ORGANOCHLORINE PESTICIDE RESIDUES IN DIFFERENT BRANDS OF TEA AVAILABLE IN ETHIOPIAN MARKET AND THEIR POTENTIAL RISK TO CONSUMERS

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Declaration

This thesis is my original work and has not been presented for a degree in any other university.

Signature ……………………………… Date…………………………

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Abstract

Tea is the most commonly consumed beverage in the world, next to water. Outdated pesticides have been documented as one of the major problems in Africa, particularly in Ethiopia. DDT and endosulfan were detected in Jimma Zone The main objective of this study was to determine the most persistent organochlorine pesticides in different brands of tea and their potential risk to consumers. A total of nineteen different tea brands were purchased purposively from shops and supermarkets found in Jimma town and Addis Ababa city. Five organochlorine pesticide residues (Aldrin (0.1465 mg/kg), γ-Chloridane (0.167 mg/kg), DDT (0.2044 mg/kg), Endrin (0.3067 mg/kg), and Dibutylchlorepoxide (0.4089 mg/kg) were detected from domestic tea samples. And four organochlorine pesticide residues (Endosulfan Sulfate (0.258 mg/kg), Methoxychlor (0.458 mg/kg) and Dibutyl Chloridate (1.427 mg/kg)) were detected from imported tea samples. Average concentration of DDT is above the MRLs and Endosulfan Sulfate is below the MRLs established in China. The EDIs of γ- Chloredane and Endrin were greater than ADIs. The findings of this study pertaining to the detection of pesticide residues in tea from shops and supermarkets in Jimma towns and Addis Ababa city. This is an indication of the widespread or historical use of organochlorine pesticides in tea as they are stable in the environment for long period of time. Strict regulation of pesticide during production, importing, their sale, and for application in the field is important for Ethiopia.

Acknowledgements

My heartiest thanks go to my first advisor Dr. Seblework Mokonen, for her support and demands in scientific contents, incisive advice and tolerant guidance beginning from title selection up to the end of my thesis. She devoted a lot of time to read all the drafts seriously, and made prompt and constructive comments. More important, she has greatly influenced me to think the sense of science. I am so lucky to have her supervision and I deeply appreciate her unselfishness support and guidance. Everything she taught me I will cherish for a lifetime.

My tremendous gratitude's go to my second advisor Mr. Higemengist Astatkie, for his guidance and encouragements in all respects of my thesis. His advice and support helped me in the evolution of my thesis work development.

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Finally, I am much obliged to my dear parents for their great support throughout my life. The deep love and practical support I enjoy from my families have always given me faith and strength. I only want to say: with all my heart, I love you!

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Abbreviation and Acronyms

AD -Alzheimer Disease

CI -Confidence Interval

CIBRC-Central Insecticides Board and Registration Committee

DDT- Dichlorodiphenyltrichloroethane

DDE-Dichlorodiphenyldichloroethylene

EDCs- Endocrine Disrupting Chemicals

EU-European Union

FDA-Food and Drug Administration

GCB-Graphite Carbon Black

GC-Gas Chromatography

GC-ECD- Gas Chromatography-Electron Capture Detector

GC/MS-Gas Chromatography–Mass Spectrometry

HCH- Hexachlorohexane

HR -Hazard Ratio

KTDA- Kenya Tea Development Agency

LD- Lethal Dose

LODs-Limits of detections

LOQs-Limits of quantification

OCPs- Organochlorine pesticides

PCBs- Polychlorinated Biphenyls

PCPB -Pest Products Control Board

PPD- Personal Protective Devices

POPs- Persistent Organic Pollutants

PPE- Personal Protective Equipments

PSA- Primary Secondary Amine

QuEChERS-Quick, Easy, Cheap, Effective, Rugged and Safe

RSD-Relative Standard Deviation

RTL-Retention Time Locked

SBSE-Stir Bar Sorptive Extraction

USA- United States of America

WHO- World Health Organization

Definition of terms

Acceptable daily intake (ADI): is the daily intake which, during an entire lifetime, appears to be without appreciable risk to the health of the consumer on the basis of all the known facts at the time of the evaluation of the chemical by the Joint FAO/WHO Meeting on Pesticide Residues.

Estimated daily intake (EDI): is a prediction of the long-term daily intake of a pesticide residue on the basis of the assumptions of average daily food consumption per person and median residues from supervised trials, allowing for residues in the edible portion of a commodity and including residue components defined by the JMPR for estimation of dietary intake.

Good Agricultural Practice: Good agricultural practice in the use of pesticides (GAP) includes the nationally authorized safe uses of pesticides under actual conditions necessary for effective pest control. It encompasses a range of levels of pesticide applications up to the highest authorized use, applied in a manner which leaves a residue which is the smallest amount practicable.

Hazard Index (HI): is a summation of the hazard quotients for all chemical to which n individual is exposed. A hazard index value of 1 or less than 1 indicates that adverse human effects are expected to occur.

Hazard Quotient (HQ): for threshold contaminants, the risk to a human receptor from being exposed to a chemical via a single pathway can be expressed as an Exposure Ratio, commonly called a Hazard Quotient (HQ)

Limit of determination (LOD): is the lowest concentration of a pesticide residue or contaminant that can be identified and quantitatively measured in a specified food, agricultural commodity or animal feed with an acceptable degree of certainty by a regulatory method of analysis.

Limit of quantification (LOQ): is the smallest concentration of the analyte that can be quantified. It is commonly defined as the minimum concentration of analyte in the test sample that can be determined with acceptable precision (repeatability) and accuracy under the stated conditions of the test.

Maximum Residue Limit (MRL): is the maximum concentration of a pesticide residue (expressed as mg/kg), recommended by the Codex Alimentarius Commission to be legally permitted in or on food commodities and animal feeds.

Pesticide: means any substance intended for preventing, destroying, attracting, repelling, or controlling any pest including unwanted species of plants or animals during the production, storage, transport, distribution and processing of food, agricultural commodities or animal feeds, or which may be administered to animals for the control of ectoparasites.

Pesticide residue: is any specified substance in food, agricultural commodities, or animal feed resulting from the use of a pesticide. The term includes any derivatives of a pesticide, such as conversion products, metabolites, reaction products, and impurities considered to be of toxicological significance.

Recovery: The proportion of analyte (incurred or added) remaining at the point of the final determination from the analytical portion of the sample measured.

Residue: - Level of the pesticide in or on foods

Standard: - A substance of known identity and purity and/or concentration.

1. Introduction

1.1 Background

Tea is made from young shoots and leaves of the plant *Camellia sinensis*. This plant is native to East and South Asia. Depending on the techniques used in the production and processing, tea can be divided into four basic types, namely green tea, black tea, oolong tea, and white tea. In the early 17th century, green tea was first introduced into Europe through Amsterdam. During the following century, tea became fashionable and was introduced in France, Germany, England, America, Russia, as well as other countries. With increasing demand, cultivation was extended into other tropical countries. Tea consumption has risen further in recent years, and has become the most popular beverage consumed worldwide, due to its special flavour and possible beneficial health effects (Meng Bian, 2013).

Tea derived solely and exclusively, and produced by acceptable processes, notably withering, leaf maceration, aeration and drying, from the tender shoots of varieties of the species *Camellia sinensis* (Linnaeus) O. Kuntze known to be suitable for making tea for consumption as a beverage. Linnaeus named the tea plant as Thea sinensis in 1753 in his Species Plantarum. However, later on in 1887, Carl Ernst Otto Kuntze transferred the species to the genus Camellia and now the accepted combination is Camellia sinensis (Linnaeus) O. Kuntze. J.W (Das and Chandra, 2016).

Tea is the most commonly consumed beverage in the world, next to water. It is also recognized as one of the most popular healthy drink in 21st century. The viewpoint of tea drinking is beneficial to human health and was accepted by the consumers. The safety and quality was paid more attention by the consumers all over the world. For the purpose of control of tea pests in the tea production, chemical pesticides are applied and their residues remain on tea plant. The following are the reasons for the contamination(Zongmao, 2011):

★The harvest portion of tea plant (tea shoot) is the pesticide direct applied portion
★Tea shoot with tender and thin leaves, the surface area per unit weight of tea plant is larger than that of other crops including the vegetable crops
★ Tea plant is a crop that harvested multi-times annually, the interval days between the pesticide application and tea plucking is shorter than other crops

★The tea fresh leaves are directly manufactured after plucking and without washing

In recent years, a body of scientific evidence has shown that regular tea drinking may have an important role in health and wellness. The potential health benefits of tea includes; reduce risk of heart disease and stroke, blood cholesterol lowering effects, inhibiting uncontrolled cell growth, offer cognitive benefits and improve mental clarity and work performance, improve weight loss and maintenance, reduce risk of type 2 diabetes, reduce risk of kidney stones and reduce risk of osteoporosis (Valerie Kulbersh, 2015).

"Tea" refers to the aromatic beverage prepared from the cured leaves by combination with hot or boiling water. Tea contains catechins, a type of antioxidant, theanine and the stimulant caffeine at about 3% of its dry weight. Tea has negligible carbohydrates, fat, and protein. Tea also called as 'health beverage' because of its antioxidant properties and beneficial effects on human health so, should be free from contaminants such as pesticides. Most tea farmers and producers use pesticides for agricultural for the protection from pests (Amirahmadi et al, 2013).

Pesticides use is central practice in the agriculture to enhance the quantity of food produced for the fast population growth and for the control and prevention of vector borne diseases. Thus the environmental effect of pesticides and the accumulation of pesticides in food chain are inevitable especially in developing countries. Organochlorines enter in to the food chain and bioaccumulate in adipose tissue due to their lipophilicity and remain in ecosystems for a long period of time. Monitoring studies from Asia revealed widespread contamination of foodstuff and animal feed with pesticide residues (Daniel et.al., 2011).

1.2 Motivation

With the increase of Tea production and the use of pesticides over recent years, residual pesticide is a cause of concern for Tea consumers. DDT and other pesticides are being used on crops in some parts of Ethiopia. Despite the ban of DDT in the 2009 in Ethiopia there are many veterinary drug retail outlets that hold and sell DDT in Southwest of Ethiopia. This availability and accessibility of DDT will lead to use and practices by farmer live around area especially in Khat production. Farmers spray unidentified pesticides on Tea. The disposal practices of left-over Tea can lead to contamination of the environment and leads to health risks. These problems needed further assessment.

Monitoring the pesticides residue of tea provides an opportunity to predict the quality of the tea product before the leaves are being plucked. It will help to minimize subjectivity by tea judges and improve tea garden management, thus assuring consumers that the tea is of expected quality and strengthening confidence in the international tea trade.

So far, no study had been conducted concerning pesticides residue in Tea and their possible risks to consumers in the country as a whole. Therefore, to fill this gap we need to do pesticide residue analysis and consumer risk assessment. This study will conducted to fill the gap and to provide data bases for further research undertakings and for policy makers as well as other interested groups.

1.3 Statement of the Problems

Chemicals play an important role in the efforts of countries to achieve economic growth and fulfil their development objectives (Moser, 2015). However, as far as they are vital for ensuring food security and economic growth, incorrect and indiscriminate use can be disastrous both for human health and the environment. In this context, chemicals can have a dual nature; they can be either beneficial or harmful, depending on numerous factors, such as the amounts to which exposure occurs (Damalas, 2009).

