

Addis Ababa University
School of Graduate Studies



***ORGANOCHLORINE PESTICIDE RESIDUES
IN ORANGE AND TOMATO SAMPLES FROM PIASA
ATEKELT TERA, ADDIS ABABA***

By: Ashenafi Geletu

June, 2009

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University in partial fulfilment of the requirement for the Degree of
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Addis Ababa University

Dedicated to

My father Late Geletu Hayi, my Mother Tsige Lemma

And

My beloved wife Eyerusalem Muhe

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List of Abbreviations

ASE	Accelerated solvent extraction
CVDs	cardiovascular diseases
DDD	dichlorodiphenyldihloroethane
DDE	dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
FAO	Food and Agricultural organisation
FDA	Food and drug Administration
g	gram
GC-MS	Gas chromatography mass spectrometry
GDP	Grosse domestic production
GPC	Gel permission chromatography
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane
ISO	International standard organisation
LCC	Lower Calibration Curve
LLE	Liquid-Liquid extraction
LSE	Liquid Solid Extraction
mg-	mile gram
mL	mile liter

MRL	Maximum Residue limit
MSPD	Matrix solid Phase Desperation
OCPs	Organochlorine Pesticides
OPPs	Organophosphorous pesticides
PAO	BLK- Piasa Atekelt Tera Orange blank
PAO	SPK- Piasa Atekelt Tera Orange spiked
PAT	BLK- Piasa Atekelt Tera Tomato blank
PAT	SPK- Piasa Atekelt Tera Tomato spiked
PCBs	polychlorinated biphenyl
PCDD	polychlorinated dibenzo- <i>p</i> -dioxins
POPs	Persistent Organic Pollutants
QSAE	Quality Standard Authority of Ethiopia
RSD	Relative Standard Deviation
S/N	Signal to Noise ratio
SANCO	South Africa National Civic Organization
SD	Standard deviation
SPE	Solid phase Extraction
U.S. EPA	united state Environmental protection Agency
WHO	World health organisation
µg/g	Microgram per gram

Abstract

The concentration levels of ten organochlorine pesticides have been investigated in tomato and orange samples purchased from Piasa Atekelt Tera market, Addis Ababa. Sampling technique was used based on the Quality and Standards Authority of Ethiopia, standard sampling method (ES ISO874:2007) and from United Nation Food and Agricultural Organization(FAO:2002).

A standard analytical method was used for extraction of the pesticides in fruits and vegetables. Since the method was out of scope it validated before application to the analysis of these pesticides. Accordingly the limit of detection (LOD), Limit of quantification (LOQ), repeatability and reproducibility, and percentage recovery were determined. Target analytes were determined using GC-MS. The Spiked recovery results for five analytes were within the acceptable international standard . The LOD value and LOQ value are below the LCL and MRL of the analyte. Precision of the method was measured in terms of repeatability and reproducibility expressed in terms of percentage relative standard deviation (% RSD) values. The %RSD of all detected analytes were less than the acceptable international standard (<20%).

The study found that both orange and tomato samples were contaminated by the five and four type of OCPs out of ten target analyte, respectively. In orange sample g- HCH, heptachlor, aldrin, endosulfan and DDT were detected while in tomato heptachlor, aldrin, endosulfan and DDT were detected. However from the detected analytes none of them above the international MRL values.

Keywords: Organochlorine pesticide, Tomato and orange, Method validation, maximum residue limit.

1 Introduction

1.1 Overview

Several hundred pesticides of different chemical nature are widely applied to crops throughout the world. These pesticides provide unquestionable benefits for increasing agricultural production. However, fruit and vegetables usually receive direct application of pesticides in the field or in post-harvest treatment and may retain a proportion as residues in or on the edible portion delivered to the consumer [1]. Therefore, public concern about the contamination of food by pesticides has been increasing over the past years due to the uncertainty about the adverse effect of those residues may pose over a long-time exposure.

The toxicity of most pesticides and the consumption of raw fruit and vegetables reinforce the concern for contamination of these food substances over other foods [2] . As a result, levels of pesticides in different food item are regulated by international and national organizations in order to protect human health [3]. That is for the protection of the public against the toxic effects of pesticides, regulatory agencies in many countries have established standards specifying the residue levels of each pesticide in various foodstuffs. At an international level, the WHO, in conjunction with the FAO, has been convening Joint FAO/WHO Meetings on Pesticide Residues annually since 1961. At these meetings, the toxicological and related data are evaluated for the establishment of an acceptable daily intake or provisional tolerable daily intake [4].

Almost every country either imports or exports food. Most countries do both [5]. Especially many African countries do not have adequate food security, resulting in a situation where at least 60% of the food supply is imported to supplement local production. Thus guaranteeing the safety of both imported and locally produced food begins on the farm or on the port and follows through the entire food chain until meals are on the table [6].

Therefore, based on these facts this research focuses on the determination of organochlorine pesticides residue, which could contaminate fruits and vegetables harvesting in Ethiopia. This research paper including this introductory chapter, it consists five chapters. Chapter one is an introduction of the thesis which describe general frame work of the thesis, the objective of the study, Significance of the study. In Chapter two give general information about fruit and vegetable, state of contamination, description about pesticide particularly organochlorines pesticide and their classification, , pesticide use and impact , possible risk in Ethiopia and finally sample preparation and method validation. Chapter three deals with the experimental part that details about chemicals , materials and instrument used, sampling of fruit and vegetable, extraction, clean up procedure of the analysis of the target pesticide. Chapter four contain result and discussion of the research. In this part major findings of the research work are presented. Finally conclusions and recommendations of the study are presented as chapter five.

1.2 Objective of the Study

1.2.1 General Objective

The main objective of this study is to determine the concentration levels of organochlorine pesticides in fruit and vegetables and establish the pollutions status of these food products.

1.2.2 Specific Objective

The specific objectives of this research are:

- Determination of level of ten organochlorines pesticide residue in commercially available orange samples
- Determination of level of ten organochlorines pesticide residue in commercially available tomato samples

- Comparison of the residue level in orange and tomato with the international Maximum Residue Level (MRL)

1.3 Significance of the Study

Ethiopia by the account of the UN is one of the least developed countries in the world. It is the third largest populous country in Africa with a population of about 70 million. Agriculture is the dominant source of foreign currency earnings accounting to about 50% to the GDP, about 90% to foreign export earnings, and 80% to employment [7,8,9]. The country have a favourable climate, abundant labour, land and water resources, most regions of the country are suitable for the production of a wide range of tropical and sub-tropical fruits, vegetables and flowers [10].

Mean while the recent report shows that about 30%-40% crop yield has lost to pest and disease annually. This and increasing population, shrinking farm size, result in food insecurity. Therefore the government of Ethiopia considered a viable option to overcome these problems by agricultural intensification includes the increased use of pesticide [11,12]. However this agricultural intensification and the threat of pesticide in its widespread use together with very little published information on the levels of pesticides in different environmental compartments [9] make individual interventions in food safety ineffective [12]. In fact there are regulations concerning registration and use of pesticides in Ethiopia, but there are still some pesticides like OCPs in use that have been restricted in some industrialised countries [12].

In Ethiopia regulating food safety is a shared responsibility of Ministry of Health, Ministry of Agriculture and Quality and the standard Authority of Ethiopia. However there is no comprehensive food safety policy in the country, but the government of Ethiopia has issued a Public Health Proclamation No. 200/2000 in which the issue of food safety is included in this proclamation [8].

The study of the levels of Pesticide residue in food item is very limited in the country. Therefore, the contamination status of fruit and vegetables by pesticide residues at all is unknown. This calls for an extensive study of the residue status of agricultural products. To this effect the main focus of this work is to determine the organochlorines pesticide residue and its degradation products (Metabolites) levels in tomato and orange samples available in market. Thus the study will be significant to show preliminary data regarding the pollution status of the orange and tomato by ten organochlorine pesticides. In addition this research work can have the following out come;

- It serves as baseline information for the development of standards regarding maximum organochlorines residue limit in fruit and vegetable grown in Ethiopia.
- To protect the society from acute and chronic health hazard the status of contamination must be known thus this research work partly addresses this issue.
- Also it contributes for the economic development of the country. That is pesticide residues in agricultural products may also have a detrimental effect on export agricultural crops Eg . Coffee case [13].
- The research could be sources of information in education. In the aspect of analysis of pesticide residue in food matrix in general and fruit and vegetable in particular.

2 Literature review

2.1 Fruits and Vegetable

For thousands of years, mankind has depended on a few crop plants for its sustenance and survival. Wheat, rice, corn and a few cereals, potato and some legumes have provided the staple food for human. As societies developed the demand for fruit and vegetable crops increased [14].

Currently the role of fresh fruits and vegetables in nutrition and healthy diets is well recognized. In recent years many countries have undertaken various initiatives to encourage consumers to eat more of these products. This, together with increasing consumer demands for variety, availability, and the changing structure of global trade, and has led to an increase in international trade in these products. For many countries, particularly developing countries, such products have become valuable, making a substantial contribution to the economy as well as to the health of a country's population. However, recent food safety problems linked to these products threaten production and consumption of fresh produce [15].

2.2 Type and Classification of Fruits and Vegetables

Fruit is defined botanically as the ripened ovary of a seed-bearing plant that contains the seed(s). By this definition, Zucchini, tomatoes, peppers, peapods, and even the seed pods of deciduous trees are fruit. In the other way fruit is more commonly defined as the sweet, fleshy, edible part of plant that contains the seeds, excluding the non-sweet [16].

Vegetables are broadly defined as the edible portions of a plant (excluding fruit and seeds) such as the root tubers stems and leaves [17]. In other word a vegetable can be defined as the edible portion of herbaceous garden plants usually grown as annuals. With lettuce, celery, potato and carrot the edible portion is the leaf, petiole, tuber and root respectively; however it is the fruit part that is eaten with tomato, melons and cucumber [16].

