Human Exposure and Consumer Risk Assessment to Pesticide Use in Ethiopia

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Dissertation in the fulfillment of the requirements for degree of Doctor (PhD) in Applied Biological Sciences

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Dutch translation of the title: Humane blootstelling en consumenten risicoanalyse van het gebruik van bestrijdingsmiddelen in Ethiopië

Cover Photo taken and designed by author

Cited as Mekonen S. Human exposure and consumer risk assessment to pesticide use in Ethiopia, PhD dissertation, Ghent University, 2016

ISBN 978-90-5989-911-7

Printing Universitypress,Zelzate:info@universitypress.be

Acknowledgments

First of all, I would like to thank and glorify to the almighty God, for taking care of me all the time and giving me health, rigor and courage to embark on the PhD work. I would like to express my deepest gratitude to my promoter Prof.dr.ir. Pieter Spanoghe, at first for his willingness to accept my request to pursue my PhD in his laboratory. His guidance, inputs and encouragement have been inspirational in shaping my PhD dissertation. I am very grateful for his devotion to correct and comment all the manuscripts I prepared for publication and the whole PhD thesis.

I am so grateful to the technical staff in the Laboratory of Crop Protection Chemistry, Lilian Goeteyn and Claudine Schollaert for their support during the experimental work. Lilian, you were very helpful to me in all aspects of academic, social and administrative processes. It is unforgettable how you helped me to socialize in the lab and around Ghent. I am also very grateful to Michael Houbraken and David Senaeve who were assisting me during instrumental analysis of my samples in the laboratory. I am also indebted to Elvina Mortier, Caroline, Davina, Kim, Wumbie, Chara, Edlbis and dr. ir. Bjorn Vandekerkhove for their support during my study and all the things we share in the coffee room.

I also like to extend my gratitude to em. Prof. Walter Steurbaut and his wife Rita, for outings around Ghent, Ostende, Brussels, and for the delicious dinner we had together, and overall support to make me feel at home during my stay in Belgium. Prof. Steurbaut did support in framing my PhD study during the inception of the PhD ideas.

I would like to forward special appreciation to my home institution, Jimma University, for giving me a leave and other supports to undertake my study. I am grateful to Mrs. Zinash Solomon, an administrator to the College of Health Sciences, for facilitating administrative issues that are related to my study. The staff in the department of Environmental Health Science & Technology were very cooperative. I would like to thank dr. Seid, Taffre, Asgdom, Higemengist, Embiale, Dessalegn, Samuel, Wuhib, Hailu, dr. Alemayehu, dr. Taddesse, dr Abebe, dr. Dante, Tebkew, Alebel, Gudina, Tesfalem, Tizita, Seyoum, Yared, Tenagne and Abeba, for the appreciations and moral support.

I am deeply grateful to Ghent University for the full financial support through BOF, a special research fund, which made this PhD study become realized.

Without the motivation, support and care of my family, this PhD study would not come true. I would like to extend my deepest gratitude to my beloved husband dr. Argaw Ambelu. Arg, your support, encouragement, love, care and guidance were very helpful for me throughout the period of my PhD study. I remember how much you were carful for our children by acting as a father and a mother in all my absence from home. My love, your usual dream and effort, to make me one of the strongest women, is always motivating me to move forward. Arg, I really do not have enough words to thank you. I understand how much my lovely children, my son Fsihatsion and my daughter Sosna Argaw, missed me during my stay in Belgium. My children, your eagerness to see my final defense and to hear your mother being called ‘doctor’, gave me the energy and additional encouragement to accomplish this work. I am also very much thankful to my sisters, Hamelmal Mekonen, Lidia and Aselefech who were taking care of my children during my stay in Belgium.

My heartfelt appreciation and gratitude also goes to my father Mekonen Shegen and my mother Masho Terefe, who were my base to reach this stage. The support and encouragement of my brothers, Amdemariam and Ayele, and my sisters, Tsehay, Abaynesh, Genet, Zebenay, Yeshihareg, Aynalem, and Kidist were very helpful to me to reach this level.

My appreciation also goes to the staff of OBSG, i.e. Marleen, Annemie, Father Charles and Isabel for their support during my stay in Ghent. Marleen, thank you so much for reserving a room to me every year and making me comfortable. I also would like to thank all those people whom I have forgotten to mention, but who directly or indirectly contributed to the successful accomplishment of this work.

Table of contents

Contents Page

Table of contents

Summary

Pesticides are indispensible to increase agricultural production and control human and animal diseases. Due to their benefits, pesticides have been continued to be used worldwide. Even though, the use of pesticides is important, their residues eventually end up in different environmental compartments (water, food, soil, air) and may negatively affect human health and the environment. Because of the harmful effects to human and other organisms in the environment, pesticides are under strict regulations starting from their synthesis and formulation, over the mixing/loading of pesticides and their application in the field.

Ethiopia's population is continuously increasing and this put pressure on agricultural activities. Due to this pressure, the government of Ethiopia use agricultural intensification with high demands for fertilizers and pesticides as one of the strategies to increase agricultural production. Additionally, unlike most developed countries, the pesticide regulation such as pesticide registration and control proclamation 674/2010 in Ethiopia, is not well enforced due to different reasons explained in **chapter one** and **chapter two**. Moreover, most of the Ethiopian farmers are illiterate and they do not know how much amount they should apply in the field, where to store the pesticides and from whom they should buy these chemical pesticides. As a result, the different consumer products may become contaminated by pesticides. Despite all these problems, there is no study done on the contamination of the commonly consumed food items (staple foods) with pesticides in the country. Additionally, there is no data in Ethiopia regarding human exposure and consumer risk assessment from pesticide exposure. Therefore, it is important to evaluate the presence of pesticide residues in commonly consumed food items and assess their risks to consumers.

Chapter three presents, the analysis of the different pesticide residues in food items in southwest Ethiopia. For this study, teff (*Eragrostis tef*), maize (*Zea mays*), red pepper (*Capsicum annuum*), coffee (*Coffea arabica*), were collected from one big central market in Jimma zone, southwest Ethiopia. This market is chosen as a sample point, because most agricultural crops are collected for sale. The selection of the pesticides for analysis in food items was done based on the information obtained from agricultural offices and farmers in the study area. The study also includes pesticides such as DDT and endosulfan which are known to be applied for indoor residual spraying (IRS) to control malaria, as Jimma zone is a malaria endemic area. For the analytical procedure, the Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) extraction method combined with dispersive Solid Phase Extraction (d-SPE) clean up was applied. Pesticides determination was done using gas chromatography with electron capture detector (GC-ECD). In this work, different pesticides such as, DDT and its metabolites, endosulfan (α and β isomers), cypermethrin, permethrin, deltamethrin and chlorpyrifos ethyl were detected at a concentration range of 0.011 to 1.115 mg/kg food. The findings of this study indicate that the commonly consumed food items were contaminated by pesticides, which may come from field application as well as from application for disease control. More than one third of the pesticides detected were above the maximum residue limit (MRL) set by Codex Alimentarius. This indicates that there is overuse of the pesticides in the study area. DDT and endosulfan which are not authorized for agricultural use were also detected in the food items. This may be due to, cross contamination during spraying for malaria control or may be due to their persistent (non-degradable) nature in the environment. The detection of these pesticide residues in the commonly consumed foods may affect human health and food safety in general.

Chapter four presents, the risk of DDT residues in maize to infants in the southwest Ethiopia. In this region, maize is the commonly produced agricultural crop which is also used as a major component in infants' food. To work out a risk assessment of this pesticide to infants, a consumption and residue data base is needed. Therefore, a consumption survey was undertaken for 6-12 month infants, from randomly selected households, using the 24 hour recall method. The maize samples were collected from the households selected for the consumption survey, as well as from markets in the surrounding area. During screening of the maize samples, DDT and its metabolites were detected. To assess the exposure of infants, the estimated daily intake (EDI) was determined using the deterministic and probabilistic exposure analysis method based on the recommendation of the European Food Safety Authority (EFSA). The EDI was compared with the health based reference values such as provisional tolerable daily intake (PTDI) to characterize the risk. From the results, it was found that about three fourth of the maize samples were contaminated with DDT residues above the MRL norm set by Codex Alimentarius. This may be due to illegal application of this pesticide in agriculture or due to previous application for malaria control which is discussed in chapter three. The results of the exposure estimation revealed that the mean and the high consumer (97.5 percentile) EDIs of the total DDT for the infants were above the PTDI. This is an indication of chronic health problems for the infants consuming maize as a complementary food. The health problem may be severe for this vulnerable group of the population. This may be due to their metabolic mechanism to detoxify the chemical hazards is not well matured at that age. In addition to this, the infants' food intake per body weight is higher compared to adults. Therefore, continuous monitoring of DDT and other pesticides in infants food is recommended in Ethiopia.

Several studies report that food processing such as washing, peeling, baking, cooking, roasting, boiling, fermentation, drying etc. have an effect on the reduction of pesticide residues in different food commodities. Our study in **chapter five** presents, the effect of household processing such as washing, roasting, brewing, doughing and baking on the level of pesticides in coffee and teff. The previously detected pesticides in teff and coffee (**chapter three**), such as DDT, deltamethrin, cypermethrin, permethrin, endosulfan (α and β) and chlorpyrifos ethyl were spiked in blank samples of teff and coffee beans. Following this, the household processing methods were undertaken and the effect on the pesticide residues was determined by calculating the processing factor (PF). According to the joint meeting on pesticide residues (JMPR) of FAO/WHO, PF is defined as the ratio of the residues in the processed food commodity to the raw commodity before processing. The result of this study indicates that the PF for each household preparation of coffee and teff is less than one. This means that there is a reduction of pesticide residues due to the household processing of these two food commodities. From the processing methods studied, roasting (reduction of 99.8%) and brewing (reduction of 100%) of coffee and baking (reduction of 90.2%) of teff were the most effective methods to reduce pesticide residues of the raw food items. Therefore, in countries like Ethiopia, who do not have well enforced pesticide regulations and proper pesticide monitoring programs, food processing may be used as a solution to minimize exposure and the risks associated with pesticide residues in food.

Human exposure to chemical hazards such as pesticides may not happen only from consumption of food but also from drinking water. Most of the water sources are located in the vicinity of agricultural fields where pesticides are applied. The water sources is often contaminated due to runoff, environmental drift, erosion and diffusion processes. This may affect human health and the environment as well. **Chapter six** presents the evaluation of different pesticides in water sources around Jimma zone, southwest Ethiopia and Addis Ababa which is the capital of Ethiopia. Water samples were collected from springs, wells, river, pond, the distribution reservoir and community taps. For the extraction of water samples liquid-solid and liquid-liquid analytical methods were applied. The results of this study reveal that the water sources were contaminated with organophosphate pesticides (diazinon, malathion and pirimiphos methyl), a herbicide (2,4-D) and a fungicide (fenpropimorph) at varying concentration (from 0.109-138 µg/l) from source (river, spring, and well) up to the point of consumption (community taps). The concentration of the detected pesticides in the water was higher in the untreated water sources such as the well, the spring, and the river. This may indicate that the conventional treatment process such as coagulation/flocculation, filtration and chlorination may have some effect on the reduction of pesticide level in the water. From our findings, the estimated daily intake (EDI) of the detected pesticides from drinking water was less than the acute reference dose (EDI < ARfD) for all the pesticides detected in water. Although, the concentration for diazion and fenpropimorph was above the acceptable daily intake (ADI). This may result in chronic health problems for consumers. This suggests also a need for continuous monitoring of pesticides in the water bodies in Ethiopia.

