.0 -> 156.0) WorklistData02-r...
7.069 min.
+ MRM (311.1 -> 156.0) WorklistData03-r...
8.660 min.
+ MRM (254.1 -> 92.0) WorklistData03-r0...
8.433 min.
Counts
Acquisition Time (min)


+ MRM (254.1 -> 92.0) WorklistData03-r0...
8.433 min.
+ MRM (281.1 -> 108.0) WorklistData03-r...
7.785 min.
+ MRM (256.0 -> 156.0) WorklistData03-r...
7.076 min.
Counts
Acquisition Time (min)


+ MRM (254.1 -> 92.0) WorklistData03-r0...
8.433 min.
+ MRM (265.1 -> 92.0) WorklistData03-r0...
7.398 min.
+ MRM (251.1 -> 156.0) WorklistData03-r...
6.845 min.
Counts
Acquisition Time (min)

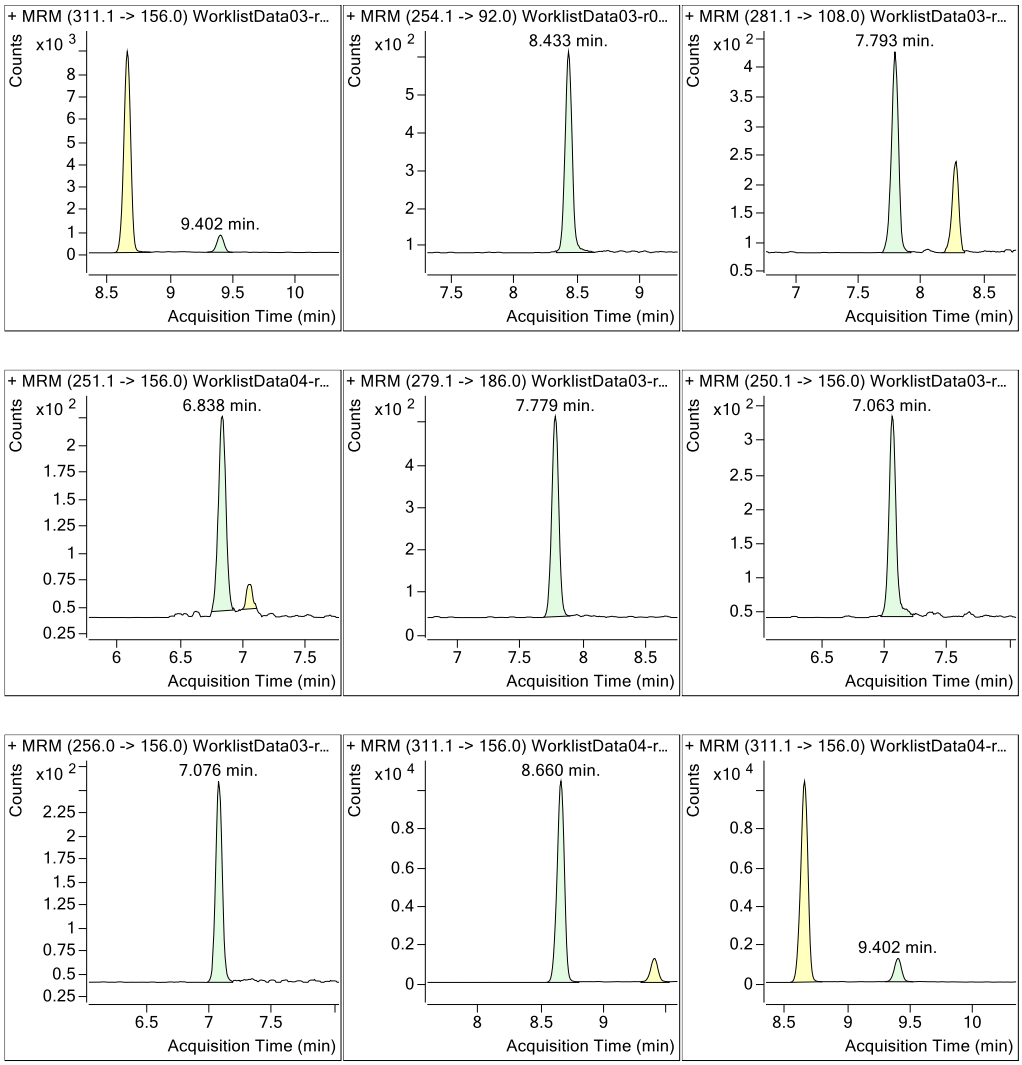


Figure A.14 Chromatograms for sulphonamides

***Table A6**: LOD, LOQ and RSD% for Veterinary drugs*

Serial No.	**Compound Name**	**LOD (ng/ml)**	**LOQ (ng/ml)**	**RSD%**
1.	Sulfachloropyradazine	0.02	0.02	2.1
2.	Sulfadiazine	0.02	0.02	1.5
3.	Sulfathiazole	0.03	0.04	2.4
4.	Sulfapyridine	0.002	0.04	3.5
5.	Sulfamerazine	0.02	0.02	1.6
6.	Sulfaguanadine	0.04	0.02	2.4
7.	Sulfamethoxazole	0.03	0.05	3.8
8.	Sulfamethazine	0.04	0.04	3.7
9.	Sulfamethoxypyradine	0.04	0.03	3.2
10.	Sulfadimethoxine	0.03	0.05	1.4
11.	Sulfadoxine	0.03	0.04	3.6