When we see the classification of fruits and vegetables because mankind used hundred of thousands of plants, it is impossible to talk about each one individually. But, Plants are grouped or classified by various common characteristics such as by their use , climatic requirements, ecological adaptations, stem and leaf texture [16] genetic, morphological or the plant parts eaten etc. Based on these classification criteria vegetable are classified [18] as:

Root crops (plants grown for various fleshy underground organs), *Bulb crop* (hardy and belongs to the genus *Allium* in the lily family), *Solanaceous fruit* (the major bulb crop is onion but also include garlic, leek, shallot and chive the three major vegetable in this group tomato, pepper and eggplant are tender), *Salad crop* (include lettuce, celery escarole and a variety of miscellaneous green vegetables), *Cole crops* (cabbage, broccoli, cauliflower and Brussels sprouts), *Vine crops* (cucumber, muskmelon, watermelon, pumpkin, and squash), *Bean and peas*: (the two major type of bean used as vegetable are the snap bean and the lima bean both are legume), *Green* (spinach is the only green of major commercial) and *Perennial crop* (Asparagus, Artichoke.) [18].

The most important classification of fruit is climatic classification. ***Temperate fruit*** are deciduous and tolerate or even require a cool period each year such as *Pomes fruit* (Apple, pear, quince etc), *Stone fruit* (Peach, nectarine, cherry, and plum, apricot) and *Small fruit* (Strawberry, blueberry, cranberry, raspberry, blackberry, and grape). ***Tropical fruit and sub tropical fruit*** are evergreen can withstand no temperature below freezing and may like banana suffer chilling injury as temperature above freezing. *Citrus*: (orange, grape fruit, lemon, tangerine (mandarin)), *Avocado*, *Olive*, *Date* (palmae), *Pineapple* (Bromeliaceae), *Banana* (Musaceae), *Papaya* (Caricaceae), *Mango* (Anacardiaceae) are tropical and sub-tropical fruit [18].

2. 3 Importance of Fruits and Vegetables in Human Diet

Fruit and vegetables are an important component of a healthy diet and, if consumed daily in sufficient amounts, could help prevent major diseases such as cardiovascular diseases (CVDs)

and certain cancers. According to the WHO Report 2002, low fruit and vegetable intake is estimated to cause about 31% of ischaemic heart disease and 11% of stroke worldwide. Overall it is estimated that up to 2.7 million lives could potentially be saved each year if fruit and vegetable consumption was sufficiently increased [19].

Consuming vegetables and fruit can be dietary sources of fibre, Vitamins, proteins and protective micronutrients. The recent report of Joint FAO/WHO expert Consultation on diet, nutrition and the prevention of chronic diseases, recommended the intake of a minimum of 400g of fruit and vegetables per day [19,20]. Generally speaking fruit and vegetable are important sources of essential minerals, vitamins and fiber and energy as shown in *Table 1.1* in the human diet when eaten together with some root and leguminous crops in flavour and colour.

2. 4 Production and Consumption of Fruit and Vegetable in Ethiopia

Ethiopia has highly-diversified agro-ecological conditions which are suitable for the production of various types of fruit and vegetables. However, the contribution of horticultural crops both to the diet and income of Ethiopians is insignificant. In recent year with the aim of insuring food security the agricultural development of the country has been due attention. Among the sector that has been given attention at micro level is the production of fruit and vegetables [19]. Fruit and vegetable production in Ethiopia is scattered through out the country on patches of land in peasant smallholder farm. Where as large scale production and processing of fruits and vegetables is carried out only by state organisations [22].

The country has a variety of fruits, leafy vegetables, roots and tubers adaptable to specific locations and altitudes. Shallot, garlic, potatoes and chillies are mainly produced under rain fed conditions.

Table 2.1 Nutritive composition of some Fruits and Vegetables

Composition in terms of 100g edible portion										
Food	Energy (cal.)	Protein (g)	Calcium (mg)	Iron (mg)	Fibre (g)	β-carotene (µg)	Thiamine(mg)	Riboflavin (mg)	Niacin (mg)	Ascorbic acid (mg)
Apple	55.7	0.20	9.00	0.70	0.90	0.00	0.02	0.04	-	5.00
Banana	87.8	0.80	8.00	0.50	0.40	0.00	0.04	0.10	1.00	2.00
Orange	33.90	0.70	50.00	0.80	1.40	335.00	0.08	0.05	0.06	38.00
Lemon	48.50	0.40	41.00	0.50	0.90	0.00	0.05	0.05	-	51.00
Avocado	110.10	1.60	13.00	1.70	3.10	0.00	0.06	0.11	0.80	13.00
Grapes	49.9	0.60	31.00	2.40	0.70	0.00	0.07	0.05	0.05	32.00
Cabbage raw	20.50	0.90	43.00	0.80	0.90	0.04	0.04	0.01	-	9.00
Carrot raw	42.00	1.70	31.00	1.30	1.00	4780.00	0.04	0.03	0.05	3.00
Tomato raw	30.7	1.30	9.00	0.90	1.50	620.00	0.06	0.05	0.50	29.00
Onion	71.30	1.06	41.00	0.80	0.80	0.00	0.06	0.02	-	7.00

Source: EHNRI food composition table III [21]

Tomatoes, carrots, lettuce, beetroot, cabbage, spinach and Swiss chard are usually restricted to areas where irrigation water is available [23]. According to the CSA of Ethiopia the 2007/2008 annual of fruit and vegetable production by private peasant holding are described in table 1.2 above.

On the other way consumption of fruits and vegetables in Ethiopia is low compared to developed countries [24-26]. The main reason for the low consumption of fruit and vegetables is the eating habit of the society.

The Ethiopians eating habits are broadly categorized in to four types. The predominant food of 'injera and wat' which is consumed in the urban central part of the country. The root crops like potatoes, sweet potatoes, yams and enset consumed the south central part of the country. The diet of low landers which is mainly based on dairy products with some maize and or sorghum. The big towns and their surroundings which is the mixture of foreign dishes and the high landers staple food, injera and wat [23]. The average annual per capita consumption of horticultural crops estimated to be 48 kg for the rural and 37 kg for the urban population [23].

This low level of per capita consumption is accounted by the traditional eating habit of the population, the low level of per capita income, the generally high price level of fruits and vegetables, the lack of knowledge about certain types of vegetables and the supply shortage of some preferred vegetables [23].

Table 2.2 Area, production and yield of Horticulture crops for private peasant holdings for Meher Season 2007/2008(2000E.C)

Crop	Area in Hectare	Production in quintal	Yield (qt/ha)
Vegetables	119,091.43	4,719,664.46	
Ethiopian Cabbage	28,470.69	2,383,602.95	83.72
Tomatoes	4,800.07	338,380.91	70.49
Green peppers	7,952.35	623,209.04	78.37
Red pepper	75,340.93	1,223,996.86	16.25
Root crops	184,329.45	15,309,489.12	
Beetroot	1,840.47	169,479.87	92.09
Onion	18,012.55	1,751,061.71	97.21
Potatoes	50,488.00	4,025,080.08	79.72
Sweet potato	62,357.65	5,264,870.43	84.43
Fruit crops	62,731.42	4,621,475.23	
Avocado	6,473.22	428,292.20	66.19
Banana	39,425.90	2,610,592.27	66.22
Orange	3,396.81	428,072.76	126.02
Pineapples	86.74	448.95	5.18

Source: CSA of Ethiopia [10]

2. 5 Export capacity of Fruit and Vegetables in Ethiopia

Agricultural commodity export is almost the only source of export earnings for Ethiopia. Commercial horticultural crop production is carried out mainly in the central rift valley and eastern part of the country. Most of the vegetables and fruit produced in the eastern region are exported to Djibouti and small amounts of fruit and vegetables are also exported to Europe, Pakistan, Saudi Arabia and Yemen [19, 27].

When we see the commodity specific export performance of Ethiopia, coffee is largest share in the total exports value. Chat is the second largest agricultural commodity. The export of hide and skin share in the total export income became the third. Oil seeds, pulses and cereals rank fourth, fifth and sixth respectively in the total share. Fruits vegetables and flowers appeared to be in the seventh place [27].

Recently, promoting the production and export of horticultural products (fruits, vegetables and flowers) has caught the attention of the federal government of Ethiopia. Yet, the share of horticultural export income is one of the lowest in the total export earnings. But there is a tendency of positive growth in the export of this sub sector, i.e., from 0.80% of the total export value in 1997/1998 to 2.21% in 2001/2002 [27].

To enhance the export capacity of fruits and vegetables one of the required quality is the degree of their contamination with hazardous chemicals.

2. 6 Contaminant of Fruit and Vegetables

Food contamination is an ongoing public concern. The contamination can take place at any point between the fields where food is grown and food consumption. Fruits and Vegetables are susceptible to insect and disease attacks easily when it is in the farm and in store. Thus to protect from damage pesticides are widely used. However some of the pesticides have residual effect and may pose serious threat to the health of the consumers [28]. Generally in any food items including fruit and vegetable three categories of hazards are known. These are biological, physical and chemical hazards [29].

Many of the problems involve microbiological contamination incidents, which in most cases are preventable. Most microbial contamination of leafy greens and fresh vegetables is stated to be associated with improperly composted manures, irrigation water containing manure or

sewage, contact with domestic animals, wild animals, or contamination during packaging, slicing or shredding, and food preparation [29].

Physical hazards are the second category of food contaminant. In which foreign materials that can lead to injury or serve as a carrier of a microorganism. The more common physical hazards are metal, glass, wood, rocks, insects and worms, and in some situations dirt [30].

The third food contaminant are chemical contaminants .This type of contaminant have special concern due to widespread occurrence and potential for toxic effects. It also includes metabolites of past-use chemicals and mixtures. These classes of contaminant are classified mainly into five categories that are persistent organic pollutants, pharmaceuticals, inorganic, nanotechnology particles, and radioactive [31].