In addition to the contamination of the above mentioned conventional edible food commodities and drinking water, pesticides may also detected in other agricultural crops such as khat (*Catha edulis*). Khat is consumed in many parts of East Africa (Ethiopia, Somalia, Djibouti, Eritrea and Kenya) and Arabian countries (Saudi Arabia, Yemen, and Tunisia). Within the communities in these different countries, khat chewing is considered a social habit. In Ethiopia, about half of the population consumes the khat plant. However, chewing khat causes different health problems such as euphoric effect, excitement, constipation, mydriasis (dilated pupil of the eye), hyperactivity, and suppressed apatite. If khat also contains pesticides it causes a double burden to consumers, as pesticides have also their own health effects. **Chapter seven** presents the human exposure to the commonly detected and highly persistent pesticide DDT from the consumption of khat in southwest Ethiopia. The chewable part of the khat plant (leaves with tender stems) was collected from the central market in Jimma zone, southwest Ethiopia. The khat samples were transported to the laboratory and dried under shade to prevent photodegradation. Solid phase extraction with column chromathography clean up procedures was undertaken to extract the khat samples. DDT and its metabolites were detected in 80% of the khat samples. To assess the human health risk of DDT exposure in khat, a probabilistic exposure analysis method was applied and compared with the health based reference value (PTDI). The results indicate that the estimated daily intake (EDI) of DDT was lower than PTDI. However, this does not guarantee 100% safety as this plant is consumed without any preceding processing steps. Therefore, creating awareness for consumer population about the double health burden of chewing khat and continous monitoring of DDT and other pesticide in this plant is important.

In conclusion, the PhD research provides evidence on the level of contamination of the commonly consumed food commodities, drinking water, and khat by pesticides and associated risk to human health. Mutual consumption of these commonly consumed food items may expose the Ethiopian population to multiple pesticides which results in a cumulative risk. **Chapter eight** presents, the general discussion and implication of the findings for human exposure and consumer risk, conclusions and recommendations for further research.

Samenvatting

Bestrijdingsmiddelen zijn onmisbaar om de landbouwproductie te verhogen en voor het beheersen van ziektes bij mens en dier. Omwille van hun voordelen worden bestrijdingsmiddelen wereldwijd gebruikt. Hoewel het gebruik van bestrijdingsmiddelen belangrijk is, blijven hun residuen in verschillende milieucompartimenten aanwezig (water, bodem, lucht) en kunnen zij de menselijke gezondheid en het milieu negatief beïnvloeden. Omwille van hun schadelijke effecten voor mens en andere organismen, staan bestrijdingsmiddelen onder strikte regulering vanaf hun synthese en formulering over het aanmaken en laden van het spuitmengsel en hun toepassing op het veld.

Door de steeds groeiende populatie in Ethiopië staat de landbouw onder druk. Daardoor maakt de regering van Ethiopië gebruik van intensifiëring van de landbouw, wat gepaard gaat met een hoge vraag naar meststoffen en bestrijdingsmiddelen als een van de strategieën om de landbouwopbrengst te verhogen. Bovendien wordt, in tegenstelling tot de meeste ontwikkelde landen, de wetgeving i.v.m. bestrijdingsmiddelen, waaronder registratie en controle (Proclamation 674/2010), in Ethiopië niet zo goed afgedwongen door verschillende redenen die beschreven worden in **hoofdstuk 1** en **hoofdstuk 2**. Daarenboven zijn de meeste telers in Ethiopië analfabeet en weten dus niet goed hoeveel bestrijdingsmiddelen ze dienen toe te passen op het veld, waar ze deze middelen dienen te stockeren en waar de middelen aan te kopen. Bijgevolg kunnen verschillende consumentenproducten gecontamineerd geraken met schadelijke bestrijdingsmiddelen. Niettegenstaande deze problemen, is er in het land nog geen studie uitgevoerd waarbij de contaminatie van vaak geconsumeerde voedingsmiddelen met bestrijdingsmiddelen werd onderzocht. Bovendien is er in Ethiopië geen data beschikbaar i.v.m. humane blootstelling en consumenten risico analyse van blootstelling aan bestrijdingsmiddelen. Daarom is het belangrijk om de aanwezigheid van residu's van bestrijdingsmiddelen in vaak geconsumeerde voedingsmiddelen te evalueren en hun risico's voor de consument in te schatten.

Hoofdstuk 3 handelt over de analyse van verschillende bestrijdingsmiddelenresidu's in het zuidwesten van Ethiopië. In deze studie werden teff (*Eragrostis tef*), maïs (*Zea mays*), rode peper (*Capsicum annuum*) en koffie (*Coffea arabica*) verzameld in een grote centrale markt in de zone Jimma, in het zuidwesten van Ethiopië. Deze markt werd gekozen als locatie voor staalnames omdat de meeste gewassen er te koop worden aangeboden. De selectie van bestrijdingsmiddelen voor analyse in voedingsmiddelen gebeurde op basis van informatie bekomen via landbouwkantoren en telers in het studiegebied. De studie omvat ook bestrijdingsmiddelen als DDT en endosulfan, welke gekend zijn omwille van hun gebruik binnenshuis (IRS) om malaria te bestrijden, Jimma is immers een gebied waar malaria heerst. De analytische procedure werd uitgevoerd met behulp van een 'Quick, Easy, Cheap, Effective, Rugged and Safe' (QuEChERS) extractie methode gecombineerd met vastefase extractie (d-SPE) opzuivering De bepaling van bestrijdingsmiddelen werd uitgevoerd met behulp van gas chromatografie met elektronendetector (GC-ECD). In dit werk werden verschillende bestrijdingsmiddelen, zoals DDT en zijn metabolieten, endosulfan (α en β isomeren), cypermethrin, permethrin, deltamethrin en chloorpyrifos-ethyl gedetecteerd aan een concentratie-interval van 0,011 tot 1,115 mg/kg voedsel. De bevindingen uit onze studie geven aan dat de courant geconsumeerde voedingsmiddelen gecontamineerd waren met bestrijdingsmiddelen, afkomstig uit zowel toepassing in het veld als uit toepassing ter bestrijding van ziekten. Meer dan een derde van de gedetecteerde bestrijdingsmiddelen overschreden de maximum residu limiet (MRL), bepaald door de Codex Alimentarius. Dit geeft aan dat er te veel bestrijdingsmiddelen worden gebruikt in het bestudeerde gebied. DDT en endosulfan, welke niet toegelaten worden voor landbouwkundig gebruik, werden ook teruggevonden in voedingsmiddelen. Dit kan te wijten zijn aan kruis-contaminatie tijdens het spuiten tegen malaria of aan hun persistent karakter in het milieu. Detectie van deze residuen in vaak geconsumeerde voedingsmiddelen kunnen de menselijke gezondheid en de voedselveiligheid in het algemeen, nadelig beïnvloeden

Hoofdstuk 4 beschrijft de risico's van DDT residuen in maïs voor jonge kinderen in het zuidwesten van Ethiopië. Maïs is in dit gebied een veelvuldig geproduceerd gewas dat ook een belangrijk bestanddeel is in de voeding van jonge kinderen. Om een risicoanalyse van dit middel uit te werken voor kinderen zijn data over consumptie en residuen nodig. Een consumptie enquête werd dus uitgevoerd bij jonge kinderen tussen 6-12 maanden oud uit willekeurig geselecteerde gezinnen, gebruik makend van de methode waarbij men bijhoudt wat men de afgelopen 24 u heeft geconsumeerd. De maïsstalen werden verzameld vanuit de huishoudens en vanuit lokale markten in het omliggende gebied. Bij screening van de maïsstalen werden DDT en zijn metabolieten gedetecteerd. Om de blootstelling van jonge kinderen in te schatten werd de geschatte dagelijkse inname (EDI) bepaald, gebruik makende van de deterministische en probabilistische blootstellingsanalyse methode gebaseerd op aanbevelingen van het EFSA (European Food Safety Authority). De EDI werd vergeleken met referentiewaarden zoals de toelaatbare dagelijkse inname norm (PTDI) om het risico te bepalen. De resultaten gaven aan dat ongeveer ¾ van de maïsstalen gecontamineerd waren met residuen van DDT boven de MRL norm, zoals bepaald door de Codex Alimentarius. Dit kan het gevolg zijn van illegale toepassing in de landbouw of van eerdere toepassingen tegen malaria (hoofdstuk 3). De resultaten van de blootstellingsschatting toonden aan dat de gemiddelde en hoge consumenten (97,5 percentiel) EDI's van het totale DDT voor de kinderen boven de PTDI zaten. Dit is een indicatie voor chronische gezondheidsproblemen voor de jonge kinderen die maïs eten als aanvullende voeding. Het risico kan ernstig zijn voor deze kwetsbare populatiegroep, mogelijks doordat hun fysiologie op die leeftijd nog niet in staat is om deze chemische gevaren te metaboliseren en efficiënt uit te scheiden. Bovendien is hun voedselinname per lichaamsgewicht hoger dan bij volwassenen. Bijgevolg is de continue monitoring van DDT en andere bestrijdingsmiddelen in Ethiopië aangewezen.

Verschillende studies rapporteren dat de huishoudelijke verwerking van levensmiddelen, zoals wassen, schillen, bakken, stoven, roosteren, koken, fermenteren, drogen, enz. een effect hebben op de vermindering van residuen van bestrijdingsmiddelen in voedsel. Onze studie in **hoofdstuk 5** geeft het effect aan van verwerkingsmethoden als wassen, roosteren, brouwen, maken van deeg en bakken op de hoeveelheid bestrijdingsmiddelen in koffie en teff. De voordien gedetecteerde bestrijdingsmiddelen in koffie en teff (hoofdstuk 3), waaronder DDT, deltamethrin, cypermethrin, permethrin, endosulfan (α en β) en chloorpyrifos-ethyl werden in gekende hoeveelheden toegevoegd aan teff en koffiebonen. Hierop volgend werden de huishoudelijke verwerkingsmethoden uitgevoerd en de residuen bepaald door de verwerkingsfactor (PF) te berekenen. Volgens de joint meeting over bestrijdingsmiddelenresiduen (JMPR) van het FAO/WHO wordt de PF gedefinieerd als de verhouding van residuen in verwerkt voedsel tot de rauwe voedingsgrondstof voor de verwerking. Het resultaat van de studie geeft aan dat voor zowel de verwerking van koffie en teff de verwerkingsfactor (PF) lager is dan één, wat een indicatie is voor een reductie van bestrijdingsmiddelenresiduen vanwege de toegepaste voedselverwerking. Van de bestudeerde huishoudelijke bewerkingsmethoden waren voor koffiebonen het roosteren (99,8% residudaling) en het koffiezetten (100% residudaling) en voor teff het bakken (residudaling van 90.2%) het meest effectief om de residuen van bestrijdingsmiddelen te doen afnemen in de rauwe voedingsmiddelen. Daarom kan in landen als Ethiopië, die niet beschikken over een sterke en gehandhaafde bestrijdingsmiddelen regelgeving en monitoringprogramma's, de verwerking van levensmiddelen één van de oplossingen zijn om de risico's in verband met bestrijdingsmiddelen te beperken.