The occurrence of harmful chemicals in the environment has become an issue of great debate in recent decades. Pesticides and other foreign substances in food products and drinking water pose an immediate threat to human health, whereas other contaminants gradually build up in the environment and in the human body, causing disease long after first exposure(Gavrilescu, 2015). It is also well known that many pesticides can accumulate in living species causing long-term and chronic effects. But there are difficulties in defining chronic exposure and disease outcomes, given the existence of a large series of variables of interest, such as lifestyle, occupation, diet preferences, and smoking. All of which must be taken into account to establish a disease-exposure relationship in the epidemiological investigation (Colosio, 2013).

Pesticides are common chemicals used to eliminate a great variety of unwelcome living organisms, particularly in agriculture. They are widely used in agriculture for the purposes of crop protection and in public health to control vector-borne infectious diseases. Because of high biological activity, and, in some cases, long persistence in the environment, pesticides may cause harmful effects to human health and to the environment (M. Maroni, Fanetti, A., Metruccio, F. , 2006). Improper handling may result in severe acute poisonings; in some cases, adverse health effects may also result from long-term, low-level exposures. As a result of widespread diffusion of pesticides, a great part of the population may be exposed to pesticides due to occupation. Several groups of people, characterized by quite different patterns and degree of exposure, face the risk of adverse effects. Occupational exposure typically occurs in workers involved in the manufacture of pesticides and among specific users in public health (e.g., exterminators of house pests). In the agricultural sector, exposure to pesticides typically occurs among farmers and professional applicators of pesticides (Ye, 2013).

Regarding the general population, individuals may be exposed to pesticide residues in food and drinking water on a daily basis or to pesticide drift in residential areas close to spraying areas.The greatest amount (about 75%) of pesticide use in the USA occurs in agriculture (Calvert, 2008). In Europe, despite international efforts to promote the sustainable use of pesticides in agriculture and an actual reduction in use in several countries, overall pesticide use did not decline substantially in the WHO European Region during the period of 1990s (Robertson, 2004).

In developing nations, many old, non-patented, more toxic, environmentally persistent, and inexpensive types of chemicals are used extensively, which can able to cause significant acute health problems and environmental contamination (Aktar, 2009). Farmers and farm workers face greater risk of exposure to pesticides than typical non-agricultural workers,. The type of pesticides, the frequency of use, and the application method are usually different according to the farm type and the specific crops grown. Pesticide use is a seasonal, but intermittent task, covering only one of a wide range of tasks undertaken by farmers. Consequently, the frequency and the total time of exposure for most farmers are generally lower than for pesticide applicators in other industries. Dedicated pesticide applicators in the agricultural sector are exposed to pesticides more frequently than farm operators, even though they may have fewer years of pesticide use. Many studies of pesticide exposure have focused mainly on farm workers who are certified pesticide applicators. However, there is enough evidence that pesticide exposure may not be universal among farm workers and that a great proportion of farmers may not be exposed to pesticides directly (Aktar, 2009).

Recently, the potential of certain pesticides to act as endocrine disrupting chemicals (EDCs) has been assessed (Ewence, 2015). These pesticides include certain organometallic compounds and many older organochlorine compounds that are also toxic and persistent. The latter were banned from use in many countries in the 1970s and 1980s, so exposures to these compounds have been decreasing ever since. However, these chemicals are highly persistent and small amounts are still present in the environment. Due to the uncertainty surrounding how much of a chemical is needed to have an impact, further research is required to allow determination of the best management approach. Other pesticides such as organophosphates, carbamates, triazines, and pyrethroids that are less persistent and less toxic than the organochlorines were used to replace them, but many are now confirmed or suspected to act as endocrine disrupting chemicals (Andersen, 2002). Some endocrine disrupting pesticides show toxic effects that decrease as the dose decreases, until, at very low doses (often as low as parts per billion or even parts per trillion), their effects increase (Welshons, 2003).

Though no section of human population is completely immune to pesticide exposure yet much higher level of risk is associated in case of occupationally exposed groups including those involved in agricultural activities and pesticide manufacturing etc... Exposure to chemical pesticides may result in a number of serious and chronic health problems such as birth defects, nerve damage, cancer, skin diseases and many other that might occur over a long period of time (Pratibha et al, 2015). However, health effects of pesticides are greatly dependent on factors such as their chemical nature and toxicity as well as dose and length of exposure. According to EPA (EPA, 2007) the risk is directly proportional to toxicity as well as exposure.

Worldwide tea production has increased significantly over the past 10 years, growing from 3.89 million tons in 2006-2008 to 5.06 million tons in 2013, a 30 % increase. World tea exports, with China, India, Kenya, and Sri Lanka as the major exporters, reached 1.77 million tons in 2013, a five percent increase over 2012. World tea consumption continues to surge, with Russia, the United Kingdom, Pakistan, and the U.S. as the leading importers in this market. Tea imports into the U.S. have nearly tripled over the past 15 years alone, according to the U.S. Department of Agriculture (USDA) (Nikita, 2015).

In Ethiopia, Tea production increased dramatically from the year 1976 until 2013, which is from 15 tons to 7,400 tons, respectively. In 2012, the highest Tea Production year in Ethiopia for the indicator which results in 7,500 Tons and year 1976 is the lowest year for the indicator. Ethiopia is number 23 in the world in quantity of Tea production (FAO, 2013).

Tea is cultivated in about 36 countries all over the world, but production is heavily concentrated in just a handful. In 2000, five countries (India, China, Sri Lanka, Kenya and Indonesia) together produced almost 80% of the world's tea. Some contaminants are important in tea, from food safety aspects, such as heavy metals and pesticides. The unsafe use of chemicals not only puts the workers and the environment in danger, but also leaves traces of harmful pesticides and insecticides in the processed tea (Amirahmadi et al, 2013).

Outdated pesticides have been documented as one of the major problems in Africa, particularly in Ethiopia. In Jimma Zone, some banned pesticides and those not authorized for use in cereals, vegetables, and coffee, such as organochlorines (e.g., DDT and endosulfan), were detected.. The Ethiopian Federal Ministry of Health uses indoor residual sprays such as DDT and dieldrin to protect people against mosquito transmitted diseases. These activities could potentially contaminate different food items produced in Ethiopia, which in turn may impact public health(Mekonen et. al., 2014).

Thus, studies focused on pesticides residue level are highly required in order to understand the Maximum Residue Level regarding this and at the same time to assess the effects of common agricultural practices on consumer health. There is no study done on the pesticide residues in tea in Ethiopia. Further, such studies may also prove useful to design and establish the regulatory policies concerning chemical inputs in agriculture with an aim to develop safe and sustainable agricultural practices.

Therefore, the main aim of this study is to investigate the residue of organochlorine pesticides in different brands of tea available in Ethiopian market and its potential risk to the consumers.

1.4 Research Questions

1. What is the type and level of pesticides in different brands of Tea available on the market?
2. Is the maximum residue limits obtained from the tea in line with internationally acceptable maximum residue limits?
3. Do the studied pesticides have a health risks to the tea consumers?

2. Literature Review

2.1 Pesticide Toxicity and Risk (Hazard)

Pesticide toxicity depends on the compound family and are responsible for acute poisonings as well as for long term effects, including cancer and adverse effects on reproduction in humans and wildlife (Maroni, 2006). Toxicity of a pesticide is a measure of the capacity or ability of the pesticide to cause injury or illness and is determined by subjecting test animals to varying dosages of the pesticide active ingredient. Toxicity pathways are normal pathways for maintaining cellular functions but when sufficiently perturbed, will lead to an adverse health outcome and the magnitude of the adverse effect is related to dose at the cellular level, the timing of the perturbation and the susceptibility and life stage of the host (Linda, 2009).

Harmful effects due to acute toxicity is injury from a single high exposure and generally of short duration via any route of entry while chronic toxicity and chronic effects occur from exposure to small doses of the active ingredient repeated over a long period of time. Chronic effects from exposure to pesticides include birth defects, severe depression, irritability, confusion, delayed reaction time, drowsiness, insomnia and production of benign or malignant tumors, genetic changes, birth defects, nerve disorders, endocrine disruption and reproduction effects (Linda, 2009).

In terms of pesticide safety, there is a difference between the words “toxicity” and “risk”. Toxicity refers to inherent poisonous ability of a material. The toxicity of a material is evaluated in toxicology laboratories and is expressed in quantitative terms, such as LD_{50} or LC_{50} (lethal dose or concentration 50%, i.e., the dose or concentration at which a material will kill 50% of a reference organism). The risk (or hazard) depends not only on the toxicity of a material, but also on the possibility of exposure when used. In simple terms, toxicity is the capacity of a substance to produce illness or even death, whereas risk (hazard) is a combination of toxicity and exposure (Figure 1). Therefore, the risk (hazard) from a specific pesticide depends on the toxicity of the specific product used and the amount and form of exposure experienced (Frank, 2011).

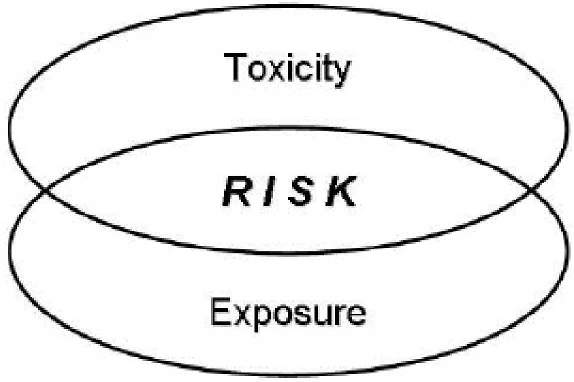
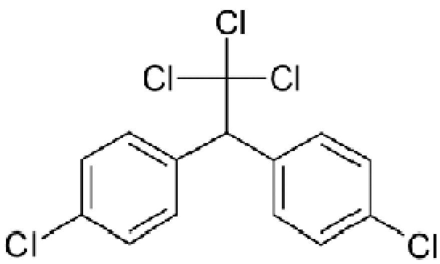


Figure 1: Risk is a combination of toxicity and exposure.

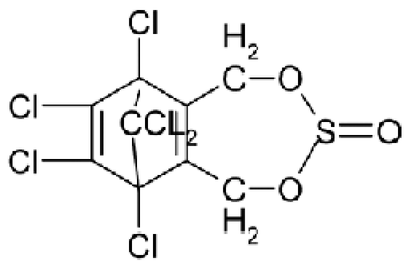
Information about both toxicity and exposure is required to determine risk (hazard). Normally, the potential for adverse effects to humans from highly toxic pesticides is greater than that from pesticides that are less toxic (Frank, 2011). However, other factors, such as the concentration of the pesticide in a formulation, the length of exposure to the pesticide, and the route of entry into the human body, are of major importance in the potential for poisoning. Evidently, a pesticide applicator can have limited control over the toxicity of a pesticide, but significant control over risks that are associated with the use of this pesticide can be expected. For example, a container of a highly toxic pesticide that is sealed presents little risk before the seal is broken. Even in the case when the container is opened, the risk remains small, unless the end user is not wearing protective gear. However, if the container is cracked or leaking, or if proper protective gear is not used, the risk can be serious (Sarwar, 2015).