In recent years, contamination of surface and groundwater by pesticides has been recognized as a serious and growing problem in agricultural regions [32].On the other hand fresh fruit and vegetables are grown, harvested and handled under a wide range of climatic conditions, using a variety of agricultural inputs and technologies on a wide range of farm sizes. As a result chemical hazards identified in fruit and vegetable production are due to incorrect applications by the producer; also it can occur via run-off during floods or from contaminated soil or water [30].

Pesticides contaminate fruit and vegetables during different phases of production. Some pesticides are used before blooming, some while fruit are growing, and others after harvesting. Post-harvest pesticides are the major source of synthetic pesticides in food [33, 34]. Thus in each production unit or area it is necessary to consider the specific agricultural practices that promote the safety of fresh fruit and vegetables [35]. Current report indicate that some pesticides like OCPs categorized as persistent organic pollutant.

2. 6.1 Persistent Organic Pollutants

Persistent Organic Pollutants (POPs) are organic compounds that, to a varying degree, resist photolytic, biological and chemical degradation. POPs are characterized by low water solubility and high lipid solubility, leading to bioaccumulation in fatty tissues. Pesticides now classified as POPs started to be used on a large scale after World War II in agriculture and for disease vector control [35]. There are twelve POPs known as the dirty dozen that have been selected under Stockholm Convention in 2001. Eight of them are pesticides, such as Aldrin, Chlordane, DDT, Dieldrin, Endrin, Heptachlor, HCB and Toxaphene; PCBs, mirex as well as certain by-products such as polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzo-*p*-furans [36].

These chemicals are also known for their potential of long range transport. They are not contained within the geographic area of their release or application. The substances generally attach themselves with such media as air, water, soil, sediments etc. to circulate around. POPs chemicals even take the agency of migratory species such as birds and mammals to travel long distances as the animals move into their seasonal abode. This makes the problems they cause transboundary in nature [37].

2.7 Pesticides History and Uses

Pesticide means any substance intended for preventing, destroying, attracting, repelling or controlling any pest including unwanted species of plants or animals during production, storage, transport, distribution and processing of food, agricultural commodities or animal feeds or which may be administered to the animals for the control of ectoparasites. The term includes substances intended for use as a plant growth regulator, defoliant, desiccant, fruit thinning agent and substances applied to the crops either before or after harvest to protect

the commodity from deterioration during storage and transport. The term normally excludes fertilizers plant and animal nutrients, food additives and animal drugs [38,39].

In history it is known that chemicals have been used to kill or control pests for centuries. The Chinese used arsenic to control insects, the early Romans used common salt to control weeds and sulphur to control insects. In the 1800s pyrethrin was found to have insecticidal properties. Another material developed for insect control in the 1800s was Paris green, a mixture of copper and arsenic salts. Fungi were controlled with Bordeaux mixture, a combination of lime and copper sulphate [40].

However, it was not until the 1900s that the compounds we identify today as having pesticidal properties came into being. Petroleum oils, distilled from crude mineral oils were introduced in the 1920s to control insects and red spider mites. In 1940s saw the introduction of the chlorinated hydrocarbon insecticides such as DDT and the phenoxy herbicides were created to eradicate the Japanese rice crop, and later used as a component of Agent Orange to defoliate large areas in jungle warfare. After World War II these chemicals have been providing enormous benefits for increasing agricultural production [41,42].

2. 7.1 Use of Pesticides

Pesticides have numerous beneficial effects these include crop protection, preservation of food and materials and prevention of vectorborne diseases. Thus, from their importance the application of pesticide basically grouped in to two form that is, for agriculture pest control and for public health programmes.

Pesticides are used in areas of agriculture, horticulture, fish farming, forestry, homes and gardens, food and commodity storage, animal husbandry, etc for pest control. Several insect and other arthropods, fungi, molluscs, and bacteria attack crops and result in quantitative

and qualitative losses. Globally, the use of synthetic pesticides has increased rapidly in the last fifty years due to intensification of farming in order to obtain higher yields [42].

Pests do not affect only the quantitative yields of the crops also they affect the public health. Many of the most important human disease in the tropics are transmitted by vectors or intermediate hosts, such as insects or molluscs that can be killed with insecticides and molluscicides. A WHO study show that the greatest demand for pesticide in urban vector control was for insecticide [42].

2. 7.2 Classification of Pesticides

Pesticides can be broadly classified according to their *intended target pest* and also by their *chemical structure and properties* [43]. Based on the intended target pest it is classified as *Herbicide*: weed control in agriculture, right-of-ways, and gardens designed to kill plants rather than animals, *Fungicides*: used extensively in agriculture and around homes to control molds and mildew, *Fumigants*: Used to sterilize soil and in structural pest control and etc [44, 45]. In this research the main focused pesticide was insecticide it could have a potential to contaminate fruit and vegetables.

Insecticides are a chemical used specifically to kill or control the growth of insects. Mainly it is classified as; *Organophosphate (OPs)*: are currently the most widely used class of insecticides. Introduced to replace organochlorines, they are generally shorter lived in the environment but more acutely toxic. Some commonly used organophosphates include malathion, methyl parathion, chlorpyrifos, and diazinon. *N-methyl carbamates* are often grouped together with OPs because they act similarly. Which is active against a relatively narrower range of target organisms than the organophosphates, but they are highly toxic to such beneficial insects as honeybees. Common N-methyl carbamates include aldicarb and carbaryl [46,47].

The other types of insecticide are *synthetic pyrethroid* which used primarily in structural pest control and agriculture, function much like organochlorines. However they are fairly short-lived in the environment and are less acutely toxic to humans. Typical pyrethroids include permethrin, cypermethrin, cyfluthrin, lambda-cyhalothrin [45]. As it described above the major study area of this research were contamination of fruit and vegetables by insecticide. From this class of pesticide particularly organochlorine insecticide were explore as the contaminant of fruit and vegetables.

2.7.3 Organochlorine Pesticides (OCPs)

Organochlorines (OCPs) are chlorinated organic compounds used predominantly as insecticides. It is mainly classified into three categories; namely **diphenyl aliphatics** such as DDT and its metabolite, **cyclodienes** which includes aldrin, dieldrin, endrin, heptachlore, endosulfan, and endosulfan sulfate. **Hexachlorocyclohexanes** exist in several structural isomers such as α -HCH, β -HCH, γ -HCH, and δ -HCH are the most known common organochlorine insecticides [48].

These pesticides are typically very persistent in the environment, and are known for accumulating in sediments, plants and animals. Most of them break down slowly and can remain in the environment long after application and in organisms long after exposure [49].

Organochlorine pesticides are broad spectrum insecticides active against a great variety of pests. They vary in their chemical structures. The OCPs and their metabolites are mainly classified into three categories; namely diphenyl aliphatics, cyclodienes and hexachlorocyclohexanes [48].

i. Diphenyl aliphatics include *p,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD and methoxychlor. DDT successfully controlled spreading of malaria, a disease still plaguing large parts of the human population in Africa, and crop destroying insects [50].

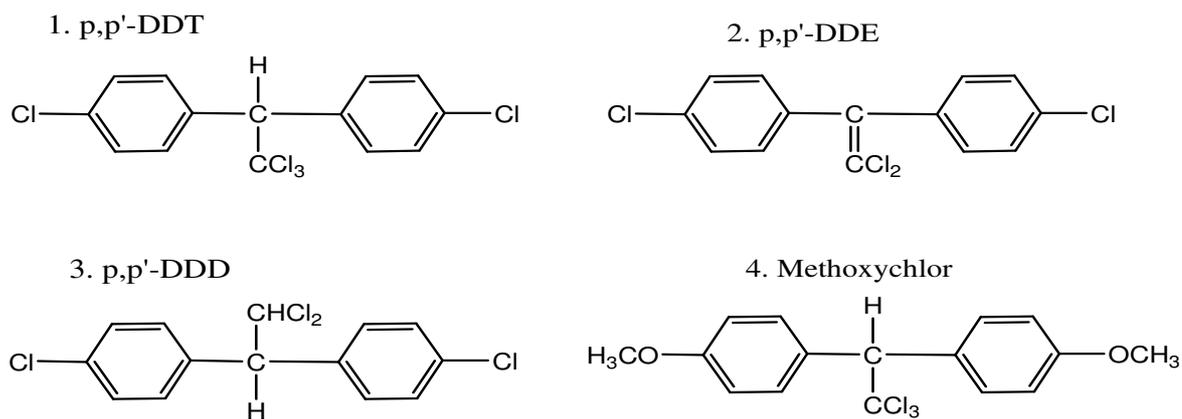


Fig. 2.1 structure of DDT and its metabolite

ii. Cyclodiene compounds are collective group of synthetic cyclic hydrocarbons, which includes aldrin, dieldrin, endrin, endrin aldehyde, endrin ketone, heptachlore, heptachlor epoxide, α -endosulfan, β -endosulfan, and endosulfan sulfate. They are particularly effective where contact action and long persistence is required; for example endosulfan acts as a contact poison on sucking, chewing and boring insects of field crops [50].