Humane blootstelling aan chemische gevaren waaronder bestrijdingsmiddelen kan niet alleen gebeuren via voedsel maar ook via drinkwater. De meeste waterreservoirs liggen in de nabijheid van landbouwgronden, waar bestrijdingsmiddelen worden toegepast. Het oppervlaktewater is veelal gecontamineerd via uitspoeling, milieudrift, erosie of diffusieprocessen. Dit kan zowel het milieu als de volksgezondheid schaden. **Hoofdstuk 6** evalueert verschillende bestrijdingsmiddelen in waterbronnen uit de Jimma zone, in zuidwest Ethiopië, en Addis Ababa, de hoofdstad van Ethiopië. Waterstalen werden gecollecteerd uit bronnen, putten, een waterzuiveringsinstallatie (voor en na zuivering), en verdeelreservoirs en aftakpunten voor drinkwater, gebruik makende van de "grab sampling" methode. Voor de extractie van de waterstalen werd gebruik gemaakt van vaste stof-vloeistof en vloeistof-vloeistof analytische methoden. De resultaten van de studie tonen aan dat de waterstalen gecontamineerd waren met organofosfaten (diazinon, malathion en pirimifos-methyl), een herbicide (2,4-D) en een fungicide (fenpropimorph) in variabele concentraties (0,109 - 138 µg/l) vanuit oorsprong (rivier, bron en put) tot aan het aftakpunt voor drinkwater. De concentratie van gedetecteerde bestrijdingsmiddelen in het water was hoger in de ongezuiverde waterbronnen zoals de put, bron en rivier. Dit kan aangeven dat conventionele zuiveringsprocessen zoals coagulatie/flocculatie, filtratie en chlorinatie een zeker effect hebben op de reductie van de concentratie bestrijdingsmiddelen in het water. Uit onze bevindingen bleek de geschatte dagelijkse inname (EDI) van deze bestrijdingsmiddelen uit drinkwater lager dan de acute referentie dosis (EDI<ARfD) voor alle bestrijdingsmiddelen gedetecteerd in het drinkwater. Echter de concentratie diazinon en fenpropimorf waren hoger dan de toelaatbare dagelijkse inname (ADI). Dit kan leiden tot chronische gezondheidsproblemen voor consumenten. Dit suggereert tevens een nood aan frequente monitoring van verschillende waterbronnen in Ethiopië op aanwezigheid van bestrijdingsmiddelen.

Naast contaminatie van bovenvermelde conventioneel eetbare voedingsmiddelen en drinkwater, kunnen bestrijdingsmiddelen ook gedetecteerd worden in andere landbouwgewassen zoals khat (*Catha edulis*). Khat wordt in vele gebieden van Oost-Afrika (Ethiopië, Somalië, Djibouti en Kenia) en Arabische landen (Saudi-Arabië, Jemen en Tunesië) geconsumeerd. Binnen de gemeenschappen in deze landen wordt het kauwen van khat beschouwd als een sociale gewoonte. Deze stimulerende plant wordt door ongeveer de helft van de Ethiopische bevolking geconsumeerd. Echter, het kauwen van khat veroorzaakt verschillende gezondheidsproblemen waaronder euforische effecten, opwinding, constipatie, mydriasis (verwijding van de oogpupil), hyperactiviteit en verminderde trek. Als khat ook bestrijdingsmiddelen bevat, heeft dit een dubbele impact op de consument daar bestrijdingsmiddelen ook hun eigen effect hebben op de gezondheid. **Hoofdstuk 7** gaat over de humane blootstelling aan het courant gedetecteerde en zeer persistente bestrijdingsmiddel DDT ten gevolge van de consumptie van khat in het zuidwesten van Ethiopië. De eetbare delen van khat (bladeren met zachte steel) werden verzameld in de centrale markt in de Jimma zone, in het zuidwesten van Ethiopië. De khat stalen werden naar het laboratorium gebracht en werden gedroogd in de schaduw om fotodegradatie tegen te gaan. Vastefase-extractie opzuiveringsprocedures werden toegepast om de khat stalen te extraheren. DDT en diens metabolieten werden in 80% van de khat stalen gedetecteerd. Om het gezondheidsrisico van DDT blootstelling in khat in te schatten, werd een probabilistische blootstellingsanalysemethode toegepast en vergeleken met de gezondheidsgebaseerde referentie waarde (PTDI). De resultaten gaven aan dat de geschatte dagelijkse inname (EDI) van DDT lager was dan PTDI, hoewel dat geen 100% veiligheid garandeert, gezien de plant vers wordt geconsumeerd. Daarom is het belangrijk om de consumenten bewust te maken van de dubbele gezondheidsimpact die het kauwen van khat met zich meebrengt en om DDT en andere bestrijdingsmiddelen in deze plant te monitoren.

Om te besluiten, het doctoraatsonderzoek voorziet bewijs van de hoeveelheid contaminatie van courant geconsumeerde voedingsmiddelen, drinkwater en khat door bestrijdingsmiddelen en de daarmee geassocieerde risico’s voor de volksgezondheid. Consumptie van deze courante voedingsmiddelen kan de Ethiopische bevolking blootstellen aan verschillende bestrijdingsmiddelen, wat resulteert in een gecumuleerd risico. **Hoofdstuk 8** behandelt de globale discussie en implicatie van de resultaten i.v.m. humane blootstelling en consumentrisico’s, conclusies en aanbevelingen voor verder onderzoek.

List of abbreviations

ACN	Acetonitrile
ADI	Acceptable Daily Intake
ARfD	Acute Reference Dose
BW	Body Weight
CRA	Cummulative Risk Assessment
CAG	Cumulative Assessment Group
CMG	Common Mechanism Group
DDD	Dichloro-Diphenyl –Dichloro -Ethane
DDE	Dichloro-Diphenyldichloro-Ethylene
DDT	Dichloro-Diphenyl- Trichloroethane
EC	European Commission
ECD	Electron Capture Detector
EDI	Estimated Daily Intake
EFSA	European Food Safety Authority
EPA	Environmental Protection Agency
EEPA	Ethiopian Environmental Protection Authority
ERA	Environmental Risk Assessment
ESI	Electron Spray Ionization
FAO	Food and Agricultural Organization
FFQ	Food Frequency Questionnaire
GAP	Good Agricultural Practice
GC	Gas Chromatography
GPS	Geographical Positioning System
HCH	HexaChloroHexane
HPLC	High Performance Liquid Chromatography

IRS	Indoor Residual Spray
ISARC	International Sociological Association Research Committee
KOW	Octanol-Water Partition Coefficient
LC	Liquid Chromatography
LOD	Limit of Detection
LOQ	Limit of Quantification
LOAEL	Lowest Observed Adverse Effect Level
MCRA	Monte-Carlo Risk Assessment
MRL	Maximum Residue Limit
MS	Mass Spectrometer
ND	Non-Detected
OCPs	Organochlorine Pesticides
PF	Processing Factor
POPs	Persistent Organic Pollutants
POD	Point of Departure
PPEs	Personal Protective Equipments
PSA	Primary Secondary Amine
PTDI	Provisional Tolerable Daily Intake
QuECHERS	Quick, Easy, Cheap, Effective, Rugged and Safe
RA	Risk Assessment
RSD	Relative Standard Deviation
RPF	Relative Potency Factor
RV	Reference Value
SPE	Solid Phase Extraction
WHO	World Health Organization

Chapter One: Framework, research objectives and outline of the dissertation

1.1. Framework of the research

Food safety regarding pesticide residues is an important issue of concern considering consumers health (PAN UK, 2002). In developing countries, the main concern of the government is to increase agricultural production by the application of external inputs such as fertilizers and pesticides (Rundgren, 2006). Access to safe and sufficient food is a basic requirement for human health. However, ensuring food safety and security becomes a worldwide problem for governments, commercial organizations and individuals (Brijnath et al., 2014; Kaferstein, 1997). Pesticides have a great positive impact to control pests and to increase agricultural production; but they are also identified as a treat to food safety due to their potential harmful effects to human health and the environment. Some of the general harmful health effects are, damage to immune system, endocrine disruption, neurodevelopment delays, cancer and respiratory distress (Andersson et al., 2014).

In most developing countries, human exposure to pesticides may be aggravated due to different reasons. One of the main reasons is the legislation and regulations with regard to pesticide use which is not well enforced in developing countries. In Ethiopia there is a regulation regarding pesticide importation, handling and use which is called the 'pesticides registration and control proclamation no.674/2010' (Yohannes, 2010). However, there is an inadequate implementation, weak monitoring and follow-up activities, a lack of integration, weak institutional setup, and poor networking and exchange of information among stakeholders to enforce the regulation. Additionally, there is illegal use of non-authorized pesticides such as DDT which were banned for agricultural use. The farmers do not have enough training about how to use pesticide, where to store, how much amount they have to apply in the field and the precautions before application (Mekonnen and Agonafir, 2002). The farmers buy cheap peasticides such as DDT from illegal retailers because they can get it from neighbouring countries.

Moreover, as Ethiopia is one of the tropical countries, insects are common pests that can affect agricultural crops. According to Aktar et al. (2009), the commonly applied pesticides in tropical countries are insecticides which are more toxic compared to fungicides and herbicides. Vector borne diseases such as malaria is also the most prevalent in Ethiopia and insecticides including DDT were used as indoor residual spray (IRS).

Despite all these problems, there is no study that addresses the consumer exposure to pesticides and associated risks to their health. Therefore, it is the intention of this PhD study to perform a detailed analysis of pesticide residues in commonly consumed food commodities and drinking water and undertaking human exposure assessment. For this purpose, the commonly consumed food items such as teff (Eragrstis tef), red pepper (Capsicum annuum), maize (Zea maize), coffee (Coffea arabica) and khat (Catha edulis) were collected from the central market in Jimma town, southwest Ethiopia and water samples were taken from different sources (river, spring, well, pond and community taps). Following this, a proper analytical method which is environmental friendly Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method with dispersive Solid Phase Extraction (d-SPE) clean-up was applied for the analysis of food samples. Liquid-solid and liquid-liquid extraction methods were applied for water samples and solid phase extraction was used for the pesticide extraction from khat samples. The pesticides to be analysed were selected by interviewing stakeholders (agricultural officers or farmers) about the commonly applied pesticides in the study area. Some of the pesticides were also included in the study based on the condition of the study area. As Jimma zone, which is located in the southwestern Ethiopia, is indeed a malaria endemic area, pesticides such as DDT and endosulfan may be used for vector control. The determination of pesticides was done using gas chromatography with electron capture detector (GC-ECD) and liquid chromatography with double mass spectrometer (LC-MS/MS) analytical instruments.

To understand and make informed decisions to reduce food safety related problems, risk analysis has become the main focus in food science in recent years. Risk analysis involves three components: risk assessment (scientific advice and analysis of information), risk management (regulation and control) and risk communication.

In this study, deterministic and probabilistic exposure assessment models were used to determine the exposure and risks of pesticides to human health, based on the recommendation of the European Food Safety Authority (EFSA, 2012a). The data required for these models are the residues of pesticides in food and the amount of food consumed. The consumption data were collected using the 24 hour recall method for two non-consecutive days.

After determining the presence of pesticide residues in food and their possible health risks for consumers, this study also considers whether household food processing such as washing, roasting, brewing, doughing, and baking has an effect on the pesticide residues in food. Two commonly consumed and exported crops (coffee and teff) were selected and household processing were undertaken. The mutual consumption of the different food items may cause a cumulative effect on the health of consumers. Therefore, cumulative risk assessment was also addressed. Finally, the results were discussed, concluded and a recommendation for future research was forwarded

1.2. Research objectives

The overall objective of the study is to investigate human exposure and consumer risk assessment to pesticide use in Ethiopia.

Specifically the study aimed to:

- give the state of the art on pesticide contamination in agricultural crops and water sources,
- evaluate the presence of different pesticide residues in commonly consumed food commodities and drinking water in southwest Ethiopia,
- assess the exposure of human beings to pesticides in food, drinking water and khat,
- identify the human health risk by comparing the exposure level with the health based reference values such as acceptable daily intake (ADI) and acute reference dose (ARfD),
- identify the cumulative risk of multiple pesticides from mutual consumption of food commodities under study, and
- investigate the effect of household food processing on pesticide residues.