Pesticides may harm humans via poisoning or injuries. Poisoning is caused by pesticides that affect organs or systems inside the body, whereas injuries are usually caused by pesticides that are external irritants. Some pesticides are highly toxic to humans; only small amounts can cause highly harmful effects. Other active ingredients are less toxic, but overexposure to them also can be detrimental. Toxic effects by pesticide exposure can range from mild symptoms, like minor skin irritation or other allergic symptoms, to more severe symptoms, like strong headache, dizziness, or nausea. Some pesticides, e.g., the organophosphates, can cause severe symptoms, like convulsions, coma, and possibly even death. Pesticide toxicity in humans can be categorized by the nature of exposure, the route through which exposure occurs, or the body system affected. As a general rule, any poison is more toxic if ingested than if inhaled and more toxic if inhaled than if absorbed by the skin (dermal exposure). Some toxic effects by pesticides are temporary, given that they are quickly reversible and do not cause severe or permanent damage. Certain pesticides may cause reversible damage, but full recovery may take long periods of time. Still other poisons may have irreversible effects, although the exposure is not fatal (A. D. Christos, , Spyridon, D. and Koutroubas,. 2016).

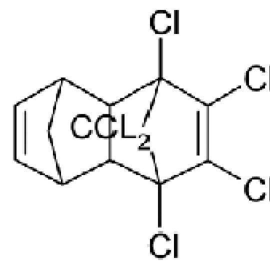
2.2 chemical structure of interested pesticides



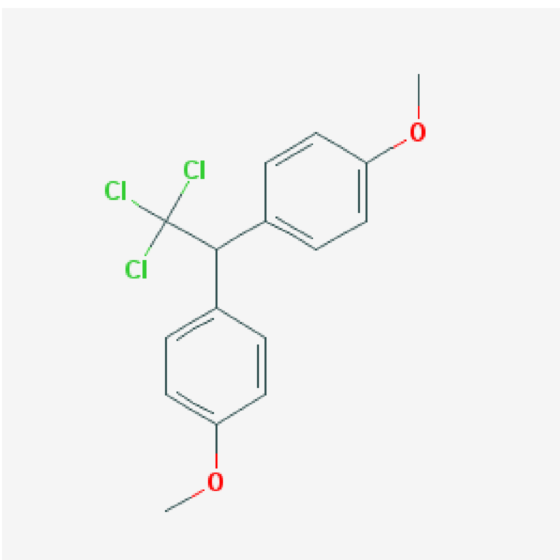
DDT



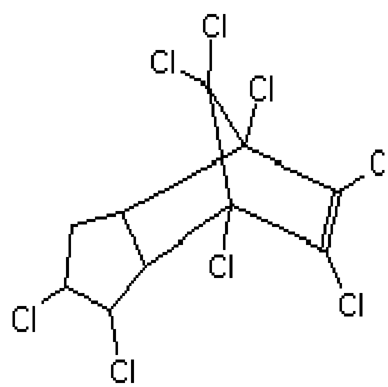
Endosulfan



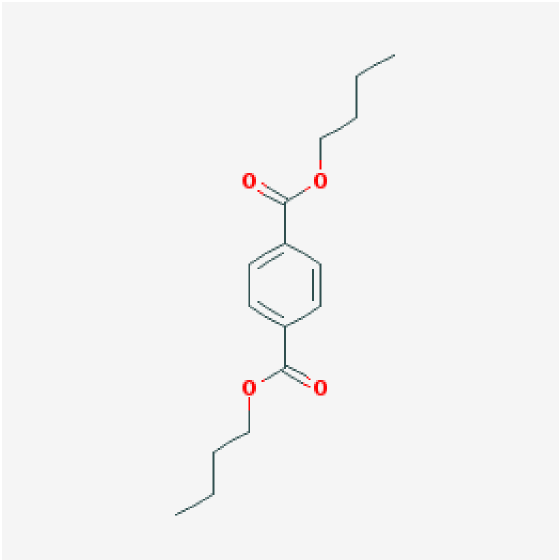
Aldrin



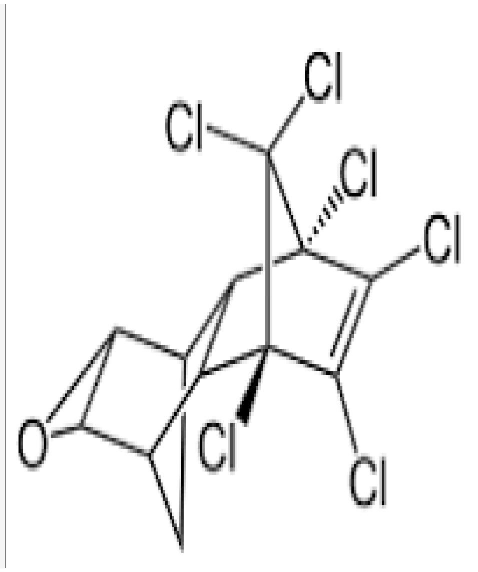
Methoxychlor



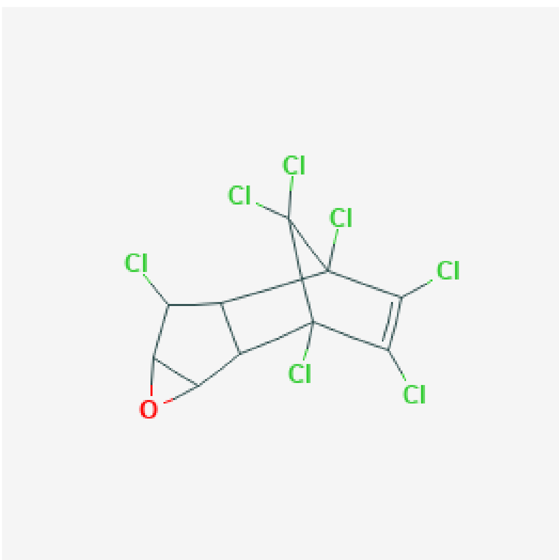
Chloridane



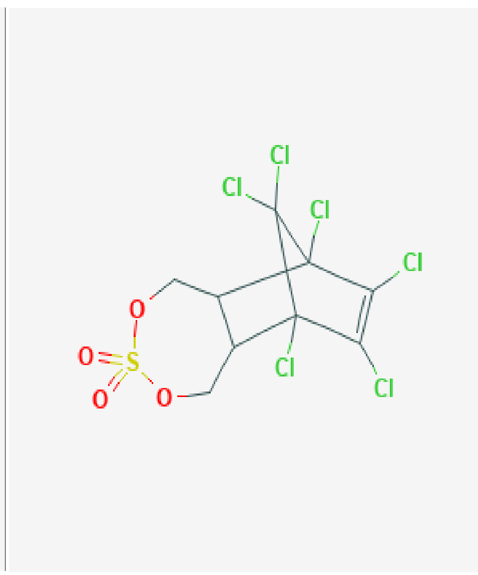
Dibutylchlor Epoxide



Dieldrin



Heptachlor Epoxide



Endosulfan sulfate

2.3 Human Exposure to Pesticides and Factors Affecting Exposure

Human exposure to pesticides may occur through occupational exposure in the case of agricultural workers in open fields and greenhouses, workers in the pesticide industry, and exterminators of house pests (Martínez, 2009). However, irrespective of whether the occupation involves the use of pesticides, the presence of such chemicals in the working environment constitutes potential occupational exposure. Evidently, workers who mix, load, transport and apply formulated pesticides are normally considered to be the group that will receive the greatest exposure because of the nature of their work and are therefore at highest risk for possible acute intoxications. In some situations, exposure to pesticides can occur from accidental spills of chemicals, leakages, or faulty spraying equipment. The exposure of workers increases in the case of not paying attention to the instructions on how to use the pesticides and particularly when they ignore basic safety guidelines on the use of personal protective equipment and fundamental sanitation practices such as washing hands after pesticide handling or before eating (Fenske, 2005).

Several factors can affect exposure during pesticide handling. The form of formulation of pesticide products may affect the extent of exposure. Liquids are prone to splashing and occasionally spillage, resulting in direct skin contact or indirect skin contact through clothing contamination. Solids may generate dust while being loaded into the application equipment, resulting in exposure to the face and the eyes and also respiratory hazards. The type of packaging of pesticide products can also affect potential exposure. For example, the opening of pesticide bags can result in some kind of exposure depending on the type of packaging in combination with the formulation of the active ingredient. Also, the size of cans, bottles, or other liquid containers may affect the potential for spillage and splashing (Fenske, 2005). Moreover, adjuvant chemicals used in pesticide formulations to enhance their efficiency in terms of biological activity (e.g., enhance the contact between the active ingredient and its specific molecular target) as well as to facilitate application and reaching target species, may show toxicity themselves, thus contributing to the overall effect of exposure to a commercial pesticide product (Surgan, 2010). Weather conditions at the time of application, such as air temperature and humidity, may affect the chemical volatility of the product, the perspiration rate of the human body, and the use of personal protective equipment by the users. Wind increases considerably spray drift and resultant exposure to the applicator. The amount of pesticide that is lost from the target area and the distance the pesticide moves will increase as wind velocity increases, so greater wind speed generally will cause more drift. In addition, low relative humidity and high temperature will cause more rapid evaporation of spray droplets between the spray nozzle and the target than high relative humidity and low temperature (Gil, 2008).

General hygiene behaviour of workers during pesticide use can also have substantial impact on exposure. For example, workers who avoid mixing and spraying during windy conditions can reduce the exposure. Proper use and maintenance of protective clothing are considered important behaviours associated with reduced chemical exposures. Furthermore, the frequency and duration of pesticide handling both on a seasonal and lifetime basis affects the exposure. In particular, the exposure of an individual farmer that applies a pesticide once a year is lower than that of a commercial applicator that normally applies a pesticide for many consecutive days or weeks in a season (Fenske, 2005).

Exposure of the general population to pesticides occurs mainly through eating food and drinking water contaminated with pesticides, whereas substantial exposure to pesticides can also occur when living close to a workplace that uses pesticides or even when workers bring home contaminated articles. Non-occupational exposure originating from pesticide residues in food, air and drinking water generally involves low doses and is chronic (or semi-chronic). However, clear links between individual pesticides and individual health effects can only be shown in animal studies, but the doses used in these studies are far higher than the enforced legally pesticide limits. Therefore, the risk to human health from these studies appears to be negligible. The actual acute exposure, however, may be higher than that anticipated due to certain food preferences, residue variability between individual food items and the greater than average consumption of a particular food item only at one sitting. As a result of pesticide use in or around the home, individuals can be exposed during the preparation and application of pesticides or even after the applications are completed, whereas delayed exposure can occur through inhalation of residual air concentrations or exposure to residues found on surfaces, clothing, bedding, food, dust, discarded pesticide containers, or application equipment. Also, accidental poisoning with pesticides in the home is a possibility from pesticide use around the house or garden. Exposure is likely to occur from pesticide spills, improper use, or poor storage as a result of use without reading or accounting to the pesticide label. Pesticide mishandling such as transferring the products from their original packages into household containers and also the lack of compliance with instructions of the label can be also sources of exposure (A. Christos, Damalas L, and Ilias G. Eleftherohorinos,. , 2011).