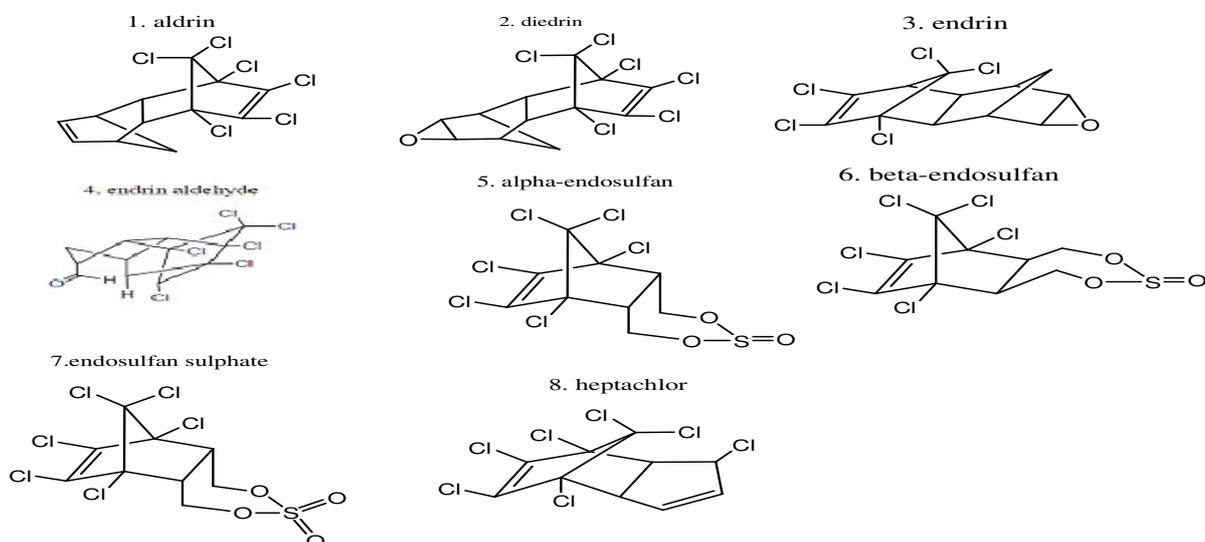


Fig. 2.2 Structure of cyclodienes pesticide

iii. Hexachlorocyclohexanes (HCH) are manufactured chemicals that exist in several structural isomers such as α -HCH, β -HCH, γ -HCH, and δ -HCH. Only one of these forms, γ -HCH (commonly called lindane) has insecticidal activity and used as an insecticide on

fruits, vegetables, in forestry and animal husbandry. Lindane is also used as active ingredient in lotion, cream, or shampoo to treat head and body lice, and scabies [50].

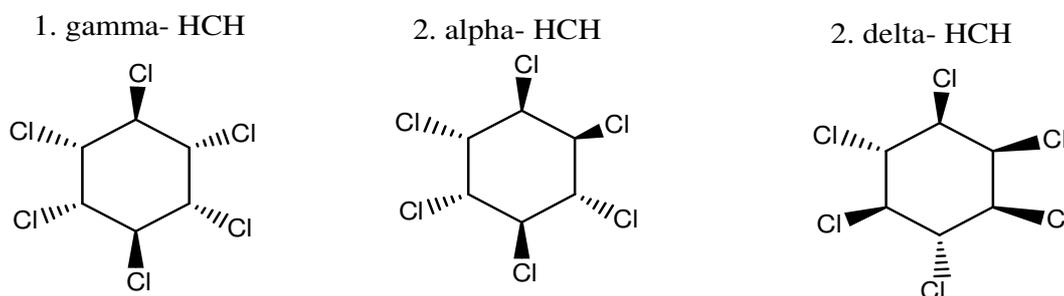


Fig 2.3 Structure of Hexachlorocyclohexanes pesticide

2.7.3.1 Properties of Organochlorine Pesticides

Organochlorine pesticide involve organic molecule that contain several halogenated atoms. They are chemically stable and do not degrade in environmental conditions. OCPs are fat soluble. Most have a $\log P_{ow}$ over 5 (Aldrin, DDE, DDT, Dieldrin, Endrin, and heptachlor) and the HCH isomers have a $\log P_{ow}$ in the 3-4 range. P_{ow} Noted that a connection between hydrophobicity and fat soluble partition coefficients (n-octanol/water). The ratio is reported as a logarithm ($\log P_{ow}$) that can be considered a quantitative measure of the hydrophobicity of a compound [51].

Organochlorine (OC) contaminants generally have chemical characteristics such as non-polarity, lipophilicity, and low volatility due to their multi-chlorinated structure. The following tables are shown some of the physical properties of OCPs [51].

Table 2.3 Physico-chemical properties of representative OCPs

			Solubility					
Pesticide	M.Wt.	M.P (°C)	At 25 ⁰ c in water (mg/L)	Benzen e (g/L)	Acetone (g/L)	Hexane (g/L)	Methano l (g/L)	Logp _{ow} (g/L)
Aldrine	364.93	104-105	<0.05	3500	1590	980	90	5.0-7.4
P,p'- DDE	318.00	—	—	Soluble	Soluble	Soluble	Soluble	5.69-6.09
P,p'-DDD	320.00	112	—	Soluble	Soluble	Soluble	—	—
P,p'-DDT	354.51	108.5-109	1.2X10 ⁻³	770	500	—	40	6.19-6.91
Dieldrin	380.93	176	0.19	400	220	—	10	4.32-5.40
a- Endosulfan	406.96	109.2	0.32	—	—	24	—	—
b- Endosulfan	406.96	213.3	0.33	—	—	24	—	—
a-HCH	209.8	—	—	—	—	—	—	3.78-3.81
g-HCH	209.8	112.5-113.5	10	289	435	—	74	3.66-3.72
Heptachlor	373.34	95-96	0.056	750	1060	—	—	5.27-6.06

M. Wt : Molecular weight, M.P: melting point, Logp_{ow}: partition octanol- water logarithm

2.7.3.2 Fate of Organochlorine Pesticides in the Environment

Organochlorines are noted for their persistence, bioaccumulative and toxicity characteristics in the environment. Due to their widespread use, these compounds are detected by determination of their residues in various environmental matrices such as water, air, sediments, soil, vegetation and biota. An organochlorine pesticides (OCPs) residue reaches the aquatic environment through direct run-off, leaching, equipment washing and careless disposal of empty containers etc [52]. For instance DDT and its metabolites are persistent in the environment and resistant to complete degradation by microorganism, although photochemical degradation does occur.

2. 8 Impact of Pesticide

Pesticides are used to control or eliminate pests, weeds, fungi and other unwanted species in the agricultural system and in public health program. However, when they are applied, even non-target organisms, the environment they are in and the users are also affected. The overuse and misuse of pesticides are detrimental to the health of users, consumers and the environment [53].

The use of large quantities of pesticides has affected the atmosphere, water body, soil, ecosystem resulting in the destruction of fauna and flora and pollution of the environment. Some report from FAO says many countries in Africa stocked pesticide (aldrin, chlordane, DDT, dieldrin and heptachlor) in certain areas and these became waste dump sites [53].

The other main impact is to human health whether contact with pesticides is direct or indirect. There are many ways that man may be directly exposed to pesticides. Population groups directly exposed to pesticide manufacturers, formulators, mixers, applicators, suicides and mass poisoning. Exposure to pesticides has been documented to cause health problems and defects such as, attention deficit and hyperactivity disorders in children, birth defects, brain damage, cancer, chronic neurotoxic effects, infertility, miscarriages, and Parkinson's [53]. Indirect impacts of pesticides on humans include consumption of food contaminated with pesticides as well as contact with pesticide residues in the air, water, soil, sediment, food materials, plants and animals [53].

When the indirect impact of pesticides are discussed mainly pesticides residues are point out Pesticide residue means any specified substance in food, agricultural commodities, or animal feed resulting from the use of pesticide. The term includes any derivatives of a pesticide, such as conversion products, metabolites, reaction products and impurities considered to be of toxicological significance.

Crops treated with pesticides invariably contain small amount of these chemicals and the hazard depends on the amount of pesticide residues that remain on the crop and their toxicity. The amount of the residue that may remain on the crop or commodity depends on the nature of the pesticide, crop, cultural practices and various other environmental conditions under which the crop is grown or a treated commodity is stored [45].

Pesticide residue research supports the establishment and control of safe levels of pesticides in food. It is important not only for trade purposes but also for ensuring human health. For this reason, maximum residue levels (MRLs) are set in order to ensure appropriate agricultural practices [54].

Maximum residue Limit is the maximum concentration of a pesticide residue (expressed as mg/kg), permitted in or on food commodities and animal feeds. MRLs are primarily a check that Good Agricultural Practice is being followed and to assist international trade in produce treated with pesticides. MRLs are not safety limits and exposure to residues in excess of an MRL does not automatically imply a hazard to health [55].

2. 9 Pesticides use in Ethiopia

Over 85 percent of Ethiopia's population live in rural areas and depend on agriculture for food and other basic necessities. Population growth and land degradation contribute most to the increasing risk of food insecurity and famine in Ethiopia. On top of these obvious factors, the average crop loss due to pests was estimated to reach between 30 and 40% annually. These problem and agricultural intensification increased use of pesticides. Actually chemical pesticide use in Ethiopia was historically low, recent developments in increased food production and expansions in floriculture industry have resulted in higher consumption of chemical pesticides [56].

Pesticide use in Ethiopian State farms is estimated at 7.76kg/ha/yr, and less than 0.1kg/ha/yr in smallholder farms. Cotton on commercial farms uses 90% of imported insecticides. Government extension services promote packages of chemical inputs to improve the productivity of smallholder agriculture and achieve food security [11].

The Pesticide Registration Council of Ethiopia has registered a total of 171 pesticides consisting of 86 insecticides, 45 herbicides, 22 fungicides and 18 miscellaneous groups [57]. Of these, 159 are currently in use. The largest proportion of pesticide use in Ethiopia has been for the control of bollworms and other pests such as the cotton aphid and the cotton whitefly in cotton. Horticultural crops such as vegetables and fruits have also become heavy users of pesticides in recent years [58].

In fact Ethiopia is signatory to the Stockholm Convention on POPs (ratified through Proclamation No. 279/2002) and other international conventions such as the Rotterdam Convention (ratified through Proclamation No. 278/2002). The country also has pesticide regulations enacted in 1990 through a Special Decree No. 20/1990. Pesticide registration process has been effective but enforcement of other regulations has not moved as well as expected due mainly to lack of trained personnel, facilities and inadequate funding [58].

But POPs in general Organochlorine pesticides in particular pollution in Ethiopia is anticipated mainly from two sources namely obsolete pesticides and agricultural uses. These are significantly affected the ecosystem and the health of the people [50].