1.3. Hypotheses of the PhD study

The following hypotheses have been put forward:

- Pesticide use in Ethiopia increases. High concentration of pesticide residues can be detected in commonly consumed food commodities such as teff, maize, coffee and red pepper.
- Infants are the most vulnerable groups of the population. Exposure to pesticides in their complementary food has a health risk.
- Household processing of coffee and teff such as washing, roasting, brewing, doughing and baking reduce pesticide residues.
- Drinking water sources such as springs, wells, rivers and tap water may be contaminated with pesticides. A health risk for consumers is expected.
- Khat consumption is a common practice in Ethiopia. Hence, khat chewers are exposed to pesticide residues to a level which causes a health risk.

The PhD study intended to answer a couple of research questions (RQ): What is the problem related to pesticide use in developing countries, particularly in Ethiopia (RQ1)? Does the commonly consumed food commodities produced in southwest Ethiopia contain pesticide residues (RQ2)? Are people exposed to different types of pesticides detected in the agricultural crops (RQ3)? How safe is the level of pesticide exposure from food, water and khat consumption (RQ4)? Do pesticides cause a health risk for short and long term exposure when residues are compared to health based reference values (RQ5)? Does the detection of pesticides in drinking water cause a health problem to consumers (RQ6)? Is there a human exposure from consumption of conventionally non-edible plant such as khat (RQ7)? Does household processing such as washing, roasting and brewing of coffee and, doughing and baking of teff has an effect on residues of pesticide (RQ8)? Is there a cumulative risk for consumers from mutual consumption of different food items (maize, teff, red pepper, coffee and khat) and drinking water in Ethiopia (RQ9)? If the use of pesticides causes human risks, what will be the next step (RQ10)?

1.4. Outline of the dissertation

Chapter one deals about the framework of the research which explains the concerns of food safety in relation to pesticides use, objectives of the research including hypotheses of the study, research questions and dissertation outline. Chapter two describes an overview of pesticide use in developing countries, pesticide residues in food and exposure assessment to pesticides. Chapter three deals with a case study about evaluation of pesticide residues in commonly consumed food items in southwest Ethiopia. Chapter four describes the risk of DDT (the most persistent and bioaccumulative pesticide in human body) to infants from consumption of maize as a complementary diet. Chapter five describes the effect of household food processing such as washing, roasting, brewing of coffee, and doughing and baking of teff on pesticide residues. Chapter six elaborates an assessment of pesticide residues in drinking water and associated consumer risks. Chapter seven gives human exposure to DDT residue in khat (*Catha edulis*) in Southwest Ethiopia. Lastly, chapter eight integrates the findings from previous chapters, elaborates possible implications of the findings for human exposure to pesticides in relation to the findings of other scholars, conclusions and suggestions for future research directions. Schematic outline of the dissertation is depicted in **figure 1.1.**

Chapter One:
Research objectives and outline of the dissertation
Aim: Identify the goal of the research, concepts of the whole thesis and outline of dissertation

Chapter Two:
Introduction and general background
Aim: Identify the problem regarding pesticide contamination in consumer products
Method: Review of literatures (Web of Science – grey literature)

Chapter Three:
Pesticide residues in commonly consumed foods in Ethiopia
Aim: To evaluate the presence of different pesticides in food
Method: Extraction was done using QuEChERS method with dispersive Solid Phase Extraction (d-SPE)

Chapter Four:
Risk of DDT to infants from maize consumption
Aim: Investigate the risk of DDT residue to infants from maize consumption
Method: Deterministic and probabilistic method using Monte-Carlo simulation model

Chapter Five:
Effect of household processing on pesticide residues
Aim: Evaluate the effect of household processing on level of pesticide
Method: Household processing such as washing, roasting and brewing for coffee and doughing and backing for teff were undertaken.
Calculating processing factor (PF) to determine the effect of food processing

Chapter Six:
Pesticide residues in drinking water and associated risks
Aim: Investigate estimated daily intake of pesticide from drinking water
Method: Liquid- solid and liquid- liquid extraction

Chapter Seven:
Human exposure to DDT from consumption of khat
Aim: Determine human exposure to DDT from consumption of khat
Method: Probabilistic exposure analysis using Monte-Carlo simulation model

Chapter Eight:
General discussion, conclusions and recommendations for future research
Aim: Elaborate main findings in relation to other studies, conclude major results and recommend for future research

Figure 1.1. Schematic outline of the dissertation

Chapter Two: Introduction and general background

2.1. Pesticide use in developing countries

For decades, pesticides protect agricultural crops against damages from pests and diseases in the field or in storage areas. In addition, pesticides are of paramount importance to control vector borne human and animal diseases (Handford et al., 2015). As a result, the use of pesticides has become a common practice in vector control and in modern agriculture to enhance crop yields, protect the nutritional integrity of food, to facilitate food storage, to assure year-round food supplies, and to provide attractive food products (Wong et al., 2014).

At the present time, pesticides are more valued in developing countries than before, particularly the tropical countries who seek to enter the global market by providing valuable crops (Ismail et al., 2011). However, these goals perhaps may not be easily achieved without the use of additional input such as pesticides, principally insecticides, herbicides and fungicides, which were not used in traditional agricultural practices (Ecobichon, 2001). Due to the needs, many of the older, non-patented, not expensive chemical pesticides such as DDT, is still illegally used by small scale farmers in most developing countries. Some African countries also move back to DDT to reduce malaria incidence (Hecht, 2004). DDT is banned as agricultural insecticide because of its persistent and bio-accumulative nature in the food chain, causing negative human health and environmental impacts. The Stockholm convention which was held in 2001, included DDT as one of the 12 persistent organic pollutants to be banned worldwide for agricultural use, while the use for disease vector control is only possible under strict guidelines (UNEP, 2001).

Moreover, in developing countries, there is a shift from extensive to more intensive land use (Nesheim et al., 2014). This development was usually associated with the simplification of ecosystem, irrigation of agricultural lands and use of fertilizers and pesticides. From a study done in Thailand (Riwthong et al., 2015), the process of agricultural intensification in developing countries indicates that farmers who cultivate their agricultural lands more intensively use a larger number and quantity of synthetic pesticides than farmers who do not intensify their agricultural lands. As a result of intensive use of pesticides some farmers experience adverse health effects such as dizziness, nausea and vomiting.

Pesticides should be regulated to protect the risks to human being, animals and the environment (Handford et al., 2015). Even though, pesticides are important in developing countries, the need to ensure local agricultural production and food security while simultaneously protecting the population against health effects, emerge as a major public challenge. Developing countries use only 20% of the world's agrochemicals, while they suffer 99% of the deaths (Donald et al., 2007). Improper pesticide use, such as haphazard application on agricultural fields, or crop harvesting without respecting pre-harvest intervals lead to pesticide contamination of the agricultural produce. Due to these problems, there is human exposure to pesticides through ingestion of food, drinking water and animal products because of bioaccumulation of specific pesticides, through breathing of contaminated air as well as to skin contact (Ennaceur et al., 2007; Issa et al., 2010; Trautmann et al., 2012; Gebremichael et al., 2013; Andersson et al., 2014; Bakırcı et al., 2014). Most developing countries experience poor application techniques, inappropriate spraying equipment and improper storage practices (Damalas and Eleftherohorinos, 2011).

In Africa, the usage of pesticides continuously increases in recent years (Williamson et al., 2008). In comparison to other parts of the world, a low volume of pesticide use was noticed in Africa. This does not mean low environmental contamination or low health risks, as some of the most toxic pesticides are still often applied under extremely dangerous conditions. Furthermore, the problem of pesticides is aggravated due to less enforcement of regulation as the national regulatory agencies are underfunded and lack of resources is available and some African countries also may not properly register or authorize the pesticide products.

Moreover, there is a lack of guidelines for the control and monitoring of pesticides. Insufficient knowledge about pesticides, poor equipped laboratory facilities, and inadequate storage are some of the additional problems in Africa (Matthews et al., 2011). In general, developing countries lack awareness about proper management and associated risks of pesticides, have a lack of trained man power, a lack of disposal facilities, etc. which result in adverse impacts of pesticides on human health and environment.

2.2. Pesticide use and impacts in Ethiopia

Ethiopia is a country of huge biodiversity and agricultural complexity. As many developing countries, agriculture is the foundation of Ethiopian economy which supports the livelihood of the people. Agriculture accounts for 46.3% of the gross domestic product, 60% export and 80% of employment (FAO, 2011). The agricultural production in Ethiopia focuses mainly on cereals (84.55%), followed by pulses (11.13%) and other fresh produce (4.32%) such as fruits and vegetables (FAO, 2006). The cereals are mainly produced for domestic consumption with only small market surpluses. The five most commonly produced cereal crops account for almost a quarter of the cultivated land and about 68% of the total production are teff, maize, wheat, sorghum and barley (Taffesse et al., 2014).

Ethiopia is also known to be the world fifth and Africa's top producer of coffee. The country leads Africa's domestic consumption. Half of the coffee produced is locally consumed (Amamo, 2014). This reflects the cultural importance of coffee for the population of Ethiopia. On the other hand, coffee is considered as the main export commodity in the country, which provides 31% of the foreign exchange. About 25% of the Ethiopian population is directly or indirectly dependent on coffee for their income.

In Ethiopia, farmers practiced rain feed agriculture and their dependency on rain may have an impact on the agricultural production and also on food security (Food and agricultural organization of the united nation, 2014). Ethiopia is the second most populous country in Africa with a total estimated population of more than 99,465,819 in July 2015 (CIA, 2015). The national grain production is not enough to feed the whole population (Stokes et al., 2010). Ethiopian agriculture is highly influenced by geographical locations. The landscape in the country defines the potential of agricultural production, the access to input and output markets and the density of the local population, which determine the local demand in food (Chamberlin and Schmidt, 2011). Due to this, the government of Ethiopia applies agricultural intensification as one of the strategies which demands the application of fertilizers and chemical pesticides.

Chemical pesticides were introduced in Ethiopia since 1960s and the introduction was in connection to the development of commercial farms (EPA, 2004). Currently, the Ethiopian government initiated to make the country food self-sufficient and to increase agricultural export commodities like coffee, flowers and vegetables, to maximize the diversity by use of agricultural intensification. Additionally, pressure exercised by pesticide sale supporting groups makes the farmers to believe that pesticides are the only way to avoid crop losses.

According to Abiye and Hadera, (2005), Ethiopia imports more than 3800 tons of pesticides per year and the country also obtains pesticides by donations. From all the pesticides imported about 72% are insecticides, 25% are herbicides, 2.6% are fungicides and 1.3% are other products such as rodenticides and disinfectants. The country's legalization for pesticide registration and a monitoring system were introduced in the late 1990's. Although there is legislation governing pesticide registration, clear guidelines on the importation, testing, and use of pesticides have not been effectively enforced. Therefore, it is common to find restricted or banned pesticides widely used in Ethiopia. There is also poor application practice without using personal protective equipments (PPEs). This results in unprotected spreading of pesticides into the environment and which causes human exposure to pesticides. Some bad practices of bystander exposure and unprotected spray operators are shown in **figure 2.1**.