2.4 General signs/symptoms of Pesticide Poisoning

Symptoms due to pesticide poisoning can range from mild skin irritation to coma or even death. These vary with classes of chemicals, individuals, duration of exposure and route of entry. It is not surprising therefore that some people may show no reaction to an exposure that may cause severe illness in others. Some of the symptoms which have been reported include central nervous system symptoms like dizziness, headache, confusion and respiratory depression. Muscarinic effects include increased glandular secretions, smooth muscle dysfunction manifested as diarrhea, miosis, blurred vision, involuntary micturation and bradycardia. Nicotinic effects include hypertension, tachycardia, muscle fasciculation, weakness and paralysis. The recovery from acute poisoning depends on the severity of the poisoning and on the availability of treatment, and may last from one day up to a few weeks (Miranda, 2004).

2.5 Pesticide-Related Work Tasks

Pesticide use is typically associated with three basic stages: (i) mixing and loading the pesticide product, (ii) application of the spray solution, and (iii) clean-up of the spraying equipment. Mixing and loading are the tasks associated with the greatest intensity of pesticide exposure, given that during this phase farmers are exposed to the concentrated product and, therefore, often face high exposure events (e.g., spills). However, the total exposure during pesticide application may exceed that incurred during mixing and loading, given that pesticide application typically takes more time than the tasks of mixing and loading. Pesticide drift is also a permanent hazard in pesticide use, because it exists even in the most careful applications, and therefore, can increase the possibility of detrimental effects of pesticide use on the users and the environment (Damalas, 2015). There is also evidence that cleaning the equipment after spraying may also be an important source of exposure.

The level of pesticide exposure to the operator depends on the type of spraying equipment used. Hand spraying with wide-area spray nozzles (when large areas need to be treated) is associated with greater exposure to the operator than narrowly focused spray nozzles. When pesticides are applied with tractors, the application equipment is mounted directly on the tractor and is associated with a higher degree of operator exposure than when the spray equipment is attached to a trailer. Pesticide deposition on different parts of the operator's body may vary largely due to differences in individual work habits. Several studies on the contamination of the body in pesticide applicators showed that the hands and the forearms suffer the greatest pesticide contamination during preparation and application of pesticides. However, other body parts such as the thighs, the forearms, the chest, and the back may also be subject to significant contamination (A. D. Christos, , Spyridon, D. and Koutroubas,. 2016).

Clean-up of the spraying equipment is an important task in the use of pesticides. The time given to the task of cleaning may occupy a considerable part of the basic stages of pesticide handling. Despite considerable variation among farm workers, equipment cleaning has been found to contribute greatly to workers' daily dermal exposure. Unexpected events, such as spills and splashes, are also a major source of dermal contamination for pesticide applicators, and often the exposure from these events can result in significant acute and long-term health effects. Spills and splashes usually occur during mixing or loading and application, but may also appear in the stage of equipment clean-up (Lebailly, 2009).

Farmers (or farm workers) who make the spray solutions and apply pesticides have been at the center of attention of most research thus far, but often farmers re-entering the sprayed fields may also face pesticide exposure, sometimes to significant levels. It is not surprising that re-entry farm workers may face even greater exposure than pesticide applicators, possibly because safety training and the use of PPE are usually less, and the duration of exposure may be greater than that of the applicators. Exposure by re-entry in the sprayed fields may become a serious problem if farm workers re-enter the treated fields soon after pesticide application. Spray drift from neighbouring fields and overexposure events of this kind, each involving groups of workers, have been documented as inadvertent events of farmers' exposure to pesticides (A. D. Christos, , Spyridon, D. and Koutroubas,. 2016).

2.6 Pesticide residue levels in Tea

An analysis of the most recently published FDA data on residue levels in tea (black, green, and oolong) from 2008 to 2012 reveals a high rate of violations. Out of the 65 samples of tea analyzed over these five years, nearly 30 percent had two or more illegal residues, with one sample from 2012 containing up to 14 violations. Of the 94 violations found in these samples, 76 were listed as "no registration" and 18 as "excess of tolerance." Many of these violations are for pesticides that are currently used in U.S. agriculture, but lack a tolerance and presumably exposure data for use in tea, such as acetamiprid (a neonicotinoid), or permethrin (a pyrethroid). Other chemicals that were found to be in violation have been long banned from use in the U.S., including DDE (a DDT metabolite), carbendazim (MBC) (not allowed for use in agriculture), and heptachlor epoxide (a derivative of the pesticide heptachlor, which was banned in the U.S. for use in agriculture and home use due to its carcinogenicity and persistence in the environment) (Nikita, 2015).

In 2014, a Green peace India investigation, *Trouble Brewing: Pesticide residues in tea samples from India*, found that nearly 94% of the tea samples tested in India contained at least one of 34 different pesticides, while over half contained a toxic cocktail of more than 10 different pesticides. The report relied on tests of 49 branded and packaged teas. Eight of the top 11 companies that make up a large part of the tea market in India were represented, including Hindustan Unilever Limited, a subsidiary of the global multinational company Unilever. Popular brands included in the study are Twinings and Lipton (Greenpeace India, 2014).

The residues found include DDT, which has been banned for use in agriculture in India since 1989, and endosulfan, which was banned in India in 2011. Over half of the samples testedcontained illegal residues either those that are not approved for use in tea cultivation or exceed allowed limits. In addition to registered pesticides that have been long banned from agricultural use in tea cultivation in India (DDT, and triazophos), also found were (i) suspected mutagens and neurotoxicants (monocrotophos), and (ii) insecticides associated with the global decline in bee populations (neonicotinoids like thiacloprid and thiamethoxam). Some of the most frequently detected pesticides include thiamethoxam (78%), cypermethrin (73%), acetamiprid (67%), thiacloprid (67%), DDT (67%), deltamethrin (67%), dicofol (61%), imidacloprid (61%), and monocrotophos (55%) (Greenpeace India, 2014).

The Greenpeace India report also provides several concrete examples of tea with residues of pesticides that are not registered for use in India. According to the report, 68% of the 34 detected pesticides were not registered at the time of publication for use on tea by the CIBRC (Central Insecticides Board and Registration Committee), although some appeared on lists of pesticides recommended for use on tea at the state level, indicating inconsistencies in regulations or recommendations at the regional and national levels. Of the neonicotinoid insecticides detected in the samples, only thiacloprid and thiamethoxam are registered for use on tea production in India. Two neonicotinoids, acetamiprid and imidacloprid, not approved for use in tea cultivation, are among the most commonly found residues in the report. Other illegal pesticide residues detected include the insecticide tebufenpyrad, a pyrazole miticide/insecticide, which is not registered for use in India. Endosulfan was found in about 8% of tea samples in the Greenpeace India investigation, despite being banned for production, use, and sale throughout India following a 2011 Supreme Court decision, although the chemical is still registered for use by CIBRC (Greenpeace India, 2014).

Rampant contamination of tea leaves with pesticides has also been found in China. In April 2012, Greenpeace China released a report, *Pesticides: Hidden Ingredients in Chinese Tea*, which found evidence of pesticide residues in popular tea brands. The report found that all of the 18 samples tested had traces of at least three different pesticides. In total, 29 different pesticides were detected, including reproductive and developmental toxicants (carbendazim, benomyl, myclobutanil, and flusilazole) and bee-killing chemicals (imidacloprid and acetamiprid). Twelve of the samples had traces of pesticides banned for use on tea by China's Ministry of Agriculture (including methomyl and fenvalerate). Six samples contained a mix of over 10 pesticides, with one sample containing up to 17 different pesticides (Greenpeace China, *2012;* Greenpeace India, 2014).

The study in Iran which is the first attempt for monitoring of 25 pesticide residues from different chemical groups in tea samples obtained from local markets in Tehran, I.R. Iran during the period 2011 showed that a reliable and accurate method based on spiked calibration curve and QuEChERS sample preparation was developed for determination of pesticide residues in tea by gas chromatography–mass spectrometry (GC/MS). The using of spiked calibration standards for constructing the calibration curve substantially reduced adverse matrix-related effects and negative recovery affected by GCB on pesticides. The recovery of pesticides at 3 concentration levels (n = 3) was in range of 81.4 - 99.4%. The method was proved to be repeatable with RSDr lower than 20%. The limits of quantification for all pesticides were ≤20 ng/g. 53 samples from 17 imported and manufactured brand were analyzed. Detectable pesticides residues were found in 28.3% (15 samples) of the samples. All of the positive samples were contaminated with unregulated pesticides (Endosulfan Sulfate or Bifenthrin) which are established by ISIRI. None of the samples had contamination higher than maximum residue limit set by EU and India (Amirahmadi et al, 2013).

Multi-Residue Method to determine five groups of 85 pesticides- chlorinated, carbamate, phosphorous, pyrethroid and others - in vegetables, fruits and green tea has been developed using stir bar sorptive extraction (SBSE) coupled to thermal desorption and retention time locked (RTL) GC-MS showed that Pre-extraction with methanol and dilution with water prior Howed that to SBSE (60 min) were performed. Dilution of methanol extract for SBSE was examined to obtain high sensitivity and to compensate the effect of adsorption to the glass wall of extraction vessel and to sample matrix for the compounds with high log $K_{o/w}$ values (e.g. pyrethroid). The methanol extracts were diluted twofold and fivefold, and were simultaneously SBSE-enriched. The two stir bars were placed in a single glass thermal desorption liner and were simultaneously desorbed. The versatility of the method was exhibited by its good linearity (4-100 μg/kg, r^2 >0.9900) for 66 pesticides and limit of detection (LOD: < 5 μg/kg) for most of the analytes. The method enables to determine pesticides at low μg/kg in tomato, cucumber, green soybeans, spinach, grape and green tea (Nobuo, 2004).

2.7 Significance of the study

This study will be expected to have some advantage that,

- Gives valuable information for regulatory bodies on availability of banned pesticides
- Tackle the consumer exposure to a dangerous effect of pesticides in tea by explaining the residues detected in tea and Hazard Index for concerned bodies and consumers.
- Increase the acceptability of Ethiopian Tea that exported to the foreign countries through promotion of the tea products that have residues below the detection limit.
- Input data for international regulations to revise and establish the Maximum Residue Limits in tea of pesticides that are not established previously.
- Further, such studies may also prove useful to design and establish the regulatory policies concerning chemical inputs in agriculture with an aim to develop safe and sustainable agricultural practices.
- Also used to establish the Maximum Residue Limits in tea especially at national level.
- Therefore, this particular study is considered to be important in providing original information for concerned bodies as well as government officials, who are responsible in protecting the lives of the people from the harmful effect of pesticides which are hazardous to human beings and the environments.

3. Objectives

3.1. General Objective

- The main objective of this study is to determine organochlorine pesticide residues in different brands of tea available in Ethiopian market and their potential risk to consumers

3.2. Specific Objectives

- To determine the amount of pesticide residues in different brands of tea
- To compare the result of pesticide residue with international maximum residue limits
- To estimate the potential risk associated with the detected pesticides to consumers

4. Materials and Methods

4.1 Study area and Period

The study was conducted in Jimma town and Addis Ababa city market, Ethiopia. Different tea brands were purchased from different shops and supermarkets. Jimma town which consists of thirteen kebeles is located at 354 km to the southwest of Addis Ababa. It is found on approximately 7°41′ N latitude, 36° 50′ E longitude and at an average altitude of 1,780 meters above sea level. According to report of the 2014 to 2015 Central Statistical Agency population and housing census, the projected total population of the town is 170,955 (Male = 85,695 and Female = 85,260) with 85,260 number of households. Generally Jimma town is characterized by climate conditions of mean annual maximum temperature 30°C; mean annual minimum temperature of 14°C and with the annual rainfall ranges from 1138 to 1690 mm. The study was conducted from June 1, 2017 to July 31, 2017.