Pesticide which is allowed to use for public health purpose some people uses it for various purposes. For instance DDT is banned for use for agricultural purpose but recent survey conducted in the Rift Valley revealed that DDT is used as insecticides by farmers. And also their is information that has farmers spraying DDT on their fields. It was also observed that DDT is openly displayed in shops for sale [56]. Pesticides present in large amounts as

obsolete stockpiles include organochlorines, organophosphates, and pyrethroids. The information from the African Stockpiles Project shows that there is more than 350 metric tonnes of obsolete stockpiles of POPs present in Ethiopia as shown in table5 [58].

Table 2.4: obsolete stockpiles of POPs present in Ethiopia

Name of POP	Amount (MT)
BHC Oil	83.6
DDT	75.7
BHC	61.8
HCH	60.7
Dieldrin	32.9
Chlordane	26.1
Aldrin	7.3
Heptachlor	3.2
TOTAL	353.1

Source: Oljira (2006) as cited by IPM research project: Pesticide *Action Network UK* [58]

2. 10 Methods of Analysis OCPs in Food Samples

Most analysis is done by taking a part of the object under study and analyzing it in the laboratory (or at the site). The first step is sampling, where the sample is obtained from the object to be analyzed. The sampler is collected and should represent the original object [59].

A number of different styles of sampling are utilized and their choice depends on the nature of the bulk material, the system investigated, and the purpose of analysis. *Random sampling* is the bulk material is made from a large number of equally sized and discrete portions, a random sample resulting... if each portion has an equal chance of being selected. *Systematic sampling* is achieved by taking portion of the bulk material on a regular basis in space or

time. *Stratified sampling* is executed when the bulk material is distributed in a zone of priority, either in reality or schematically [59].

The next step is sample preservation. Sample preservation ensures that the sample retains its physical and chemical characteristics so that the analysis truly represents the object under study. Once the sample is ready for analysis, the next step is sample preparation for the reason that most samples are not ready for direct introduction into instruments [59].

Especially determination of trace contaminants in complex matrices, such as food, often requires extensive sample extraction and preparation regimes prior to instrumental analysis. The typical steps within sample preparation as shown in fig.5 For the determination of trace organics, the final analysis is invariably achieved using a powerful separation technique, typically chromatographic, combined with an appropriate detector [60].

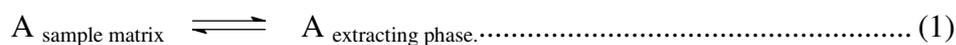
2. 10.1 Extraction of the Sample

Food samples cover a wide range of physical types from dry powders to biological matrices, such as meat, fats and liquids or solutions thus before the residues were determined, samples required extraction and purification, and in this respect, Solid-phase extraction (SPE) involves a liquid–solid partition, where the extracting phase is a solid sorbent (C8 and C18 bonded phases on silica)and it has been used extensively to remove and concentrate trace organic materials from liquid samples or solutions [61,62,63]. Accelerated solvent extraction (ASE) is an extraction technique that speed the extraction process and reduced the total amount of solvent used. The system use conventional liquid solvents at elevated temperature and pressure, which result in increasing extraction kinetics [63]. Matrix solid phase dispersion (MSPD) it provides both a porous structure to enable the solvent to penetrate the matrix and extract the analytes. The sample is mixed with a matrix, such as C18 bonded

silica, sodium sulphate or Hydromatrix, followed by washing and elution with a small volume of solvent. And liquid–liquid extraction (LLE) served as common methods [64].

2. 10.1.1 Liquid Partition Extraction

This includes liquid–liquid, liquid–solid or sometimes liquid–gas partitioning systems. Liquid partitioning means the transfer and distribution of soluble analytes in a liquid-containing phase system. *Liquid–liquid extraction (LLE)* transfers target analytes from a liquid matrix into another immiscible liquid-phase according to solubility difference. It is governed by the equilibrium distribution/ partition coefficient [65].



Where; A represents the target analyte.

The distribution Coefficient, K_d , is equal to the analyte ratio in each phase at equilibrium.

$$K_d = \frac{[A_{\text{Extracting Phase}}]}{[A_{\text{Sample matrix}}]} \dots\dots\dots (2)$$

Where; $[A_{\text{extracting phase}}]$ is the concentration of A in the organic phase at equilibrium and

$[A_{\text{sample phase}}]$ is the concentration of A in the sample at equilibrium (aqueous Phase).

The most classical extraction is performed in separating funnels to extract analytes from an aqueous biological or environmental solution into a non-polar or less polar organic solvent. *Liquid–solid extraction (LSE)* is used to isolate analytes from a solid or semi-solid matrix into solvents by liquid–solid-phase partitioning and/or desorption, which can simply be performed by stirring a solid sample in a hot or cold solvent. *Liquid–gas extraction* is widely used to capture atmospheric pollutants by dynamic or passive techniques. Sampling and preconcentration of analytes are often integrated in one step [66].

The LLE technique was selected among the methods for routine analysis of pesticide residues because it is the most commonly used extraction method with the advantage of low cost and non-specific instrumentation demands [67].

2.10.2 Clean up

Cleanup refers to a step or series of steps in the analytical procedure in which the bulk of the potentially interfering coextractives are removed by physical or chemical methods. During extraction, the solvent comes in contact with the substrate matrix, to enable extraction of the pesticide along with some of the constituents of the substrate matrix also get solubilized. The removal of these interfering coextractives from extract is called clean up. Coextractives are removed by using various separation techniques such as: Liquid-liquid partitioning, Chemical Treatment, Chromatographic techniques, Solid phase extraction cartridges [68].

2.11 Method Validation

The FDA in its most recent publication, Guidance for Industry on Analytical Procedures and Methods Validation, states that “Method validation is the process of demonstrating that analytical procedures are suitable for their intended use.” The methods validation process for analytical procedures begins with the planned and systematic collection by the applicant of the validation data to support analytical procedures [69]. The validation procedure investigated the following parameters: analytical curve and linearity, detection limit and quantification limit, precision (repeatability and intermediate precision) and accuracy [70].

3. Experimental

3.1 Materials and Methods

3.1.1 Fruit and Vegetable Samples

Samples for the study were obtained from fruit and vegetable market found in Piasa Atikelt Tera centre of Addis Ababa, Ethiopia. The samples were purchased in the month of March, 2009.

Prior to the purchase of the samples the markets were assessed for the suitability of proper sampling and the merchants from Piasa Atkilt Tera were orally interviewed on the origin of their tomatoes and oranges.

Tomato samples were purchased, first by selecting the shops using convenient sampling technique. For purchasing maximum of 6 kg of tomato, three shops were sampled from the 15 shops in found one street. Then, the samples were purchased from shops found at 5th places after starting randomly at the shop found at 3rd place. The selected shops were shop no. 3, shop no. 8, and shop no. 11. Six kilogram of tomato was purchased from these three different shops.

The Orange samples were obtained from ETRUT main distribution shop it is found at Piasa Atekelt Tera according to the sampling method set by the QSAE and FAO [71,72]. Then the samples were packed with polyethylene Plastic bag and were kept in a refrigerator for a Week until sample preparation started for the analysis.

Before sample preparation the samples were further stratified by the size (weight) into three portions as large, medium and small to make homogeneous sample. Then about 300g to 350g were taken from each portion to make a sub-sample of 1 kg.

3.1.2 Chemicals and Materials

3.1.2.1 Chemicals

i. Pesticide Standards

Organochlorine pesticide standards (α -HCH, γ -HCH, Endosulfan, P,P-DDE, P,P-DDT, P,P-DDD, Dieldrin, Aldrin, Chlordane and Heptachlor) were obtained from Dr. Ehrenstorfer Gmb H-Bgm. (Augsburg-Germany)

ii. Standard OCPs Solutions Preparation

Pesticide stock solution of 1000 mg/ L was prepared by dissolving 25 mg of each pesticide in 25ml of acetone. Then appropriate mix of each pesticide standard was made to prepare 10 mg/L concentration of the mixture. The rest of the pesticide working solutions were prepared by dilution of 10 mg /L stock solution to appropriate volumes.

iii. Organic solvents and reagents

Acetone, n-hexane, diethyl ether, and sodium chloride of Analytical grade were obtained from Sigma–Aldrich (Steinheim, Germany). Other necessary materials were Na₂SO₄ (99.0–100.5%, Merck, extrapure), Florisil adsorbent (60–100 mesh, Merck, residue grade), Diatomaceous earth was obtained from BDH chemical Ltd Poole England.

Florisil was activated by taking 1 kg of it reflex in 2.5 L of distilled water for one hour. After repeating the process two times, the water was decanted through Buchner funnel and dried in an oven at 130°C for two hours. Partially dried florisil were transferred to a crucible and kept in muffle furnace at 660°C for 3 hours. Then it was stored in glass containers. Anhydrous sodium sulfate was also activated by heating at 600 °C in a furnace for 4 h and it was stored in glass container. Purification of Celite was done by making slurry with 6M HCl while heating on a steam bath. Then the slurry was washed with distilled water several times

until neutral. Again it was washed with acetone, ethyl acetate, diethyl ether, and hexane ranging from high to low polarity and dried in an oven [73].

3.1.2.2 Materials

Blender: Waring Commercial heavy-duty blender, 1500W, Homogeniser USA, Rota evaporator: RE121 BüCHI, SWITZERLAND. Glass column 22 mm i.d 300 cm length was used for florisil packing. High retention filter paper Whatman-42 was used for all filtrations. All glassware used throughout the experiments were washed and then dried in oven at 75⁰C. They were further rinsed with acetone before use.

3.2 Sample Preparation

In pesticide residues analysis basically there are three steps which represent by the following flow diagram. Under the sample preparation step also there are sub-steps, like homogenisation, extraction, concentration and clean up. Each of them were sequentially were done in the experimental work.