Figure 2.1. Outdoor (A) and indoor or greenhouse (B) pesticide application in Ethiopia

In Ethiopia, there is a massive use of pesticides on large scale farms (Westbom et al., 2008) while, the use of pesticides in small scale farming is less. However, the government extension services promote the use of pesticides to improve productivity and to reduce food insecurity for the Ethiopian people living from small scale farming (Environment and Social Assessment International, 2006). Besides the agricultural use, pesticides have been used for public health to control vector borne diseases such as malaria. Indoor residual spraying (IRS) is used to prevent malaria in epidemic prone areas (Ministry of Health, 2006). For the last 40 years, Ethiopia has primarily used DDT for its IRS operation program (Biscoe et al., 2004). The country applies approximately 400 metric tons of the active ingredients DDT per year (Sadasivaiah et al., 2007; WHO, 2007; van den Berg, 2009). Nowadays, the use of DDT for malaria vector control is phased out due to the resistance development by the malaria vector and replaced by effective insecticides such as primiphos-methyl and propoxur (Balkew et al., 2012). Additionally, organochlorine pesticides (OCPs) such as DDT, which were used widely in agriculture and for public health purposes, became a worldwide concern due to their persistence, bioaccumulation potential through food chain, and health effects to human and non-target organisms (Jones and de Voogt, 1999; Donaldson et al., 2010). Ethiopia built-up the largest stockpiles of obsolete pesticides in Africa. These stocks have been accumulated starting from the first imports in the 1960s. The stock mostly contains old and toxic organochlorine compounds such as DDT, chlordane, dieldrin and lindan. There are different reasons for the accumulation of obsolete stocks in Ethiopia. The major reason is excessive and uncoordinated donations of pesticides by donors and purchases by Ethiopian government. For example, out of 93 tons of obsolete pesticide stocks inventoried in 2005, 67% were obtained by donation, while the rest were imported by the Ethiopian government (Abiye and Hadera, 2005). **Figure 2.2** shows obsolete pesticide stocks in different African countries in which Ethiopia holds more than 1000 tons.

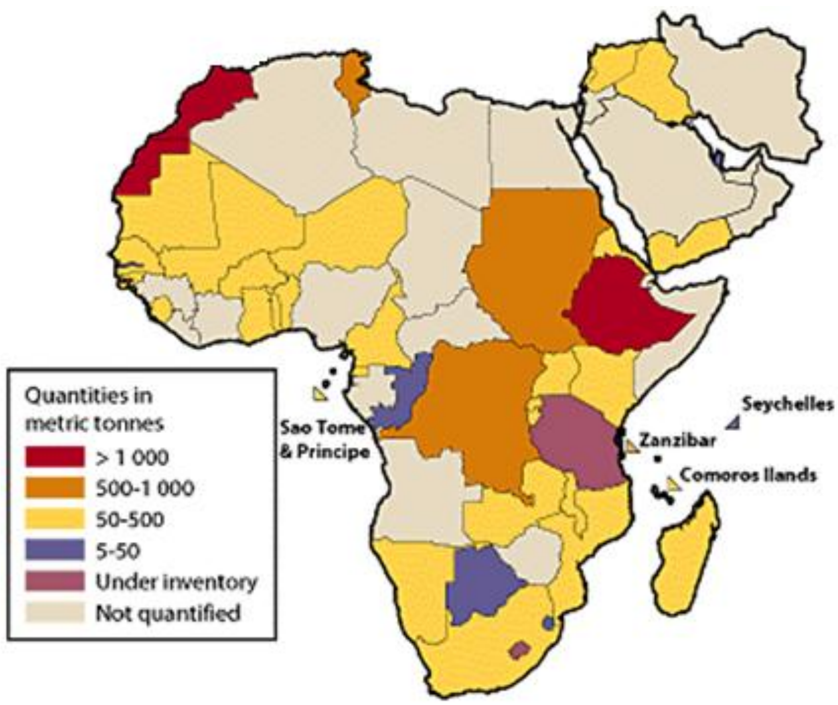


Figure 2.2. Obsolete pesticide stocks in Africa (source: (FAO, 2005)

Small scale peasant farming and some large scale mechanized agriculture in Ethiopia consider pesticides as a best choice for improving agricultural production and quality of life in general. However, evidences in the last few decades have shown that the used pesticides can also be detrimental to human health and the ecosystem (Amera and Abate, 2008). Some of the underlying reasons for these problems are:

- Lack of awareness: illiteracy of the majority of the peasants, inadequate training, lack of appropriate and timely information about the proper use and management of pesticides, inappropriate use of personal protective equipment (PPE), wrong notion that pesticides are the best solution to pest control, and poor guidance about the safe use and handling;
- Inappropriate use of pesticides: wrong mix of different types of pesticides, use of pesticides for unintended purposes, and utilization of empty pesticide containers for domestic purposes;
- Weak law enforcement: late issuance of regulations and guidelines, inadequate implementation of the issued regulations, weak monitoring or follow-up activities to control pesticide usage;
- Disposal problems: there is no disposal facility in Africa; particularly in Ethiopia due to the high cost of disposal and the cost of appropriate destruction of obsolete pesticides is too high.

Due to these problems, the contamination of the different environmental compartments (water, air, soil, food and biota) by pesticides may lead to health problems for human and other non-target organisms in the environment (EPA, 2004). The use of pesticides for different purposes in connection with contamination of environmental matrices and human exposure is illustrated in **figure 2.3**.

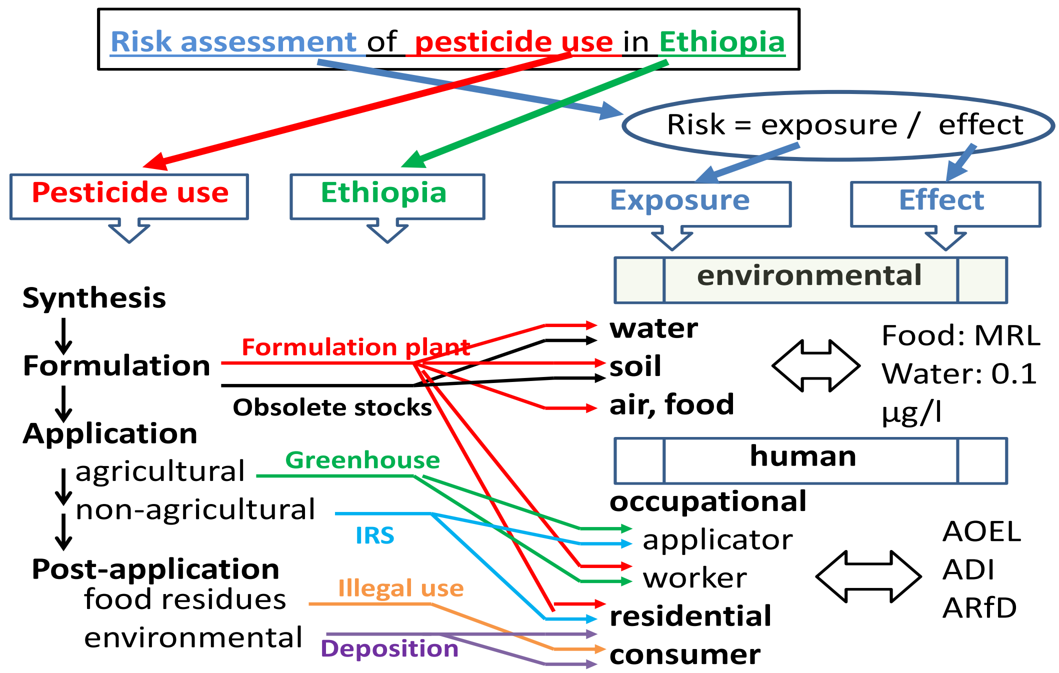


Figure 2.3. Factors determining the contamination of environmental matrices, human exposure and risks to pesticides

2.3. Pesticide residues in food

Even though, pesticides are important for agricultural productivity as well as to improve public health, there is also a great public concern on human health risks (Wanwimolruk et al., 2015). Pesticide used in agriculture have been detected in food and other environmental media, such as in streams, rivers and sediments that are located near to agricultural sites which in turn may aggravate human exposure (Dalvie et al., 2003). As a result, food stuffs such as cereals, fresh vegetables, fruits and others, can be a potential source of pesticide uptake (Radwan and Salama, 2006).

Most of the time, consumers choose vegetables based on their attractive appearance, such as proper size, colour and without damages by insects. Fresh fruits and vegetables are believed to contain a lot of vitamins and minerals. To obtain this attractive appearance, farmers apply more and more pesticides to control pests and diseases. However, the application of pesticides leaves residues on the food items. From a study done in Ghana, different vegetables were contaminated by different classes of pesticides (Bempah et al., 2012). Another study done in Thailand also indicates that 25% of the vegetables purchased from market were contaminated with different pesticides (Sapbamrer and Hongsibsong, 2014). Additionally, from a study in China, on the most widely consumed vegetable in Asia (Chine's Kala) reports that the residue of carbofuran, chlorpyrifos, chlorothalonil, cypermethrin, dimethoate, metalaxyl and profenofos exceeded the respective MRLs in 29% of the samples (Wanwimolruk et al., 2015).

In a study done in Nigeria, residues of two pesticides were detected in maize and up to 10% of these pesticide residues were above the MRL (Ogah and Coker, 2012). Similarly, a study done in Togo also describes that drinking water, maize, and cow pea were contaminated with organochlorine pesticides such as HCH, DDT, aldrin, dieldrin, endrin, heptachlor, heptachlor-epoxid, and endosulfan (α and β) (Mawussi et al., 2009). The other crop which possibly contains pesticide residues is coffee beans. A coffee crop also suffers from diseases like fungi and others. Application of pesticides often leads to a residue in the final coffee product. A fungicide, flutriafol, was found in more than 30% of the green coffee beans samples in Brazil. A similar study shows that there was a significant increase of coffee contamination by pesticides over the years (de Oliveira et al., 2016).

Pesticides are also detected in food from animal sources such as milk. A study done on cow milk collected from the market in Uganda reveals that organochlorine pesticides like lindane, aldrin, dieldrin, endosulfan, DDT and its metabolites were detected mostly above the MRL (Kampire et al., 2011). Chlorinated pesticides, mainly DDT and its metabolites were also detected in cheese, yogurts and fresh milk collected from different communities in Ghana (Darko and Acquaah, 2008). In general, chemical pesticides are detected in raw agricultural crops and also in processed foods in most developing countries (EFSA, 2009).

Contamination of food by pesticides potentially affects human health and food safety. The major health concerns from exposure to pesticides are: cancer, damage to the reproductive system, endocrine disruption, central nervous system, asthma and health problems in other body parts (Ritter et al., 2006; Gilden et al., 2010).

2.4. Human exposure and toxicity of pesticides

Pesticides are developed through strict regulation to function with reasonable certainty and with minimal impact on human health and the environment. However, major concerns have raised about health risks due to occupational exposure, residues in food and in drinking water (Damalas and Eleftherohorinos, 2011). Occupational exposure to pesticides happens during production, transportation, mixing/loading and application of the pesticides in field (Maroni et al., 2006; Damalas and Eleftherohorinos, 2011). There are different factors that determine occupational pesticide exposure such as frequency, duration, intensity, method of application and safety behaviour, as well as the physicochemical and toxicological properties of pesticides (Hoppin et al., 2006). In occupational exposure, the group of people at highest risk are persons working directly with pesticides, family members of the pesticide applicators and agricultural workers. These exposure arises from accidental spills of pesticides, leakages, incorrect use of equipment, and non-compliance with safety guidelines (Fenske, 1997; Issa et al., 2010). Compared to environmental exposure, occupational exposure is often at a relatively high dose, even if it is short or long term exposure (Damalas and Eleftherohorinos, 2011).