4.2 Study design

An experimental study was conducted to determine the organochlorine pesticide residue in different brands of tea available in Ethiopian market.

4.3 Sample collection

The sample size was all the tea brands (both imported and manufactured domestically) available in Ethiopian market during the sample collection time. Purposive sampling technique was used to collect the available tea sample at the time of study. The principal investigator decided to obtain different tea brands by purposively addressing the shops and super markets. Accordingly, different shops and supermarkets found in Jimma town and Addis Ababa were addressed in order to purchase the sample.

Also the tea brands have different quality/standards and different items with the same brand. Their quality and standards are labelled on their package and easily identified by sample collectors. Then the tea brands were purchased from different shops and super markets based on their quality/standards. Thus, a total of nineteen (19) different tea brands were purchased from shops and supermarkets found in Jimma Town and Addis Ababa city. Out of them, 14 were produced domestically (Midroc, Ethiopian Green Tea, Yitem, SIMBA, WushWush, Addis Gold Label, Gumaro, Addis Normal Label, Agare, Bekur, Black Lion, Abay, Good Morning and Abyssinia)and five were imported from abroad (Kericho Gold Green Tea, Qualitea, Kericho Black Tea, English breakfast Tea and Kericho Jesmine). From domestically produced items, two samples have Export Quality.

The collected sample was transported in to Jimma University, Environmental Biology laboratory for further processing and extraction. Then, the samples were milled by using juice or coffee milling machine in order to reduce their size and sieved by using 300µm mesh size. The milled tea samples were transported in to Jimma University, Analytical Chemistry laboratory for further extraction.

4.4 Chemicals and Reagents with their analytical purity

Chemical	Purity	Manufacturer/Supplier
n-Hexane	99% for HPLC	CARLO ERBA reagents
Acetonitrile	99.9% for HPLC	Loba Chemie PVT, Ltd., Mumbai, India
Magnesium sulphate	99% Laboratory reagent	CENTRAL DRUG HOUSE (P) LTD., New Delhi, India
Sodium chloride	99.5% Laboratory reagent	TECHO PHARMCHEM, Bahadurgarh, India
Methanol	≥99.9% for HPLC	Sigma Aldrich. Co, Germany.

4.5 Pesticide standards

Nine organochlorine pesticides standard were obtained from Jimma University, Chemistry Department. All the pesticides standard were produced by PIPARK Scientific Limited, Northampton, UK and have Analytical Standard grade 97.9% and above. The standards include; DDT, dielderin, endrin, γ-Chlordane, Endosulfan Sulfate, Aldrin, Heptachlorepoxide (Isomer B), Dibutylchlorepoxide, and Metoxychlor.

4.6 Preparation of standard solution

The neck of the ampoule containing the organochlorine pesticides standard was broken and 10mg from each except Dibutylchlorepoxide (20mg) weighted into 10ml biker and dissolved in 5ml methanol by using ultrasonication (Elmasonic). Then, the mixture emptied into a 10 ml volumetric flask to prepare 1000 ppm stock solution. The prepared stock solution was stored in a deep freezer at -4 °C. For Dibutylchlorepoxide, 20mg weighted into 10ml biker and dissolved in 5ml methanol by using ultrasonication (Elmasonic). Then, the mixture emptied into a 10 ml volumetric flask to prepare 2000ppm stock solution. Intermediate solution was prepared from stock solution by taking 200µL from each stock solution except Dibutylchlorepoxide (100 µL) into a 10 ml volumetric flask to prepare 20ppm Intermediate solution.

A working standard was prepared by serial dilution of the Intermediate solution with n-hexane by the following steps:

- Prepare 1ppm from Intermediate solution (20ppm)which is 50µL of 20ppm plus 950 µL of n-hexane in vial
- Prepare 100ppb from1ppm vial which is 100µL of 1ppm plus 900 µL of n-hexane in vial
- Prepare 50ppb from100ppb vial which is 500µL of 100ppb plus 500 µL of n-hexane in vial
- Prepare 10ppb from 50ppb vial which is 200µL of 50ppb plus 800 µL of n-hexane in vial
- Prepare 2.5ppb from 10ppb vial which is 250µL of 10ppb plus 750 µL of n-hexane in vial
- Prepare 0.5ppb from 2.5ppb vial which is 200µL of 2.5ppb plus 800 µL of n-hexane in vial
- Prepare 0.1ppb from 0.5ppb vial which is 200µL of 0.5ppb plus 800 µL of n-hexane in vial
- Prepare 0.05ppb from 0.1ppb vial which is 500µL of 0.1ppb plus 500 µL of n-hexane in vial

These working standard concentrations (0.05 ppb, 0.1ppb, 0.5ppb, 2.5ppb, 10ppb, 50ppb and 100ppb) were injected into a GC-ECD instrument to obtain a chromatogram of peaks which were used to plot a standard calibration curve and to calculate LOD and LOQ.

Another working standard solution was prepared by diluting the individual stock solution in n-hexane to a dilution of 5:100 for all pesticide standards. These working standard solutions were injected into a GC instrument to obtain a chromatogram of peaks which were used to identify retention time and peak area. All preparation of the standard was done at room temperature and the solutions stored in deep a freezer until required. During analysis the working standard solution together with the sample extract to be injected on that day were removed from the deep freezer and left to thaw at room temperature before injection into the GC.

4.7 Sample Extraction and cleanup

Samples of dried and homogenized, tea leaves (2g) were weighed into 50ml plastic centrifuged tubes and left to hydrate for 30 minutes after the addition of 10ml distilled water and agitated by hand for 30 seconds. Acetonitrile (10ml) was then added and the tubes agitated vigorously for 1 minute. Magnesium sulphate (4g) and sodium chloride (1g) was then added. These salts are added to remove water from the sample. After further vigorous agitation for 1 minute, the sample tube was centrifuged (Model PLC 02, Taiwan Inc. USA) for 5 minutes at 10,000 rpm. A 1ml amount of the (upper) Acetonitrile layer was then transferred to 15 ml plastic centrifuged tube. Then n-hexane (1ml) and 20% w/w aqueous sodium chloride solution (5ml) was added. The tube was agitated vigorously for 1 minute and then centrifuged for 1 minute at 10,000 rpm. An aliquot of the (upper) organic (n-Hexane) layer was transferred to vial for injection into GC. Each sample was replicated three times according to (Agilent Technologies Publications, 2012). Figure 2.representes the diagrammatic flow of extraction and cleanup of the samples:

1. Place 2g tea samples +10ml water in a 50ml plastic centrifuged tube, shake for 30 seconds &wait 30 minutes for matrix hydration
2. Add 10ml Acetonitrile; agitate 1 minute, add 4g $MgSO4$ + 1g NaCl; agitate 1 minute Centrifuge 5 minute
3. Take1ml Acetonitrile layer in to 15ml plastic centrifuged tube, add 1ml n-hexane & 5ml 20% aqueous NaCl solution, agitate 1 minute
4. Centrifuge 5 min at 10,000rpm, Upper layer taken into auto-sampler vial

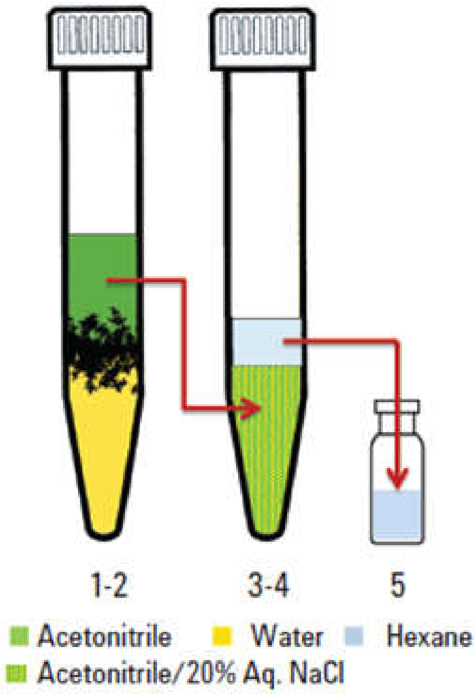


Figure 2; Flow diagram of sample extraction and clean-up regime

4.8 Pesticide identification and quantization

The determination of the pesticides under study was carried out using Gas Chromatography with an Electron Capture Detector (GC-ECD) which is the product of Agilent Technologies 7890A. An HP-5 capillary column (30mx0.25mm inner diameter; 0.25-µmfilm thickness) coated with 5% phenyl methyl siloxane (model 19091J-433; Agilent) was used in combination with the following oven temperature program: initial temperature of 80°C , ramped at 30°C min^{-1} to 180°C, ramped at 3°C min^{-1} to 205°C, held for 4 min, ramped at 20°C min^{-1} to 290°C, held for 8 min, ramped at 50°C min^{-1} to 325°C. The total GC run time was 27.92 min. Helium (99.9999% purity) was used as a carrier gas at a flow rate of 20mLmin^{-1} and nitrogen as makeup gas at a flow rate of 60mLmin^{-1}. An aliquot of 1 mL was injected in split mode at a split ratio of 10:1 and injection temperature of 280°C. The pesticide residues were detected with m-ECD operating at a temperature of 300°C. The pesticide residues in each tea sample were analyzed in triplicate, and the mean concentration was computed accordingly.

The retention times of the peaks of the sample chromatogram obtained were compared to the retention time of the corresponding peaks in the standard curves.

The sample equivalent (mg/mL) extract was calculated based on the formula suggested by (Mekonen et. al., 2014).

$$Y = \frac{a}{b} x \frac{x}{z}$$

Where,

Y is grams of sample equivalent per milliliter of extract,

a is the amount of sample analyzed (g),

b is the volume of solvent added to extract the sample (mL),

x is the amount of the cleaned extract taken for GC analysis (mL), and

z is the amount of hexane added for solvent exchange (mL).

4.9 Method validation

4.9.1 Linearity of the standard curves

All orgaochlorine pesticides were showed linearity. Linear spiked calibration curves for all the pesticides of interest were obtained with correlation coefficient >0.999.

4.9.2 Limits of detection and limits of quantification

Limits of detection (LOD) and limits of quantification (LOQ) of the method was measured in spiked serial dilution of working standards prepared for calibration curves and calculated by considering a value 3 and 10 times of background noise, respectively. Limit of detection (LOD) was determined considering it as 3 times the standard deviation. A limit of quantification (LOQ) was determined as 10 times the standard deviation.