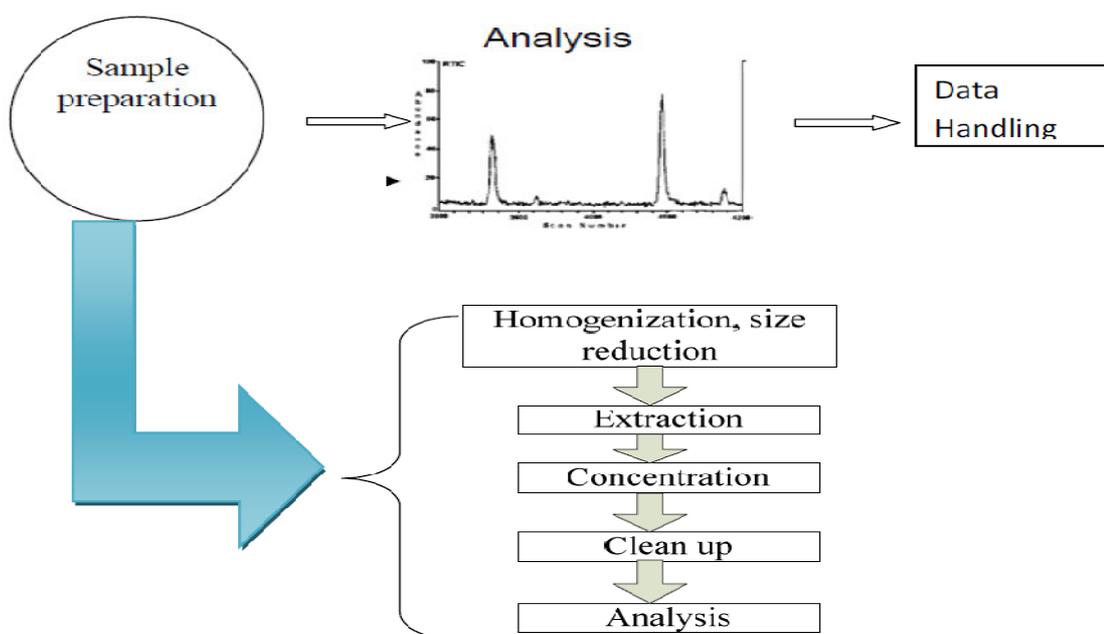


Fig. 3.1 Steps in the analysis of pesticide residue

3.2.1 Sample Extraction

An international standard method of sample extracting for fruits and vegetables was used in this study [74]. This method is described briefly as follows. A total of 1 kg of potato and orange samples was chopped and then blended for 3–5 min using Waring blender. A 20 g portion of the homogenate weighed and transferred to 250 mL beaker. This was followed by addition of 20 mL water and kept for 2 hr for equilibration. It was then extracted twice with 100 mL and 50 mL of acetone. Each time of extraction it was homogenized for 3 min using magnetic stir bar and suction filtered using No. 42 Whatman filter paper containing 1 cm diatomaceous earth. The filtrate was transferred to evaporatory flask and the solution was evaporated at 40 °C to 30 mL. The 30 mL extract was transferred to 500 mL separatory funnel containing 100 mL of 10% sodium chloride solution. It was then partitioned using 100 mL of n-hexane after shaking for 5 minutes. The n-hexane layer was transferred to a conical flask; anhydrous sodium sulfate was added until freely flowing and kept for 15 minutes by intermittent shaking. The content was suction filtered in an evaporatory flask and evaporated to dryness. The residue was dissolved in 5 mL n-hexane and proceeded to clean up step.

3.2.2 Clean-up

Florisil column preparation: a homemade glass column (1.5 cm i.d.×40 cm length) containing a piece of glass wool was filled with 10 g of Florisil in n-hexane and about 5 g of anhydrous sodium sulfate was topped on the florisil. The following clean-up process was used before chromatographic analysis. A 5 mL of the extract was transferred to the Florisil column. The pesticide residues were eluted with 200mL of n-hexane–diethyl ether (17:3, v:v) mixture and the eluate was collected in a conical evaporating flask. The eluate was

concentrated in a vacuum rotary evaporator to approximately 1mL at 40°C. Then the eluate was N₂ –gas dried and the residue was dissolved in 2 mL n-hexane for chromatographic analysis

i. Calibration curve

Standard solutions ranging from 0.005 mg/L to 1 mg/L concentrations of five points were prepared from the stock solution. These five standard solutions were injected into a GC-MS for the calibration curve.

3. 2.3 Gas Chromatographic Analysis

Various analytical methods have been reported for the determination of pesticide residues in various fruits and vegetables. Most of them used gas chromatography (GC), either with electron-capture detection, nitrogen-phosphorus detection or flame photometric detection. Other methods used GC coupled to mass spectrometry (GC-MS) to have a highly selective detection. Massspectrometry is a very sensitive and selective technique for both multiresidue determination and trace-level identification of a wide range of pesticides. It can quantify and confirm the results by its full scan or selected ion monitoring (SIM) spectra [75].

Thus, in this analysis the determination of the analyte were done by Agilent Technologies 5975 inert massselective detector (MSD), Agilent Technologies hyphenated with 6890N GC system was used for analysis, Column: HP5-MS, 0.25mm i.d x30m length, 0.25 µm film thickness, Column temperature: Initial temperature was 50 °C which was held for one minute followed by ramp of 25⁰C/min to 125⁰C then it was ramped by 10 °C/min to 300⁰C where it was held for 10 minutes, Inlet temperature was 250 °C, Auxiliary temperature was 280⁰C. Electron impact ionization with 70 eV energy was the ionization mode. The Carrier gas used was helium at a flow-rate of 1 mL/min. 2µL extract and standard sample was injected during all of the analysis. The selective ion monitoring (SIM) mode was used for

quantitation purpose and the scan mode was used for identification. Peak area or peak height was used for quantitation. The major Monitoring ions are given in the following table.

Table 3.1 OCPs and their monitoring ions in charge to mass ratio

Pesticide	Monitoring ions (m/Z)			
p,p-DDT	237	235	212	165
P,p-DDE	318	246	248	316
P,p-DDD	237	235	178	165
Aldrin	293	265	263	261
α -HCH	219	183	181	109
γ -HCH	219	183	181	238
Endosulfan	241	195	265	239
Chlordane	373	375	272	377
Heptachlor	337	272	100	270
Dieldrin	293	265	263	261

4 Result and Discussion

The main objective of this research as described in the introduction part are determination of the state of contamination of orange and tomato with organochlorines pesticides. Based on this objective the analyses were done on orange sample and tomato sample available in the market for ten target organochlorine pesticides. However, before the analyses were done the method used for the analysis were validates. For the validation purpose five common parameter were selected.

4.1 Method Validation

Method validation is one of the quality control measures that should be taken in residue analysis. Validation studies for quantitative analytical methods typically determine some or all of the following parameters: accuracy, scope, specificity, sensitivity, precision (repeatability and reproducibility), bias, linearity, detection limit, robustness, ruggedness and selectivity. In this study the accuracy in terms of percent recovery, the precision (repeatability and reproducibility), detection limit and quantification limit of the method chosen were determined for both samples.

4.1.1 Calibration Curve for the Determination of the Analytes

The calibration curves were obtained using analytical solutions of the mixture of the pesticides prepared in pure solvent and prepared in the extract of the matrix in the concentration range [70]. Acceptability of linearity data is often judged by examining the correlation coefficient and the y intercept of the linear regression line for the response versus concentration. A correlation coefficient of >0.999 is generally considered acceptable. The y intercept should be less than a few percent of the response obtained from the target level [76].

Accordingly, the calibration curves were obtained from a running of seven point calibration solutions having a concentration range of 5 ng/mL to 2000 ng/mL (in the case of heptachlor and, DDD the range were 10ng/mL to 2000ng/mL and the range of DDE was 25ng/mL to 2000ng/mL). The lowest concentration level in the calibration curve was established as a practical determination limit for the instrument. Linearity was evaluated by the calculation of a seven- point linear plots of the peak height (as observed Fig.4.1 representative chromatogram from the seven concentration range) against concentration based on linear regression and squared correlation coefficient, r^2 , which should be > 0.9900 . All of the pesticides had a good linearity except DDD which was below 0.990. The linearity range and r^2 values are given in Table 4.1.