The other route of exposure to pesticides for non-professional users is, through consumption of contaminated food. This exposure depends on the choice of food and how much of that specific food is eaten (Lu et al., 2006). Pesticide residues in food usually results from direct field application (Enault et al., 2015). The climatic conditions in Africa promote the proliferation of pests and weeds which results in a need for pesticide use which is considered as a major public health concern among the African population (Nweke and Sanders III, 2009). Human beings are on top of the food chain and it is estimated that over 90 percent, in particular chlorinated pesticides, accumulate in the human body by food consumption (Center for Food Safety, 2006). Exposure through air, drinking water and dermal contact is less. DDT accumulates in the fat tissue of the human body and excretes via breast milk which may adversely affect the infants health (Wong et al., 2005). Moreover, prenatal exposure to chlorinated pesticides has a potential adverse effect on fetal development (Guo et al., 2014).

According to Renwick, (2002), the toxicity of pesticides can be evaluated at different time frames and is classified mainly as acute (short term exposure) and chronic (long term exposure).

Intake of pesticides may not result in adverse health consequence in consumers unless it is taken in excess. So, it is important to understand how high the dose of intake is and for how long a person is exposed (Hamilton et al., 2004). Exposure to pesticide residues during one day time (24 hour exposure) is important to explain acute risk. The acute exposure can be substantially higher than average because the consumption of food on a single occasion can be very large compared with typical long-term consumption. Chronic exposure takes into account 'non-consuming' days as part of producing an 'average' diet for long-term consumer risk assessment. Organophosphates and carbamates (cholinesterase enzyme inhibitors) are the commonly reported pesticides that cause acute pesticide poisoning and sometimes death (Colovic et al., 2013). The acute effects are abdominal pain, headache, nausea, vomiting, diarrhoea etc. and the long term effects such as cancer, depression, neurological deficits, genetic disorders, neurodevelopment delays, endocrine disruption, respiratory distress, and impact on immune system (Andersson et al., 2014). The chronic or long-term toxicity refers to adverse effects occurring as a result of repeated exposure to an agent on a daily basis, or exposure to that agent over a large part of the organism's lifespan. The reference end point for acute toxicity is the acute reference dose (ARfD) while, for chronic toxicity is the acceptable daily intake (ADI) (Solecki et al., 2005). Once the human exposure level is above these two reference values, risk assessment should be an important issue of concern.

2.5. Risk assessment

To determine the possible acute and/or chronic side-effects to a given pesticide residue from consumer exposure, risk assessment needs to be done. As part of risk assessment, exposure assessment of the pesticide residues found in the food is done by combining the amount of food consumed and the amount of pesticide residues present in the food. For food safety evaluation, the obtained exposure values are compared with ADI for chronic risk assessment and with ARfD for acute risk assessment (Keikotlhaile et al., 2011). Risk assessment (RA) of a chemical is a process intended to calculate or estimate the risk for a given target system following exposure to a particular substance. RA takes into account the inherent characteristics of a substance of concern as well as the characteristics of the specific target system. The risk assessment process comprises four major steps **figure 2.4.**

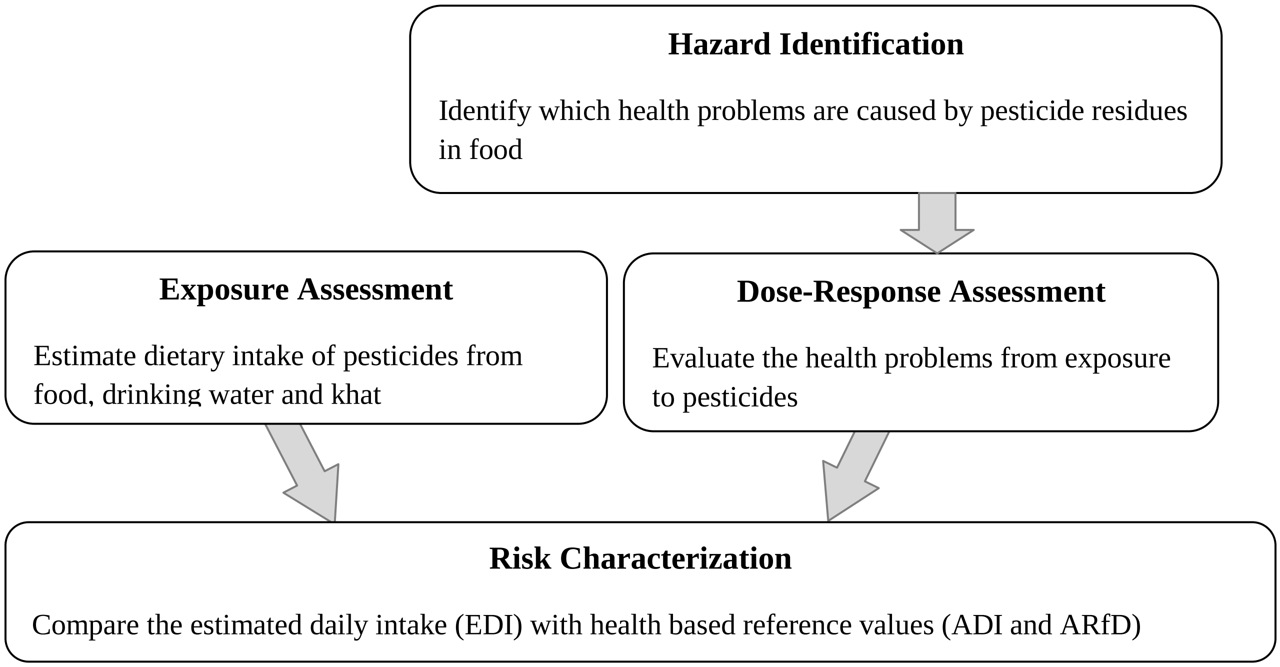


Figure 2.4. Risk assessment steps

2.5.1. Hazard identification

Hazard identification is the first step in the risk assessment process and it is the identification of a risk source capable of causing adverse effect to humans or species in the environment, together with a qualitative description of the nature of these effects. This activity is largely a qualitative evaluation of the risk issue and preliminary examination of information that can be analysed in the subsequent steps of the risk assessment. In toxicological risk assessment, the major focus of hazard identification is to determine whether sufficient evidence exists to consider a chemical as a cause of an adverse health effect (Lammerding, 2000).

2.5.2. Exposure assessment

Exposure assessment is one of the key elements in the risk assessment process. Exposure assessment is defined as "the qualitative and/or quantitative evaluation of the likely intake of biological, chemical, and physical agents via food as well as exposure from other sources". It is the estimation of how likely an individual or a population will be exposed to a chemical of concern and how much of that chemical is taken up in the body through consumption of food, drinking water and others (Lammerding, 2000). To assess exposure of chemicals in food, the three most important aspects to be considered are: (i) how to determine quantitatively the presence of a chemical in food; (ii) how to determine the consumption patterns of the individual food items containing the relevant chemicals; and (iii) how to integrate both the likelihood of consumers eating large amounts of the given food and the likelihood of the relevant chemical being present in these foods (Kroes et al., 2002). The input data used in exposure assessment is obtained from supervised field residue trials, national pesticide monitoring programs and food consumption surveys. Exposure assessment can be done for acute or chronic exposures, where acute exposure estimated for a period of 24 hours, while chronic exposure covers the average daily exposure through the entire life.

2.5.3. Food consumption data

Food consumption data are the essential part to determine the dietary exposure to pesticides. The data reflect which individuals or groups consume in terms of solid foods, beverages, including drinking-water, and dietary supplements. There are four types of dietary assessment methods to collect food consumption data, such as diet history, food frequency questionnaire (FFQ), dietary records and dietary recall (EFSA, 2009). The quality of data from food consumption surveys depends on the survey design, the data collection tools used, the motivation to respond and the memory recall ability of the respondents. The methods that are suitable for both acute and chronic risk assessment are dietary records and dietary recall. The most appropriate source is the one that measures actual consumption instead of available food supply. Dietary recall is a method used to collect consumption data, which involves asking the consumers to recall the actual food intake for the past 24 or 48 hours or previous days. The most common dietary recall method is the 24 hours recall. The data required for acute exposure assessment is, data on the consumption of large portion size (for consumer population only) for a single consumption day, for the edible part of the food and body weight of population who consume the food. While for chronic exposure the non-consumer population are also included, considering the probability of consuming through their life time (European Food Safety Authority, 2011).

2.5.4. Dose-response assessment

The dose determines the poison, is a traditional dogma of toxicology. A dose-response assessment is the determination of the relationship between the magnitude of exposure to a risk source and the magnitude and frequency of the associated adverse effects. It analyses the total amount of an agent absorbed by a group of population and the changes developed by reactions with the body. As the dose increases, the measured response also increases. The dose effect relationship for endocrine disrupting chemicals is different and the curve is non-monotonic i.e. there is non-linear relationship between the dose and the effect. Endocrine disrupting chemicals have an effect at low dose below the lowest dose at which a biological change for a specific chemical has been measured before i.e. any dose below the lowest observed adverse effect level (LOAEL). This challenges the traditional dogma of toxicology “the dose makes the poison” (Vandenberg et al., 2012; Lagarde et al., 2015). Therefore, care should be taken in dealing about the risk assessment of endocrine disrupting chemicals.

2.5.5. Risk characterization

Risk characterization is an integral component of the risk assessment process for both ecological and human health. It’s an estimate of the probability of occurrence and severity of adverse effect or event in a given population under defined exposure conditions. Or it is an integration of evidence, reasoning and conclusions collected in hazard identification, dose-response assessment and exposure assessment. It is the estimation of the probability, including attendant uncertainties, of occurrence of an adverse effect if an agent is administered, taken or absorbed by a particular organism or population. The potential risk is based on the assessment of end points such as the ADI and ARfD derived from dose-response tests. If the risk is higher than these two assessment end points, then a risk of exposure is happening (Hamilton and Crossley, 2004). The risk index (RI) for human exposure can be calculated using the following formula:

RI = Exposure (Intake)/ Effect (ADI or ARfD)

Where:

RI = Risk Index

ADI = Acceptable Daily Intake, and

ARfD = Acute Reference Dose

2.5.6. Dietary exposure models for pesticide residues in food

Consumer risk assessment is a crucial step in the regulatory approval of pesticides on food crops (Hamilton et al., 2004). The process of conducting dietary risk assessment involves the determination of chemical residues in food or beverages and the calculation of the exposure based on the consumption data for that specific food or beverages (Baker et al., 2001). To quantify dietary exposure most often exposure models are applied which are important to support dietary risk assessment. The dietary exposure models vary in complexity depending on the detail of the exposure assessment. Deterministic and probabilistic models are the two important dietary exposure assessment models.

2.5.6.1. Deterministic dietary exposure model

The deterministic exposure model can be used as a simple exposure modelling tool which relies on fixed values (point estimates) derived from data. The deterministic calculation is done by multiplying a fixed value of the food consumed and residue concentration, usually the mean or 97.5 percentile values (worst case scenario) (Kroes et al., 2002). Deterministic exposure models are used as a low tier approach to determine whether there is an indication of concern for the defined exposure. It makes part of the regulatory decision making guidelines due to their simplicity, rapid and inexpensive character (EFSA, 2012a). The deterministic model does not include information about variability in potential exposure to the exposed population. When resource allows probabilistic assessment, it is preferred than deterministic assessment.