LOD and LOQ were determined as the lowest concentrations yielding a signal-to-noise (*S*/*N*) ratio of 3 and 10, respectively.

$$LOD = 3S/N$$

$$LOQ = 10S/N$$

4.10 Recovery studies

For recovery determination, spiked tea samples at concentration levels of 1ppm was prepared in duplicates and then treated according to the procedure in sample preparation section. The recoveries were calculated using the calibration curves obtained from spiked samples. Also blank analyses was performed according to the procedure in sample preparation section to determine possible interference from the sample (Amirahmadi et al, 2013).

$$\% \text{ recovery} = \frac{\text{peak area of spiked Tea sample}}{\text{Peak area of standard}} x\ 100$$

4.11 Dietary Intake Assessment and Risk Characterization

To evaluate the toxicological significance of human exposure to the pesticide residues found in Tea, it is important to compare estimated daily intake (EDI) with the acceptable daily intakes (ADI) established by the FAO/WHO organization. The EDI was compared with the acceptable daily intake (ADI), meaning the daily dosage of a chemical which, during the entire lifetime, appears to be without appreciable risk on the basis of all the facts known at the time(WHO, 1997). Health risk estimations were done based on an integration of pesticide residue analysis data and food consumption assumptions, which aim to represent the actual residue levels in food consumed by the population, with a bodyweight of 60 kg. Tea consumption data in kg/capita/year was obtained from FAO stat which 0.058 kg for Ethiopia (FAO, 2014). Results obtained were used to calculate EDI expressed as microgram pesticides per kilogram body weight per day (µg/kg body weight/day). The EDI is a realistic estimate of pesticide exposure that was calculated for each pesticide on tea in agreement with the international guidelines (. FAO, 2002; WHO, 1997), using the following equation:

$$\text{EDI} = \Sigma C \times \frac{\text{F}}{\text{D}} \times W$$

...where

C is the mean of pesticide concentration in Tea (µgkg^{-1}),

F is mean annual intake of Tea per person,

D is number of days in a year (365), and

W is mean body weight (60 kg)

4.12 Ethical Consideration

Ethical clearance was obtained from the Ethical Review Board of Jimma University, Institute of Health Sciences. Confidentiality and privacy of the information was assured and maintained.

5. Results

A total of nine (19) different tea brands were collected from shops and supermarkets found in Jimma Town and Addis Ababa city for pesticides residue analysis. Out them, 11 brands were manufactured domestically and five brands were imported tea brands from Kenya and Sri Lanka. From domestically produced items, two samples have Export Quality. The samples were collected from June 2017 to July 2017 and were investigated for the presence of nine organochlorine pesticide residues. The investigated pesticide compounds were: Aldrin, Heptachlorepoxide, γ-Chlordane, DDT, Dielderin, Endosulfan Sulfate, Endrin, Methoxychlor and Dibuthylchlorepoxide.

5.1 Method validation results

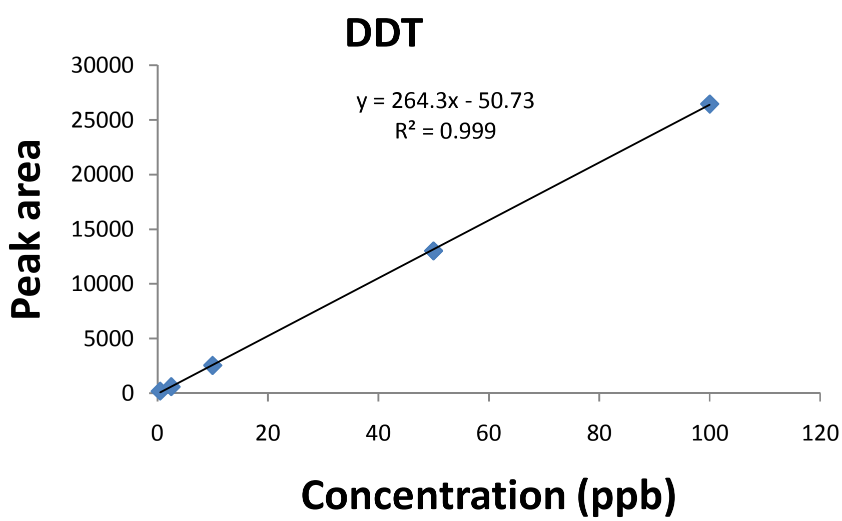
The percentage recoveries of the organochlorine pesticides were found to be acceptable ranging from % 75.87 for Endrin to 111.56% for γ-Chloridane, which indicates that the reproducibility of the method was satisfactory (Table 1). The limits of detection (LOD) and limits of quantification (LOQ) varied from 0.06-0.72 and 0.3-2.4, respectively.

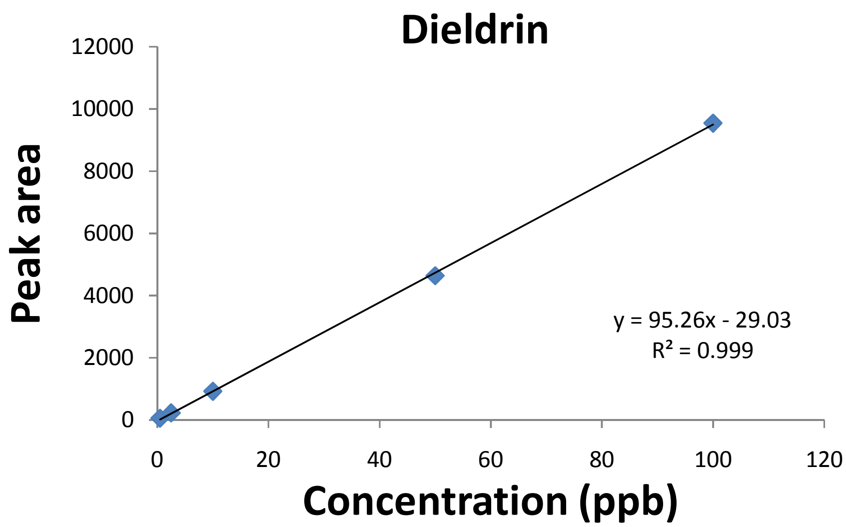
Table 1: The Percentage Recoveries and validation information of the studied Organochlorine pesticides standard

Pesticides standard	Percentage Recovery	Limits of Detection (mg/kg)	Limits Quantification (mg/kg)
Aldrin	95.57	0.10	0.34
Dibutylchlorepoxide	89.67	0.16	0.54
γ-Chloridane	111.56	0.72	2.40
DDT	102.45	0.14	0.48
Endrin	75.87	0.15	0.50
Endosulfan Sulfate	87.23	0.15	0.50
Dieldrin	78.90	0.06	0.30
Methoxychlor	82.13	0.15	0.50
Heptachlorepoxide	99.24	0.15	0.50

5.2 Linearity of the calibration curves

The entire organochlorine pesticides standard showed linearity. The calibration curves were constructed from the concentration and peak area of the chromatograms obtained from the standards of the OCP. The calibration curves were obtained by preparing serial dilution of the intermediate standard solution with n-hexane at different concentrations ranged from 0.05-100ng/ml and injecting to GC-ECD. Linear spiked calibration curves for all the interest pesticides were obtained with correlation coefficient (r^2) >0.999. The calibration curves for some of pesticide standards were presented in **Figure 3.**





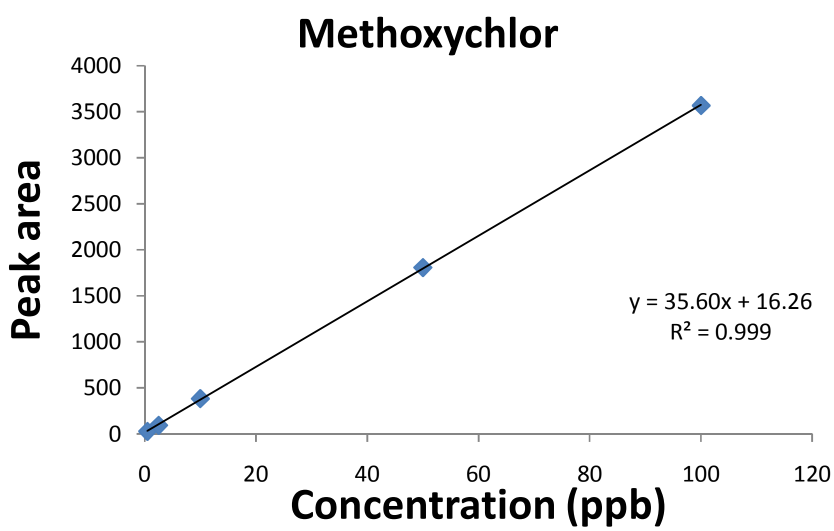


Figure 3: **Calibration curves for DDT, Dieldrin, and Methoxychlor, respectively**

5.3 Organochlorine pesticide residues

From the findings of the pesticide residues determination, a total of five organochlorine pesticide residues were detected from domestic tea samples and four organochlorine pesticide residues were detected from imported tea samples. The five organochlorine pesticide residues detected from domestic tea samples are Aldrin, γ-Chloridane, DDT, Endrin, and Heptachlorepoxide. The mean concentrations of these organochlorine pesticide residues were varied from 0.147mg/kg to 0.409 mg/kg. The mean concentration of Heptachlorepoxide was the predominant level which is 0.409 mg/kg (Table 2) and followed by Endrin and DDT with the mean concentration of 0.307 mg/kg and 0.204 mg/kg respectively. Aldrin was detected with the lowest concentration of 0.147mg/kg relative to other pesticides studied.

On other hand, four organochlorine pesticide residues were detected from imported tea samples including, Dieldrin, Endosulfan Sulfate, Methoxychlor and Heptachlorepoxide. The mean concentrations of these Organochlorine Pesticide residues were varied from 0.08mg/kg to 1.427 mg/kg. Mean level of Heptachlorepoxide in all samples analysed was 1.427mg/kg with a range of 1.222- 1.724 mg/kg in the individual samples and this is the most frequently detected Organochlorine Pesticide residues (Table 2).

The mean levels and ranges of other compounds detected in the imported tea samples analysed were: Methoxychlor with mean concentrations of 0.458 mg/kg and with a range of 0.098-1.484 mg/kg in the individual samples 0.098-1.484 mg/kg and Endosulfan Sulfate with 0.006 mg/kg and 0.067-0.733 mg/kg of mean levels and range in the individual samples respectively.

Table 2: **The concentrations of the studied Organochlorine Pesticides in domestic and imported tea samples in Ethiopia**

Pesticides	Source of Tea	
	Domestic (n=14)	Imported (n=5)
	Concentration in mg/kg (mean±SD)	Concentration in mg/kg (mean±SD)
Aldrin	0.147±0.32	0.045±0.037*
Dibutylchlorepoxide	0.112±0.162*	0.053±0.022*
γ-Chloridane	0.167±0.339	0.031±0.017*
DDT	0.204±0.2	0.031±0.022*
Endrin	0.307±0.929	0.071±0.084*
Endosulfan Sulfate	0.08±0.049*	0.258±0.316
Dieldrin	0.027±0.022*	0.08±0.049
Methoxychlor	0.098±0.124*	0.458±0.578
Heptachlorepoxide	0.409±0.102	1.427±0.262

*less than detection limit

From the total tea samples manufactured in Ethiopia and analysed in this study, about 79.58% of the tea samples were contaminated with more than one organochlorine pesticide. Out of this percent, Heptachlorepoxide was detected in most of the samples and represents more than half (26.38%) of the contaminated tea samples and followed by Endrin (19.79%), DDT (13.19%) and minimum amount of the sample was contaminated by Dieldrin (5.16%) (**Figure: 4)**. This is may be due to residues of organochlorine pesticides (OCPs) bio-accumulate in tea plants from polluted soil from agricultural applications. Bio-accumulation levels in plant tissues are greater than those in ambient environmental media such as air and water. And also there is evidence that the use of organochlorine pesticides for prevention and control malaria vector under strict condition. But they have an ability to persist in the environment for long period of time. These activities lead to the entrance of organochlorine pesticides in to food chain like tea plants.