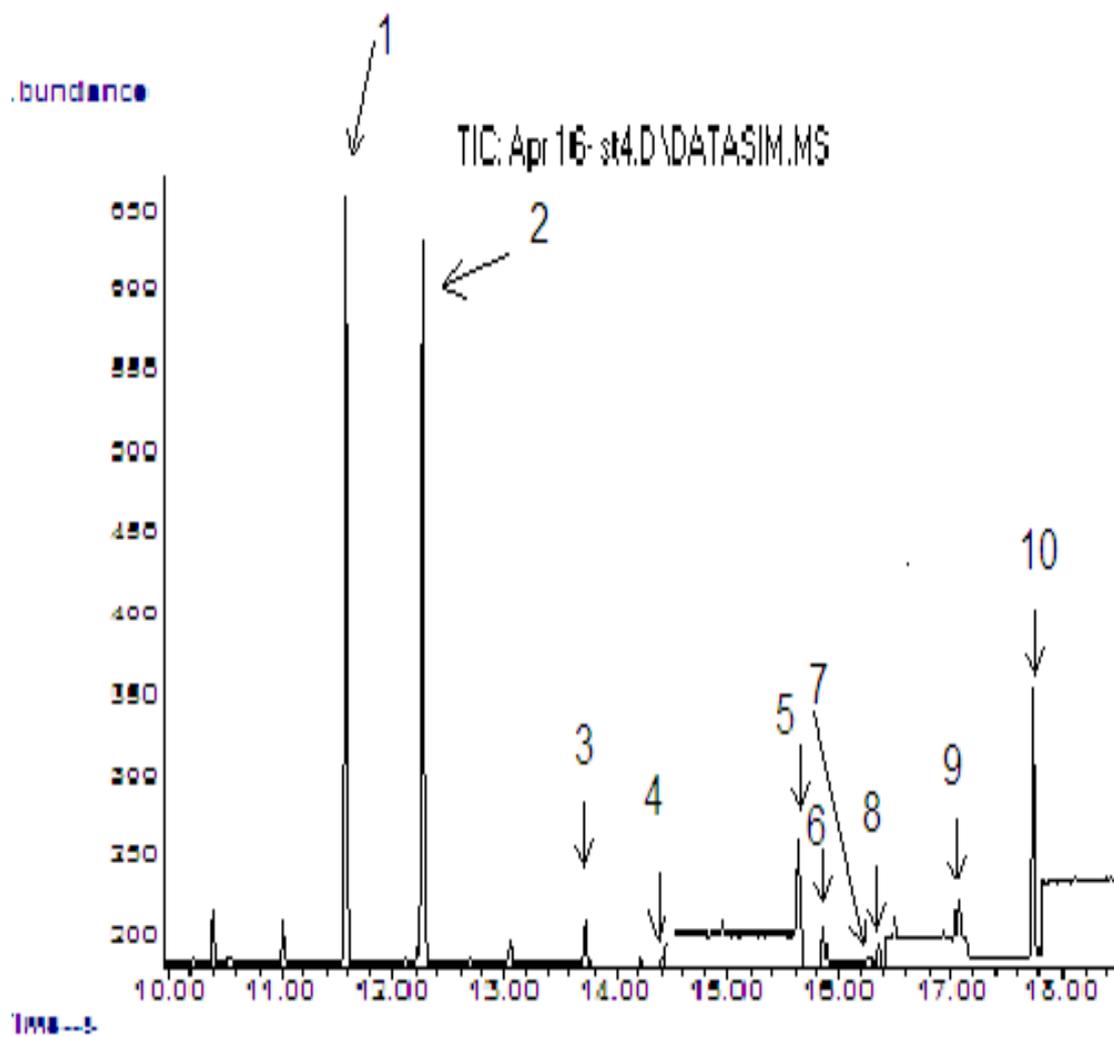


Fig.4.1 A representative chromatogram of 100 µg/L standard analyzed for 1. α -HCH; 2. γ -HCH; 3. Heptachlor; 4. aldrin; 5. Chlordane; 6. Endosulfan; 7. p,p-DDE ; 8. Dieldrin 9. p,p-DDD; 10. p,p-DDT

Table 4.1 Calibration parameters, LOD and LOQ

Pesticide	r ²	Linear range, (ng/mL)	PAO		PAT	
			LOD (mg/L)	LOQ (mg/L)	LOD (mg/L)	LOQ (mg/L)
α-BHC	0.999	5-2000	0.003	0.010	0.002	0.007
γ-BHC	0.997	5-200	0.002	0.005	0.001	0.003
Heptachlor	0.999	10-2000	0.004	0.014	0.004	0.013
Aldrin	0.998	5-2000	0.003	0.011	0.004	0.016
Chlordane	0.996	5-2000	0.004	0.013	0.004	0.015
Endosulfan	0.995	5-2000	0.003	0.008	0.002	0.005
DDE	0.995	25-2000	ND	ND	ND	ND
Dieldrin	0.990	5-2000	ND	ND	ND	ND
DDD	0.989	10-2000	ND	ND	ND	ND
DDT	0.999	5-2000	0.002	0.007	0.0003	0.001

ND- Not detected

4.1.2 Limit of Detection and Limit of Quantification

There are several terms that have been used to define LOD and LOQ. In general, the LOD is taken as the lowest concentration of an analyte in a sample that can be detected, but not necessarily quantified, under the stated conditions of the test. The LOQ is the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated conditions of test. Determination of LOD and LOQ can be done by Signal- to-noise, Blank determination and Linear regression. However in this research blank determination were used to determine LOD and LOQ [69,71].

In view of that, the values of limit of detection and limit of quantification for the detected pesticides for the proposed method are depicted in Table 4.1 above. Limit of detection is expressed as the analyte concentration corresponding to the sample blank value plus three standard deviation or sample blanks fortified at lowest acceptable concentration (i. $LOD \cong x_{ib} + 3s$ or ii. $0 + 3s$.) and Limit of quantification is the analyte concentration corresponding to the sample blank value plus ten standard deviations ($LOQ \cong x_{ib} + 10s$) where x_{ib} is the mean concentration of the fortified sample blank and s is the standard deviation of the fortified sample blank.

Thus determination of Limit of detection and limit of quantification were calculated according to EURACHEM (1998) [77] recommendations. The LOD and LOQ were determined in full scan mode from sample of tomato and orange fortified at a 10 ng/mL.

From the table (Table 4.1) it is possible to observe that the values of limit of detection for both samples were below the lowest calibration level for all of the pesticides detected in the samples. In the same manner when the limit of detection of the detected analytes compared

to the international MRL values set by codex and Japanese MRL, it was lower value. This clearly shows that it is possible to determine the residual levels of the pesticides in these food items to much lower than the international MRLs. However, the limit of detection and limit of quantification of DDE, Dieldrin and DDD were not determined because the amount might be below the detection limit.

4.1.3 Precision

The precision of a method is the measure of agreement or closeness of analyte concentrations to each other when the analyses were performed using identical conditions, i.e. the same method, same sample, same operator, and same laboratory conditions over a short period of time. This is known as *repeatability*. Mathematically it is calculated and expressed as standard deviation as shown in *equation (3 , 4)* [76]. *Reproducibility* is data collection using the same sample and the same method but a different operator, another set of laboratory conditions, and a different period of time (days or even weeks) [76]. The precision data is generally obtained from triplicate analyses of spiked samples.

$$SD = \left[\sum \frac{(x - X_1)^2 + \dots + (x - x_n)^2}{n - 1} \right]^{1/2} \dots \dots \dots (3)$$

$$RSD = \frac{SD}{X} 100 \dots \dots \dots (4)$$

4.1.3.1 Repeatability and Reproducibility

The repeatability of the method (intra-day) was studied by running five extractions which was spiked with 0.1 mg/L of OCPs mixture within one day of extraction time. The %RSD Value of all the pesticide was below 20% as shown in Table 4.2. On the other hand the reproducibility of the method (inter-day) was investigated by running five extractions of 0.1 mg/L spiked samples in different days of extraction time. The result of %RSD was also

below 20%. This indicates that method is reasonably repeatable and reproducible under the laboratory conditions available in the program [78, 79]

Table 4.2 Repeatability and Reproducibility of the studied pesticides in fortified orange and tomato samples extracted by LLE, and analyzed using GC-MS

Compound	Interaday RSD%		Intereday RSD%	
	PAO	PAT	PAO	PAT
α -BHC	7.560	9.877	5.188	11.139
γ -BHC	4.781	19.087	2.661	13.414
Heptachlor	4.702	1.729	3.372	18.483
Aldrin	3.190	3.780	6.152	12.675
Chlordane	17.786	7.564	17.346	3.537
Endosulfan	18.189	15.437	18.296	18.730
DDT	10.835	3.191	3.6134	6.876

4.1.4 Method Bias Study

Accuracy is the nearness of a measured value to the true or accepted value. It provides an indication of any systematic error or bias in the method [69]. Accuracy is a very difficult parameter to measure or validate because the analyst must consider sampling errors, errors in procedure workup, and errors from separation interferences, and the detector system. In this analysis the accuracy of the method were determined by measuring the recovery using *equation (5)*[76].

$$\%R = \frac{\text{Calc. Conc}(spiked+Sample) - \text{Sample. Conc}}{\text{Spik. conc}} \times 100 \dots\dots\dots (5)$$

4.1.4.1 Recovery

Orange and tomato samples were fortified at 0.01 µg/g of a mixed standard solution. The recovery rate was replicated and the data are presented in Table 4.3. The table shows that the recovery rate for five pesticides (α-BHC, γ-BHC, Heptachlor, Aldrin and DDT) out of seven were within acceptable range for tomato [78, 79]. Recovery for endosulfan was below the acceptable level whereas that of chlordane is above acceptable value. The low recovery for endosulfan may be due to the analyte retained in the clean-up column or during filtration or in any of the extraction step but the case of chlordane may be due some experimental error therefore these has to be checked further.

On the other hand, the recoveries of only three pesticides (α-BHC, Heptachlor and aldrin) were within the acceptable range for the orange sample [78, 79]. But γ-BHC, endosulfan, and DDT were below the standard range whereas chlordane was above the recovery range. This method is, therefore, applicable for the determination of five pesticides in both tomato and orange samples reasonably. The cause of the low recovery for other pesticides must be investigated further. Also the higher recoveries of chlordane in orange and tomato samples also investigate further. Due to shortage of time and budget it was not possible for us to look into the case.