2.5.6.2. Probabilistic dietary exposure model

Probabilistic dietary exposure models are the proper representative of the true system. These models take into account the distribution of one or more model parameters to represent real variation and generate more realistic exposure estimates. Most of these distributional models are based on Monte Carlo simulations and referred as Monte Carlo models (Hamilton et al., 2004). These distributional models provide a range of risks throughout the population distribution and provide quantitative information about variability and uncertainty. The simulation is repeated for a certain number of iterations (e.g. 100,000) using statistical software such as @risk or Monte Carlo Risk Assessment (MCRA) and results in an intake curve for the population of concern (Kettler et al., 2015). However, the model requires time and resources for additional data generation. In general, this model is important in risk assessment to assure food safety.

Chapter Three: Pesticide residue evaluation in major staple food items in Ethiopia

Redrafted from: Mekonen S., Ambelu A. and Spanoghe P. (2014), Pesticide Residue Evaluation In Major Staple Food Items in Ethiopia Using The QUECHERS Method: A Case Study From The Jimma Zone, Environmental Toxicology and Chemistry, Vol.33. No.6. 1294-1302, DOI: 10.1002/etc.2554.

Abstract

Even though, the application of pesticides is important for protection of agricultural crops from pests, their residues may remain in the crops as a contaminant. To determine the level of contamination of the different commonly consumed food commodities (staple foods) in Ethiopia, samples of maize (*Zea mays*), teff (*Eragrostis tef*), red pepper (*Capsicum annuum*), coffee (*Coffea arabica*), lentil (*Lens culinaris*) and wheat (*Triticum spp.*) flour were collected from a local market in Jimma zone, southwest Ethiopia. Samples were analysed for the presence of cypermethrin, permethrin, deltamethrin, chlorpyrifos ethyl, DDT and its metabolites, and endosulfan (α, β). For the analytical procedure, the Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) extraction method with dispersive Solid Phase Extraction (d-SPE) clean up technique was applied. The findings of this study indicate that the QuEChERS method is valid for the analysis of the pesticides under study as the recoveries of most pesticides were in the acceptable analytical range (70-120%) with a good repeatability (% RSD < 20). The limit of detection (LOD) and limit of quantification (LOQ) varied between 0.001 to 0.092 µg/g and 0.002 to 0.307 µg/g, respectively. This designates that the method can fulfil the analytical requirements for pesticide residue analysis. DDT, endosulfan, cypermethrin and permethrin were the major pesticides detected in most food items. This is an indication of high utilization of these pesticides in the study area. The concentration of the pesticides in the food items varied from 0.011 to 1.115 mg/kg food. All food items contained one or more pesticides and about one third of the pesticides were above the maximum residue limit (MRL) set by Codex Alimentarius. Such contamination is an indication of illegal use of pesticides in the study area. The organophosphate pesticides such as malathion, diazinon and chlorpyrifos ethyl were detected in dehulled lentil and wheat flour with a mean concentrations below the MRL set by Codex Alimentarius. The concentration of most environmentally persistent pesticides such as DDT in coffee pulp was significantly different (p-value < 0.01) from other food commodities except for red pepper. This indicates that, red pepper and coffee pulp were highly contaminated by DDT. Therefore, there is a need for a good pesticide monitoring program and evaluate food safety for the people in Ethiopia.

3.1. Introduction

Wide spread application of pesticides for agriculture and disease control leave residues on crops which may affect human health and the environment (Ogah and Coker, 2012). In areas where there is widespread use of pesticides, food safety has become a number one public health concern (Aktar et al., 2009). Pesticide residues can be found in different food items such as dairy products, cereals, fruits, vegetables, cash crops like coffee and others (Lesueur et al., 2008; Ahmed and Zaki, 2009; Keikotlhaile, and Spanoghe, 2011; Lozowicka et al., 2012; Bempah et al., 2012; Gebremichael et al., 2013; Kapoor et al., 2013). In most developing countries the use of chlorinated pesticides is common. For example in Nigeria, there is evidence of excessive use of organochlorine pesticides (OCPs) by farmers in production of maize (*Zea mays*) (Tijani, and Nurudeen, 2012). Due to this, about 96% of the maize samples contained residues of one or more OCPs (Ogah, 2012). Additionally, in Ghana organophosphate, organochlorine and pyrethroid pesticides were detected in vegetables and fruits (Bempah et al., 2012). Moreover, studies done in Egypt, showed that cow milk was contaminated with OCPs (Ahmed and Zaki, 2009). In addition to these, obsolete pesticides are documented as one of the major problems in Africa, particularly in Ethiopia (Amera and Abate, 2008). OCPs are often detected in different food commodities mainly due to environmental contamination from field application and leaching from dumped obsolete stocks (Darko and Acquaah, 2008). In Ethiopia, indoor and outdoor application of pesticides is a daily practice to increase productivity and to protect different food items from various pests before and after harvesting. In addition, the Federal Ministry of Health of Ethiopia is also applying indoor residual sprays (IRS) such as DDT and dieldrin to protect people against mosquito transmitted diseases (Bekele et al., 2012). These applications potentially contaminate different food items produced in Ethiopia and may have an impact on the health of the public.

The most common food items produced in Ethiopia are cereals such as maize (*Zea mays*), teff (E*ragrostis tef*), sorghum (*Sorghum bicolor*), millet (*Pennisetum glaucum*), coffee (*coffea arabica*) and dairy products (Gebremedhin et al., 2007). Maize is one of the major crops produced and a cheaper crop in Ethiopia. This may be the reason that maize is one of the major staple food commodities for millions of people in the country, in particular to the Jimma zone (Sori and Ayana, 2012). According to Gebremedhin et al. (2007), maize is the most important crop in Ethiopia for household consumption (accounting for 65%) and covers about 47% of the cultivated area in the country. In order to increase the yield of maize different hybrid varieties of maize are cropped in Ethiopia. However, the hybrid varieties of maize can easily be affected by different insects in the field and during storage after harvesting. As a result different pesticides are applied and their residue may remain on the maize crop (Demissie et al., 2008).

Teff (*Eragrostis tef*), is primarily grown in Ethiopia and has been used for centuries as the principal ingredient in the diet of Ethiopian people. The principal meal prepared from teff flour is called injera which is a big flat bread or pancake, eaten alone or with any kind of sauces prepared from meat, vegetables and other ingredients. In addition, teff flour is used for the preparation of porridge and kita (non-fermented bread) (Hrušková, 2012).

Coffee (*Coffea arabica*), is also a popular cash crop in Ethiopia. Ethiopia is Africa’s biggest coffee producer and exporter, followed by Ivory Coast and Uganda and it has been contributing to more than 4% of the world coffee production (ICO, 2012). Along with production, coffee is the most widely consumed stimulant beverage in the country as mentioned in chapter two. The annual per capita consumption of coffee in Ethiopia is about 2.4 kg which is comparable to the level of the leading coffee consuming countries (Zeru, 2006).

Red pepper (*Capsicum annuum*), is the other most ancient cultivated vegetable grown and consumed in Ethiopia (Esayas et al., 2011). For most Ethiopians, food is considered as tasteless without red pepper. The fine powder is an indispensable flavouring and colouring ingredient in a common traditional sauce called “Wat” (Nsabiyera et al., 2012). Red pepper is the major spice produced in more than nine districts of Jimma zone (Nsabiyera et al., 2012) and 50% of the production goes directly to the local consumers (Shumeta, 2012). Therefore, if this vegetable is contaminated by pesticides, there will be human exposure and a health risk can happen.

Ethiopia is also a major player in global production of legumes such as lentil (*Lens culinaris*) and mostly devoted to produce red lentil. Lentil is the most important legume for human consumption because of its high protein content (up to 35.5%) and Ethiopia produces lentil for local consumption as well as for export (Sarker and Kumar, 2011). Lentil is important to prepare a traditional sauce called “misir wot or lentil stews”. Before the preparations of misir wot, lentil is semi-processed to dehull all the upper cover and is removed as dirt (personal observation).

Pesticides may be sprayed for control of lentil from pests and their residues may remain on the crop. So analysing this type of crop is important for the safety of consumers.

Wheat (*Triticum spp.*) is also an important cereal in Ethiopia. It ranks fourth in area coverage next to teff, maize and sorghum (Central Statistical Agency, 2008). Wheat flour is used for the preparation of bread, porridge (locally called genfo), kita, traditional flat bread called injera and local traditional beer called “tella”. Even though, wheat is highly produced and consumed in Ethiopia; it may also be contaminated with pesticides sprayed for the control of weeds, insects and other pests such as fungi, rodents and others. Daba et al. (2011), analysed wheat grain collected from different regions of Ethiopia and detected organophosphate pesticides such as malathion and diazinon and organochlorine such as aldrin and DDT. The detection of these pesticides will have an effect on consumers’ health.

The agricultural office in Jimma zone reported that different types of pesticides are necessary to guarantee a high productivity in the area. Pyrethroids (permethrin, cypermethrin and deltamethrin), and organophosphates (chlorpyrifos ethyl, malathion, diazinon) are the major pesticides applied in agricultural fields. Organochlorine pesticides (endosulfan and DDT) may enter the environment by indoor residual spraying (IRS) for malaria control, from contaminated surfaces at storage places and from the presence of obsolete pesticides in areas nearby (Mustefa Temam, 2012, personal communication). The present study hypothesized that application of these pesticides and historical use of some of the persistent pesticides such as DDT may results in traces of residues in food items. The residues of pesticides can be found in all environmental compartments however, the highest risk for consumers is through consumption of pesticide contaminated food as 90% of residues, in particular organochlorine pesticides, accumulate in the human body due to food consumption (Center for Food Safety, 2006; Price, 2008; Taha et al., 2013).

From the accessible scientific literature in Ethiopia, there is almost no information regarding pesticides residues in maize, teff, red pepper and coffee except, for wheat and khat (Daba et al., 2011), cow and human milk (Gebremichael et al., 2013). This study presents data on the level of pesticide residues in selected commonly consumed food commodities sold in the local market of Jimma zone, southwest Ethiopia.

The quality of the analysis of the pesticide residues in food always depends on the available analytical methods. Determination of pesticide residues in different food items using the QuEChERS method is documented to give a better result and recovery compared to classical techniques of liquid-liquid extraction (Document N° SANCO/12495/2011, 2011). The classical extraction methods often need a large sample size because of multi-stage procedures and need more clean up steps. As a result, classical methods are time consuming, labour intensive, expensive and can produce a considerable amount of waste in the environment (Wilkowska and Biziuk, 2011). Nowadays, analytical chemists prefer to use the QuEChERS method with a dispersive solid phase extraction clean-up which is streamlined and effective for the analysis of diverse residues in food matrices (Wilkowska and Biziuk, 2011). The QuEChERS method has the following advantages: high recovery, high sample throughput, low solvent and glass ware usage, less labour and bench space, lower reagent costs, ruggedness and low worker exposure (Anastassiades et al., 2003; Lehotay et al., 2005). The aim of this study was also to evaluate pesticide residues in selected staple food items of Ethiopia using the QuEChERS method as the extraction and clean-up technique in the analysis.

3.2. Materials and Methods

3.2.1. Sampling

Samples of commonly consumed (staple food items) such as maize (*Zea mays*), teff (*Eragrostis tef*), red pepper (*Capsicum annuum*) and coffee (*Coffea arabica*) were collected from the local market in Jimma zone, southwest Ethiopia. This local market is a big market in the study area in which most agricultural crops are collected for sale. During sample collection, the retailers in the local market were interviewed about the origin of the crops and from how many farmers they bought these crops. Most of the retailers mentioned that they bought one type of food item at least from five farmers and pooled (not statistically) each food item before bringing to the market for sale. Based on this experience, a retailer was chosen as a crop sample source, only if the target crop was pooled at least from five farmers of a similar crop production location. Locations where staple food items were commonly produced and coming to the local market were mentioned in **figure 3.1.** In addition to these food items, semi-processed lentil (*Lens culinaris*) and wheat (*Triticum spp.*) flour were also collected from the shops in the same market. This is because; semi-processed foods such as dehulled lentil and ground wheat are usually sold in shops rather than open markets. The collected samples each representing 250 g were sealed and labelled with a unique sample identity and placed in a clean hard paper envelope within polyethylene plastic bags. Then the samples were transported to the Laboratory and frozen at -20 ^{0}C until the analysis was done.