Unlike the tea samples manufactured in Ethiopia, the imported tea samples were contaminated with high percent of organochlorine pesticide residues which is 90.58% of the samples. More than half of these samples were contaminated with Heptachlorepoxide (58.15%) and followed by Methoxychlor, Endosulfan Sulfate and Dieldrin which represents 18.66%, 10.51% and 3.26%, respectively. This difference might be due to most the time, the tea products that are prepared for export or import purpose are prepared from the tea shoot which have larger the surface area per unit weight of tea plant than that of other crops. This larger surface area has the potential to carry large amount of pesticides and lead to the presence of high pesticide residues in imported tea. Also the harvest portion of tea plant (tea shoot) is the pesticide direct applied portion. Since the tea products prepared for domestic market are processed from the tea leaves which have small surface and does not accumulate high amount of pesticides.

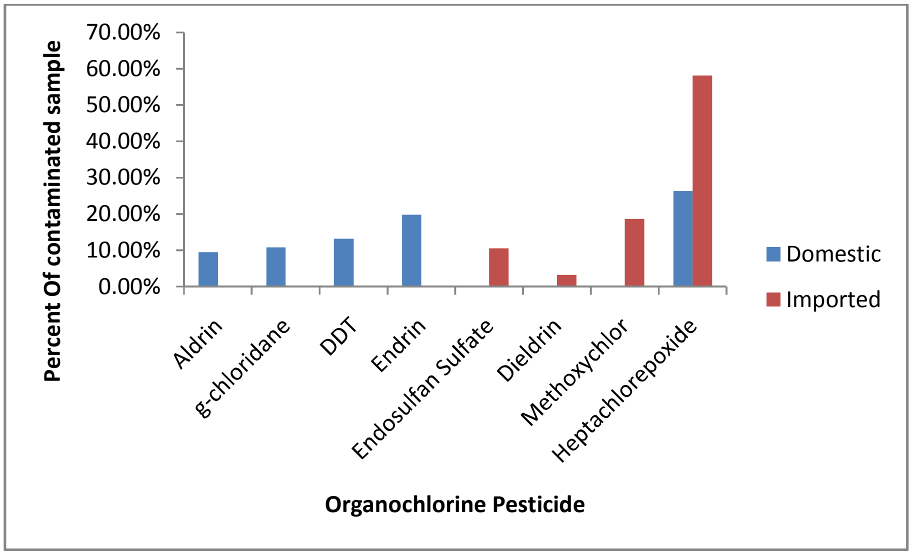


Figure 4: Contaminated samples with different organochlorine pesticides analyzed from domestic and imported tea samples in Ethiopia

The maximum residue limits (MRLs) for the organochlorine pesticides analysed in this study was presented in table 3. Most of the MRLs do not established for the organochlorine pesticides detected in this study by different International standards. Only one MRL for pesticide residue in tea was obtained from Codex Alimentarius standards and European Union which is the maximum residue limit (MRL) for Endosulfan Sulfate. The Endosulfan Sulfate residue detected in this sample was below the MRL established by both regulations. Lead to residues of an acceptable magnitude and intended to be toxicologically acceptable.

On the other hands, National food safety standard-Maximum residue limits for pesticides in tea in China established MRLs for 34 pesticides and out of them only two pesticides MRL are related to our study (China, 2015). Accordingly average concentration of DDT is above the MRLs established in China and Endosulfan Sulfate is below the MRLs established in China. This result (DDT) does not comply with the respective MRLs established in China are intended to be toxicologically unacceptable. But in order to prove sufficient consumer safety, the DDT residue is combined with cultural dietary information to estimate potential residue intake by consumers. The consumer is considered to be adequately protected when estimated dietary intake of pesticide residues does not exceed the acceptable daily intake (ADI).

Table 3: Comparisons of Average mean concentration of organochlorine pesticide residues with European Union, China and Codex Alimentareous standards

Pesticide	Source of tea		MRL ($mgkg^{-1}$)		
	Domestic (mg/kg)	Imported (mg/kg)	Codex	China	EU
Aldrin	0.147				
γ-Chloredane	0.167		ne	ne	ne
DDT	0.204		ne	0.2	ne
Endrin	0.307		ne	ne	ne
Endosulfan Sulfate	-	0.258	10	10	30
Dieldrin	-	0.08	ne	ne	ne
Methoxychlor	-	0.458	ne	ne	ne
Heptachlorepoxide	0.409	1.427	ne	ne	ne

Key: ne is not established

5.4 Dietary Intake Assessment and Risk Characterization

As indicated in table 4, the estimated daily intakes of detected pesticides like DDT and Endosulfan Sulfate were lower than ADIs, which show that tea consumption has a minimal contribution to toxicological risk for human being. In case of ϒ- Chloredane and Endrin, the estimated daily intakes were greater than ADIs, which show that tea consumption has health risk to human being. The consumer is considered to be adequately protected if the hazard index of a pesticide residue does not exceed unity. The hazard index values showed that the intakes of ϒ- Chloredane and Endrin residues remains clearly above the safe limit.

Table 4: Estimated daily intakes (EDIs) and Hazard Index (HI) of pesticide residues detected in domestic or imported tea.

Pesticide		ADI* (µg/kg body weight/day)	EDI (µg/kg body weight/day)	Hazard index HI= EDI/ADI
Aldrin		Ne	1.397	-
γ-Chloredane		0.5	1.592	3.184
DDT		10	1.949	0.195
Endrin		0.2	2.902	14.51
Endosulfan Sulfate		6	3.22	0.537
Dieldrin		Ne	0.76	
Methoxychlor		Ne	4.365	-
Heptachlorepoxide	Domestic	Ne	3.886	-
	Imported	Ne	13.605	-

[ne] not established

*Established by Codex Alimentarius Commission (Codex Alimentarius, 2017)

6. Discussion

The use of a pesticide on crops or commodities for human or animal consumption can lead to, and occasionally aims at, a residue remaining at harvest or other appropriate stage. Additionally a pesticide may move from the site of application and remain for a time elsewhere in the environment. The ability of a pesticide to persist for a certain length of time can be desirable and has been recognized as important in some situations for successful control of pests and diseases. Thus knowledge of residues of a pesticide, or arising from the use of pesticide, is useful in establishing its efficacy. However, the assessment of the human hazards arising from very small quantities of a pesticide in food and the environment has become an important part of the overall risk/benefit evaluation and is essential before a pesticide can be introduced (FAO, 1986).

In this study, from the total tea samples manufactured in Ethiopia and analysed in this study, about 79.58% of the tea samples were contaminated with more than one organochlorine pesticide. Unlike the tea samples manufactured in Ethiopia, the imported tea samples were contaminated with high percent of organochlorine pesticide residues which is 90.58% of the samples. This result is much higher than study done in Iran which reported that out of fifty three only fifteen (28.3%) samples showed contamination with pesticides (Amirahmadi et al, 2013). Also an analysis of the most recently published FDA data on residue levels in tea (black, green, and oolong) from 2008 to 2012 reveals a high rate of violations which much lower than the present study. This study also were indicated, out of the 65 samples of tea analyzed over these five years, nearly 30 percent had two or more illegal residues, with one sample from 2012 containing up to 14 violations. Of the 94 violations found in these samples, 76 were listed as “no registration” and 18 as “excess of tolerance.” Many of these violations are for pesticides that are currently used in U.S. agriculture, but lack a tolerance and presumably exposure data for use in tea, such as acetamiprid (a neonicotinoid), or permethrin (a pyrethroid) (Nikita, 2015). This variation could be due to the fact that in Ethiopia there is a high potential area in agriculture and there is a possibility of organochlorine pesticide use in the past. Another reason could be leaching and surface run-off pesticides due to most of the land of Ethiopia are rift valley.

Also the high percentage of residues in this study is similar with the study on Pesticide residues in tea samples from India, found that nearly 94% of the tea samples tested in India contained at least one of 34 different pesticides, while over half contained a toxic cocktail of more than 10 different pesticides. The report relied on tests of 49 branded and packaged teas. Eight of the top 11 companies that make up a large part of the tea market in India were represented, including Hindustan Unilever Limited, a subsidiary of the global multinational company Unilever. Popular brands included in the study are Twinings and Lipton (Greenpeace India, 2014).

In the present study DDT was detected in the domestic tea sample which also related with the study done in India that showed residues found include DDT, which has been banned for use in agriculture in India since 1989, and endosulfan, which was banned in India in 2011. In addition to registered pesticides that have been long banned from agricultural use in tea cultivation in India (DDT, and triazophos) (Greenpeace India, 2014).The most frequently detected pesticides from domestic tea in our study are Heptachlorepoxide, endrin and p,p-DDT. Also similar result reported from India that the most frequently detected pesticide is DDT (67%) (Greenpeace India, 2014).

In the current study, Endosulfan salfate was detected in imported tea a sample which is 10.51%. Different result is reported from Iran in the analysis of 53 samples in 2011 showed the presence of endosulfan sulfate in 18.9% of the tea samples with residues ranged from 5–20 μg/kg and the presence of bifenthrin in 9.4% of the samples with residues ranged from 5–35 μg/kg, these contaminated samples were lower than MRL established by regulation authorities from India and England. The difference could be attributed to variations in geographical locations, time differences in terms of the period of study and the extent of previous use of these pesticides (Amirahmadi et al, 2013).

In a study by Subbiah Seenivasan and NarayananNair Muraleedharan in 2011 a large scale survey of produced tea in the factories of south India had been carried out for a period of three years from 2006 to 2008 and 912 samples were analysed for the residues of certain pesticides such as dicofol, ethion, quinalphos, hexaconazole, fenpropathrin, fenvalerate and propargite. The analytical data showed that only less than 0.5% of samples had residues of interested pesticides. Bishnu etal, 2009 quantified the residues of 7 pesticides in tea ecosystems of Dooars regions of West Bengal, India. They found that the residues of banned pesticides like heptachlor in tasted samples may pose health hazard to the consumer. All the regulatory authorities have established the maximum residue levels (MRL) of pesticides in black/green tea since these are the commodities being traded (Greenpeace India, 2014). Also our result showed that there is high percentage of Hexachlorepoxide detected in most of the tea samples. This showed the use of illegal pesticides in our country and other part of the world.

Endosulfan was found in about 8% of tea samples in the Greenpeace India investigation, despite being banned for production, use, and sale throughout India. Although many countries have banned or severely restricted the use of most dangerous organochlorines, and pyrothroid pesticides, it is thought that many of these compounds have been or continue to be used in large quantities in some developing tropical countries for agricultural and public health reasons as control of some diseases. Presence of organochlorine residues in soils that were heavily treated years ago is suspected to be an important present day source (Greenpeace India, 2014).