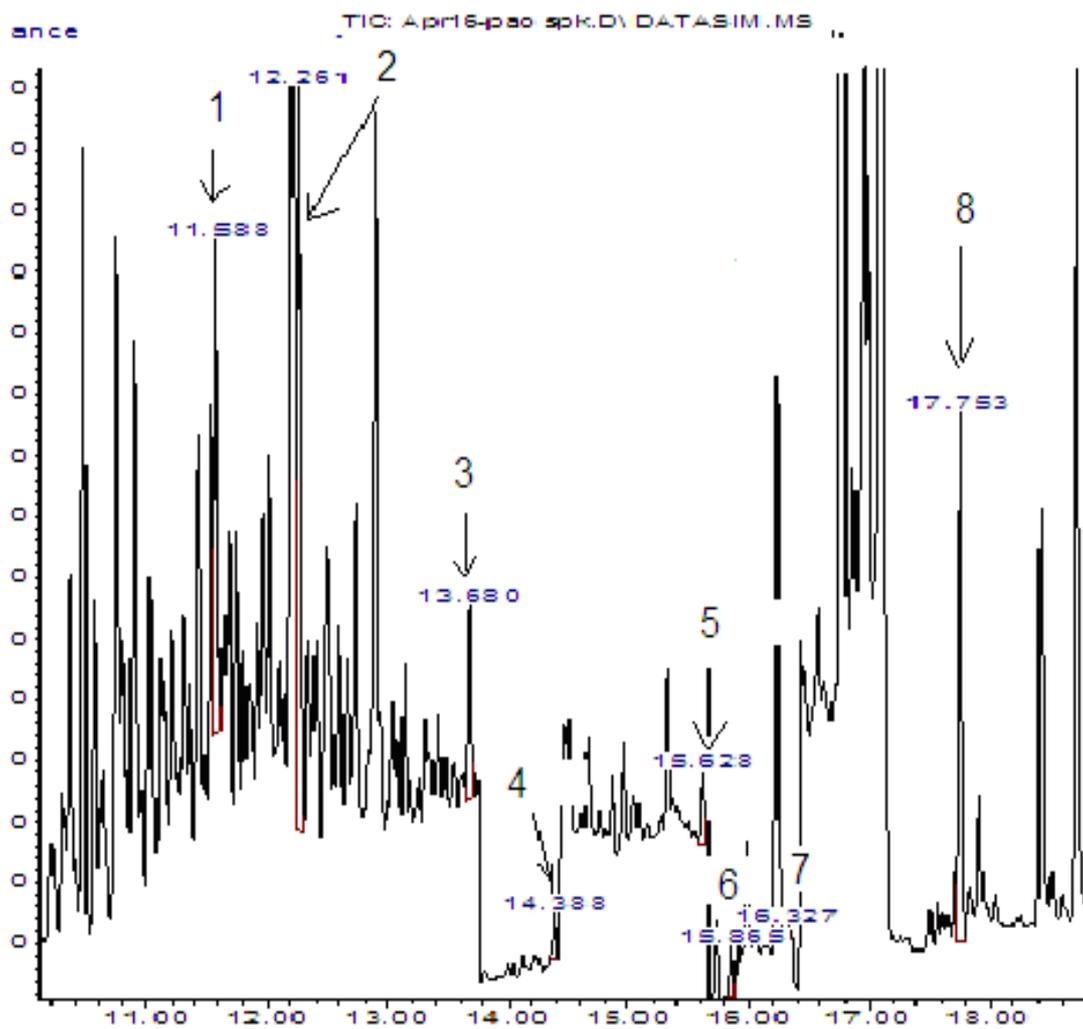


Fig. 4.2 A representative chromatogram of PAO sample spiked at 10 ng/g for the pesticides: 1. α -HCH; 2, γ -HCH; 3, heptachlor; 4, aldrin; 5, Chlordane; 6, Endosulfan; 7, DDE 8, DDT

Table 4.3 Recoveries of the studied pesticides in fortified orange and tomato samples extracted by LLE, and analyzed using GC-MS

Pesticide	Spiked Level ($\mu\text{g/g}$)	Spiked Mean Conc. ($\mu\text{g/g}$)		Blank Mean Conc. ($\mu\text{g/g}$)		Mean Recovery%	
		PAO	PAT	PAO	PAT	PAT	PAO
α -BHC	0.01	0.013	0.007	ND	ND	130	78.8
γ -BHC	0.01	0.011	0.002	0.003	ND	73	16
Heptachlor	0.01	0.030	0.080	0.018	0.069	112.3	105.3
Aldrin	0.01	0.035	0.043	0.029	0.037	70	60
Chlordane	0.01	0.014	0.02	ND	ND	140	199
Endosulfan	0.01	0.004	0.003	0.001	0.0005	37	18
DDT	0.01	0.007	0.003	0.001	0.001	60	20

ND- not detected

4.2 Sample Analysis

After the validation of the method, tomato and orange samples were collected from Piasa Atkilt Tera and analyzed for the 10 pesticide residues. Before sampling merchants and truck drivers were interviewed on the origin of their products. According to the responses of the most interviewee fruits and vegetables are coming to this market from all over the country. Some of them said that their fruits and vegetables are coming from the southern part of the

country. Only very few of them were not sure about the origin of their products. From this preliminary assessment it is possible to conclude that the Piasa Atkilt Tera is the best place to collect fruits and vegetables samples for pollution study. Therefore, it was decided to purchase fruit and vegetable samples from this market place.

Analysis were done three times and during all of the analysis γ -BHC, Aldrin, Endosulfan and DDT were detected in orange samples and heptachlor was detected only in the third and fourth analysis for orange samples. On the other way Heptachlor, Aldrin, Endosulfan and DDT were detected in the tomato samples in all of the experimental analysis. As the recovery of Endosulfan was not good in both tomato and orange samples. Whereas the recovery of DDT and γ -BHC in orange sample also below the standard. Therefore it was not possible to correctly determine what was available in the original sample.

4.3 Comparison of Results Obtained from the Sample with MRL Value of Different Source

Table 4.4 shows the mean value of the three experimental analyses against the MRL values from different sources. None of them were above the MRL of different sources as can be seen from Table 4.4. These results are an indication of there might be a proper usage of pesticides in the production of these food items or it might be low use of pesticides. However tomato and orange available for consumption were contaminated with Heptachlor, Aldrin, Endosulfan and DDT γ -BHC, Aldrin, and Endosulfan and DDT pesticides. Thus, the cumulative effect and the synergetic toxic effect of these pesticides on the consumer not yet determined.

Table 4.4 FAO/WHO and Japanese MRL of OCPs in orange and tomato and concentration of analytes in the sample

Pesticide	FAO/WHO MRLs (mg/kg)		Japanese MRLs (mg/kg)		PAO-Mean Conc. (mg/kg)	PAT-Mean Conc.(mg/kg)
	orange	tomato	Fruit	Vegetables		
α -BHC	3.0	3.0	0.01	0.01	ND	ND
γ -BHC	3.0	3.0	0.01	0.01	0.005	ND
Heptachlor	0.01	0.055	0.01	0.03	0.010	0.035
Aldrin	0.05	0.1	0.05	0.02	0.020	0.010
Chlordane	0.02	0.02	0.02	0.02	ND	ND
Endosulfan	2.0	2.0	0.5	0.5	0.0007	0.001
DDE	3.5	3.5	0.5	0.2	ND	ND
Dieldrin	0.05	0.1	0.05	0.02	ND	ND
DDD	3.5	3.5	0.5	0.2	ND	ND
DDT	3.5	3.5	0.5	0.02	0.002	0.003

Source: codex (2009) , JFCRF (2006) and codex(1986)

5 Conclusions and Recommendations

5.1 Conclusions

Pesticide residues in or on plants may be unavoidable even when pesticides are used in accordance with Good Agricultural Practice. That is why in this research the main purpose are determination of these pollutants particularly in orange and tomato. However, before the determination of target contaminant the methods used for the analysis were validated to test whether or not they were fit for the intended purpose. Accordingly, the validation parameters were LOD, LOQ, %recovery, repeatability and reproducibility were conducted because these parameters are common than the others. The result of these parameters indicated that the values of LOD for all analytes were lower than the LCC. And also all the LOD value of the detected analytes were less than the MRL set by codex and JFCRF as shown in table 4.4. Therefore this method can be used to detect five organochlorine pesticides found in orange and tomato sample even below from their MRL.

The mean recoveries were also within the standard range for five organochlorine pesticides in tomato sample and three for orange sample. However, the recoveries of γ -BHC, chlordane, endosulfan and DDT in orange sample whereas chlordane and endosulfan in tomato sample are out of the standard range as the result were indicated in table 4.4. The repeatability and reproducibility of the data in both analysis showed that the method is fit for the analysis that is the intraday and interday %RSD value are <20% as shown in table 4.2. Therefore these results depicted that the analysis method is appropriate for five organochlorine pesticides out of ten targeted pesticides.

The sample analysis result shows that commercially available orange fruit ready for consumption in Piasa Atekelt Tera were contaminated with γ -BHC, Aldrin, Heptachlor,

Endosulfan and DDT. And also the analyses for the same target pesticide in tomato sample collected from Piasa Atekelt Tera revealed that Heptachlor, Aldrin, Endosulfan and DDT were found as the contaminant as shown in table4.4. However the contamination status of these pesticides in both samples were lower than when compared to the codex and Japanese MRL value.

Moreover, the analysis have indicated that these fresh produces are contaminated with five organochlorine pesticides so it is clear that consumption of foods containing unsafe amount of pesticide residues are of public health concern, consequently entailing additional health cost. By the same token, this may affect economic development.

Thus, all concerned bodies of the country need to play their crucial role of ensuring that foods consumed by the general public are not of health concern. Even though, the concentrations of the detected analytes are lower than the reference MRL. Therefore, in order to have safe fresh produce, a variety of measures such as laws, regulations, standards, and a system of effective inspection and laboratory analysis are urgently needed.

5.2 Recommendations

The quality perception of the any fresh products by the consumer at the market is based on visual appearance and physical conditions. Damaged or decayed produce are perceived as low quality produce and rejected by the consumer. Consumers in every place usually think fresh fruits and vegetables are safe, especially those grown locally. However, most of the time chemical contaminations are not perceived by observation and even by test. Similarly, in this research, it was observed that fresh oranges and tomatoes which were not decayed and damaged were however contaminated by organochlorine pesticides. Thus, several issues and questions that need to be addressed were drawn from this research and are described as follows.

- Public awareness should be initiated about the pollution status of fruits and vegetables.
- The MRL level of different pesticide residues should be set and enforced.
- Further studies beginning from the origin of the fruits and vegetables to market level is required to map the pollution status of these very important food items.
- In this research five pesticides in orange sample and four pesticides in tomato sample were detected, future study should include the daily intake and their toxicological effect on the consumer.
- Alternate way of combating pests, such as IPM, should be thought of.

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DECLARATION

I, the undersigned, declare that this thesis is my original work and that all sources of materials used for the thesis have been correctly acknowledged.

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