In this study, most of the MRLs do not established for the organochlorine pesticides detected in this study by different International standards. Only one MRL for pesticide residue in tea was obtained from Codex Alimentarius standards and European Union which is the maximum residue limit (MRL) for Endosulfan Sulfate. According to Codex Alimentarius standards, DDT may be present in tea as a result of its presence as a contaminant in the technical Dicifol. The Endosulfan Sulfate residue detected in this sample was below the MRL established by both regulations. On the other hands, National food safety standard-Maximum residue limits for pesticides in tea in China established MRLs for 34 pesticides and out of them only two pesticides MRL are related obtained. Accordingly average concentration of DDT is above to MRLs established in China and Endosulfan Sulfate is less than the MRLs established in China. This result (DDT) does not comply with the respective MRLs established in China are intended to be toxicologically unacceptable. But in order to prove sufficient consumer safety, the DDT residue is combined with cultural dietary information to estimate potential residue intake by consumers. The consumer is considered to be adequately protected when estimated dietary intake of pesticide residues does not exceed the acceptable daily intake (ADI).

In the present study, the estimated daily intakes of detected pesticides like p,p-DDT and Endosulfan Sulfate were lower than ADIs, which show that tea consumption has a minimal contribution to toxicological risk. In case of Chloredane and Endrin, the estimated daily intakes (EDI) were graeter than ADIs, which show that tea consumption has a high contribution to toxicological risk. The consumer is considered to be adequately protected if the hazard index of a pesticide residue does not exceed unity. The hazard index values obtained in our study is greater than one which showed that the intakes of ϒ- Chloredane and Endrin residues remains clearly above the safe limit. It should be emphasized that dietary pesticide intakes estimated in this study considered only exposures from tea and did not include other food products such as grains, vegetables, fruits, dairy, fish, and meats. As such, estimates are not considered as total dietary exposure to the pesticides, nor do we consider drinking water, residential, or occupational exposures.

7. Conclusions and Recommendations

7.1 Conclusions

The data obtained from this study pertaining to the detection of pesticide residues in tea samples are probably an indication of the widespread use of pesticides in the area of study. About 79.58% of Organochlorine pesticide residues were detected from domestic tea samples. But high percent of residues (90.58%) were observed from imported tea samples, but the sample size is smaller (n=5).

In the present study, even if the residues were found in many of domestic and imported tea samples, though none of sample does exceed the MRL set by EU, and Codex. But DDT was above the MRL established in China. The estimated daily intakes of γ- Chloredane and Endrin were greater than ADIs, which show that tea consumption has health risk to human being. The hazard index values showed that the intakes of γ- Chloredane and Endrin residues remains clearly above the safe limit.

The results showed that the risk associated with exposure via tea consumption is high; a special precaution should be taken with the possible total exposure to these chemicals from various foods in the future.

7.2 Recommendations

- Tighter regulation in the production of pesticides, their sale, and application are needed as well as implementation of integrated pest management methods.
- Additionally, further monitoring studies must be performed to improve food safety and protect consumers' health.
- In our country, there are no maximum residue limits set by the concerned bodies. So, establishment of the national maximum residue limits (MRL) by the concerned bodies (like Ethiopian Food, Medicines and Health care Administration and Control Authority (EFMHACA) is necessary.;
- The lists of pesticides and their MRLs, according to the found data, at international regulations like Codex Alimentarious and Europen Union should be periodically revised.
- Strict quality control at the border of importing tea is important for the safety of the people in Ethiopia
- Tea processing like boiling can reduce the toxicity of the organochlorine pesticide residues in tea. So, further study on organochlorine pesticide residues tea infusion should be recommended.
- Also consumption survey and consumer risk assessment on residues in tea should be necessary.

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Annexes

Annex 1: Description of the tea brand samples included in the study

S.No	Type of tea	Brand name	Production/packing date	Expiry date	Quality/Standard	Company/place
1	Black tea	Black lion	-	-	Normal/ Domestic Quality	A product of East African Holdings Blended and packed in Ethiopia by: East African Agri Business PLC., Addis Ababa, Ethiopia.
2	Black tea	Wushwush	-	-	Normal/ Domestic Quality	Ethio Agri- CEFT, Addis Ababa, Ethiopia.
3[b]	Strawberry flavoured pure ceylon black tea	Qualitea	04/2017	04/2020	Imported	Qualitea Ceylon (PVT) LTD., Colombo, Srilanka
4[b]	Green tea and Jasmine	Kericho Gold	-	04/Apr 2020	Speciality teas/Gold standard	Gold Crown Beverages (Kenya) Ltd., Mombasa, Kenya.
5[a]	Black tea	MIDROC	July 2015	July 2017	Gold label tea/ Export Quality	Ethio Agri- CEFT, Addis Ababa, Ethiopia.
6	Green tea	Ethiopian green tea	01/08/2017	01/08/2018	-	Ethio Agri- CEFT, Addis Ababa, Ethiopia.

7	Black tea	Good morning	June 2015	May 2018	Normal/ Domestic Quality	A product of EAST AFRICAN HOLDINGS Blended and packed in Ethiopia by: East African Agri Business PLC., Addis Ababa, Ethiopia.
8[a]	Black tea	SIMBA	April 2017	April 2018	Export quality/ premium Gold tea	A product of EAST AFRICAN HOLDINGS Blended and packed in Ethiopia by: East African Agri Business PLC., Addis Ababa, Ethiopia.
9	Black tea	Bekur	-	-	1st level	Ethio Agri- CEFT, Addis Ababa, Ethiopia.
10[b]	Black tea	Kericho Gold	-	April 2020	Speciality teas(Gold Standard)	Gold Crown Beverages (Kenya) Ltd., Mombasa, Kenya.
11[b]	Green tea, Passion and Jasmine	Kericho Gold		June 2020	Gold	Gold Crown Beverages (Kenya) Ltd., Mombasa, Kenya.
12[b]	Black tea	English Breakfast Tea (Qualitea)	04/2017	04/2020	Premium quality tea	Packed by Qualitea Ceylon (PVT) LTD., Colombo, Srilanka
13	Black tea	ABAY Tea			Normal label	Blended and Packed in Begi/Asosa by Yonas Ethiopia
14	Black tea	Addis Tea			Gold label	Ethio Agri- CEFT, Addis Ababa, Ethiopia.

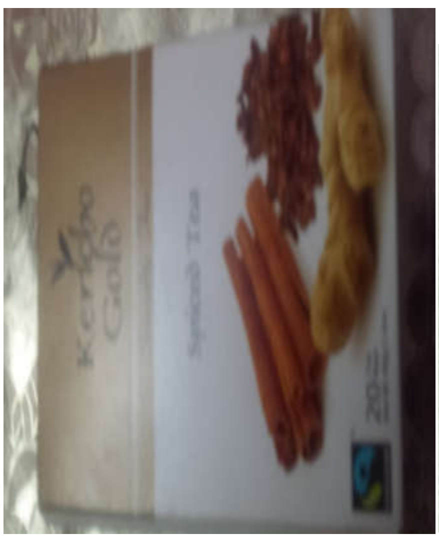
15	Black tea	ADDIS	Mar 2017	Mar 2019		Ethio Agri- CEFT, Addis Ababa, Ethiopia.
16	Black tea	ABYSSINIA Tea			Red label quality tea	Blended and Packed in Ethiopia by Great Abyssinia PLC
17	Black tea	Yitem		Mar 2018	Red label	Blended and Packed in Ethiopia by Yitem PLC
18	Black tea	Agare				Jimma, Ethiopia
19	Black tea	Gumaro				Ethio Agri- CEFT, Addis Ababa, Ethiopia.

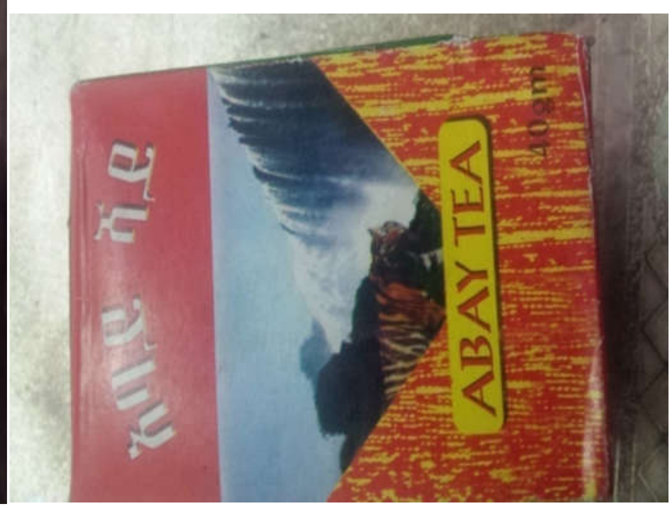
[a]indicated that domestically manufactured brands for Export Quality

[b]showed that imported tea brands from abroad.

The rest were produced for domestic market

Annex-2: Photographical image of some tea brands included in the study






Ageria Tea
BMF Tea


SIMBA
PREMIUM GOLD TEA

Highland Grown
Ethiopian Green Tea
Green Tea

Good Morning


MIDROC
GOLD LABEL TEA
BLACK TEA


Qualitea
STRAWBERRY
MIDROC
GOLD LABEL TEA
Green Tea & Jasmine

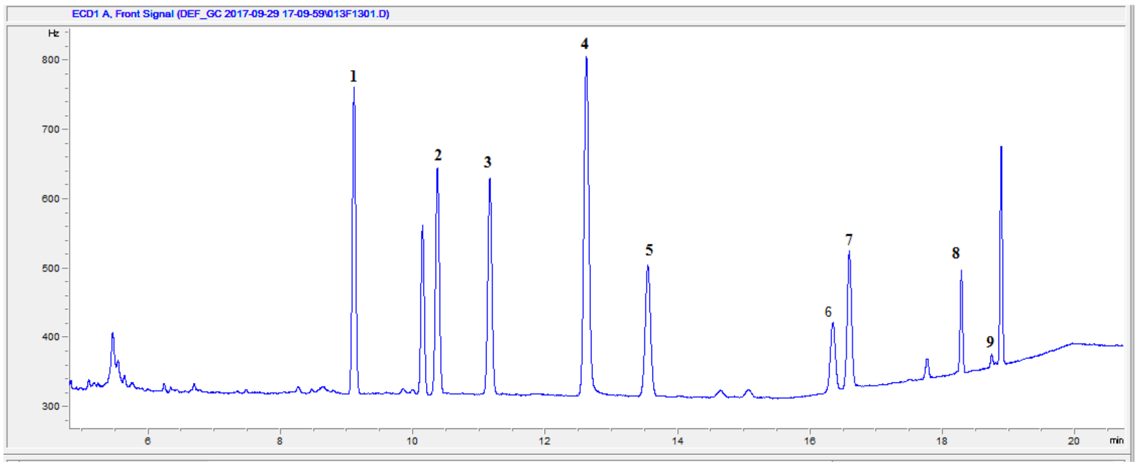

ADDIS TEA
Ethio
25


ETHIOPIAN
BLACK
LION
TEA
100 g Net


Addis Tea
Gold Label
Addis Tea
Gold label
For each cup put one tea spoonful of this high quality Addis Tea in a pot
Pour boiling water into the tea pot
Wait for two minutes stir and serve
Produced By Tea Processing and Packing Factory


HIGHLAND GROWN
WUSH WUSH TEA

Annex 3: Organochlorine pesticide standard chromatographic image



Annex 2: Chromatographic picture for tea sample analyzed