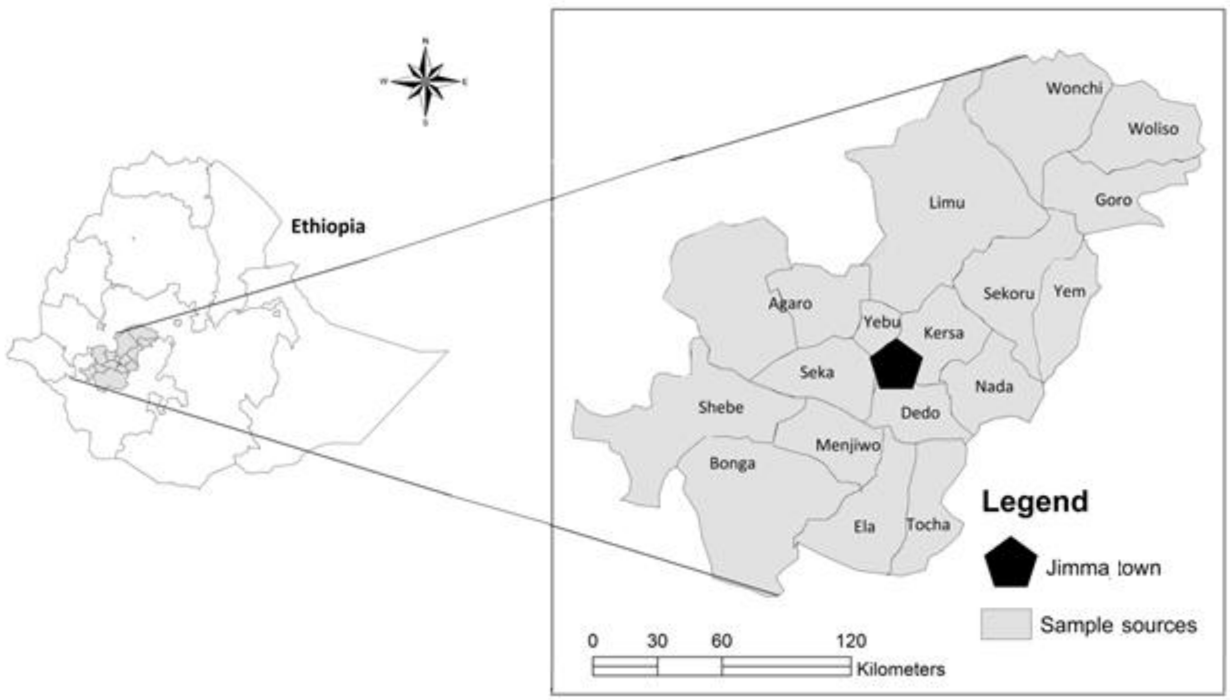


Figure 3.1. Map of the study area indicating the food sample sources

3.2.2. Sample preparation

A 250 g of each sample was first ground and homogenized using a household mill equipped with stainless steel knife (Fritel quality grinder OZX48-6cups) following the procedures used by (Dasika, et al., 2012). After each sample was ground, the grinder was cleaned by washing to avoid cross contamination. The ground and homogenized samples were packed in plastic bags and stored at -20 °C until extraction was done.

3.2.3. Reagents and materials

Analytical grade acetonitrile was supplied by VWR-PROLABO and HPLC grade n-hexane and acetone were obtained from ALLtech. Thermo Fisher Scientific supplied: magnesium sulphate

($MgSO_4$) to remove water from organic solvent, sodium acetate (NaAc) to absorb remaining water content in samples, 50 mL polypropylene centrifuge tube, 15 mL dispersive solid phase extraction tube (d-SPE tube) packed with primary secondary amines (PSA) for the removal of organic acids and polar pigments among other compounds, and octadecyl (C18) to remove lipids and sterols from the matrix. Pesticide standards, DDT (p,p'-DDE, p,p'-DDD, o,p'-DDT and p,p'-DDT), endosulfan (α and β), cypermethrin, permethrin, deltamethrin and chlorpyrifos ethyl with highest analytical purity were obtained from Supelco and delivered by Sigma-Aldrich logistic. The respective purity of reagents, materials and pesticide standards are presented in **table 3.1.**

Table 3.1. Pesticides standards, reagents and QuEChERS materials with their respective analytical purity

Pesticides standards	Purity (%)	Reagents	Purity (%)
p,p'-DDE	99.9	Acetonitrile	99.9
p,p'-DDD	99.3	n-hexane	95
o,p'-DDT	100	Acetone	99
p,p'-DDT	99	glacial acetic acid	98.5
endosulfan α	98.5	MgSO4	98
endosulfan β	98	NaAC	99
deltamethrin	99	PSA	100
permethrin	98		
cypermethrin	98		
chlorpyrifos ethyl	99.5		

MgSO4 = Magnesium Sulphate, NaAC = Sodium Acetate, PSA = Primary Secondary Amine, C18 = Octedecyl

3.2.4. Analytical equipment

The pesticides were determined by gas-liquid chromatography with electron capture detector (GC-ECD, Agilent Technologies 6890N) with an auto sampler. HP-5 capillary column of 30 m x 0.25 mm i.d. x 0.25 µm film thickness coated with 5% phenyl methyl siloxane (Model number Agilent 19091J-433) was used in combination with the following oven temperature program: initial temperature was 80°C, ramp at 30°C min^{-1} to 180°C, ramp at 3°C min^{-1} to 205°C, held for 4 min, ramp at 20°C min^{-1} to 290°C, held for 8 min, ramp at 50°C min^{-1} to 325°C. For deltamethrin the oven temperature was maintained initially at 130°c, held for 1 min, ramp at

30°C min^{-1} to 280°C, held for 16 min and ramp at 50°C min^{-1} to 325°C, held for 3 min. The total GC run time was 27.92 min. Helium (99.999% purity) was used as a carrier gas at a flow rate of 20 mL min^{-1} and nitrogen as a makeup gas at a flow rate of 60 mL min^{-1}. An aliquot of 1µL was injected in split mode at Split ratio of 50:1 and injection temperature of 280°C. The pesticide residues were detected with electron capture detector (µ-ECD) operated at a temperature of 300°C. For the determination of pesticides, each food samples was analysed in triplicate and the mean concentration was computed accordingly.

3.2.5. Analytical method validations: limit of detection (LOD), limit of quantification (LOQ), repeatability (% RSD), linearity and recovery

The LOD and LOQ were determined by preparing matrix spikes at a low level near the expected detection limit. The LOD and LOQ in this study were determined by spiking a different concentration of the pesticides under study (chlorpyrifos ethyl, cypermethrin, permethrin, deltamethrin, DDT and its metabolites (p,p'-DDE, p,p'-DDD, o,p'-DDT and p,p'-DDT), and endosulfan (α and β)) in blank samples of maize, coffee beans, teff and red pepper in six replicates. The LOD and LOQ were calculated by multiplying the standard deviation from the replicates by 3 and 10, respectively (Butler et al., 2008). The accuracy and precision i.e., % recovery and % RSD, respectively were determined with recovery experiments by spiking pesticide standards in to laboratory blank sample of crops in six replicates with the same concentration of each pesticide. Afterwards, the % RSD was calculated by dividing the standard deviation with the average concentration. The recovery was calculated by dividing the recovered concentration by the spiked concentration. The spiked samples were left for one hour before extraction to allow the pesticides partition into the food matrices (Bempah et al., 2012).

The linearity was determined by preparing a stock solution of pure standards of the studied pesticides and diluting them to produce different concentrations. The standard solutions of the pesticides ran on GC-ECD under the set chromatographic conditions to produce five point calibrations ranking from 0.01 to 1 mg/L. All the standard curves for each pesticides show, $r^2 > 0.995$ which fulfil the requirements of good analytical method.

3.2.6. QuEChERS extraction method

The extraction and clean-up of the spiked samples and blank samples for each matrix was done using the modified QuEChERS procedure with dispersive Solid Phase Extraction clean up (d-SPE) method. The procedures were based on the AOAC official method 2007.01 (Lehotay, 2007) with slight modifications. This method gives high quality results for the analysis of many pesticides in food (Dasika, et al., 2012). The Procedure for spiking and extraction is described as follows: (i) 10 g of comminuted and homogenized blank sample of maize, coffee beans, teff and red pepper was weighed in 50 mL centrifuge tube on an analytical balance (Sartorius AG): (ii) 10 mL of deionized water was added; (iii) blank samples were spiked with 25 µL of each pesticide standard in each matrix in six replicates; (iv) 15 mL of acetonitrile (ACN) containing 1% glacial acetic acid (v/v) in each sample was added using a solvent dispenser; (v) the tube was tightly caped and shaken gently for 1 min to facilitate contact between the solvent and the sample; (vi) 6 g anhydrous $MgSO_4$ and 1.5 g NaAc was added and the sample was shaken by hand vigorously for 5 min to increase sample through put (Lehotay et al., 2005)); (vii) the sample was centrifuged at 3000 rpm for 5 min.; (viii) to clean the extract, the upper organic layer was taken into a dispersive solid phase extraction tube (d-SPE) containing 300 mg PSA, 900 mg $MgSO_4$ and 150 mg C18, shaken by hand for 30 s and then centrifuged; (ix) a 5 mL aliquot of cleaned extract was then taken and evaporated to dryness using a rotavapor (Rotary evaporator N18673, Switzerland) at a temperature of 40°C; (x) the cleaned extract was reconstituted with 2 mL n-hexane/acetone (9:1v/v) for solvent exchange; and (xi) then put into vial for GC analysis. The major procedures are shown in **figure 3.2.**

Figure 3.2 QuEChERS extraction and clean-up procedures

3.2.7. Analysis of food samples

The extraction and clean-up of teff, maize, red pepper, coffee beans, coffee pulp, coffee beans (after removal of the pulp), wheat flour and dehulled lentil, were done following similar procedures for the above method validation tests (section 3.2.6) except spiking. From 250 g of ground and homogenized samples, a representative 10 g subsample was weighed in a 50 mL centrifuge tube on an analytical balance. Then, 10 mL of deionized water was added to the sample and shaken for one minute. Then step iii-xi of the procedures was followed. The cleaning procedure between samples was done by injecting a blank (n-hexane), pesticides standards for calibration and n-hexane again to assure data quality. The sample equivalent (mg/mL) extract was calculated based on the formula suggested by Schenck and Howard-King, (2000).

$Y = a/b*x/z$

Where:

Y= g sample equivalent/ mL of extract

a = g sample analysed

b = mL of solvent added to extract the sample

x = mL of the amount of the cleaned extract taken after the evaporation until dryness

z = mL of hexane/acetone (9:1v/v) added for solvent exchange

3.2.8. Data analysis

The wilcoxon signed rank sum test was performed to evaluate whether there is a significant difference of pesticide residue between the food items. Box and whisker plots were used to present the results. Statistical analysis considered significant at p-value < 0.05.

3.3. Results

3.3.1. Method validation results

The method validation results of the pesticides under study in replicate tests are shown in **table 3.2**. From our findings, the calibration curves obtained for a mixture of ten pesticide standards including isomers and degradation products over a concentration range of 0.04-1 mg/L (chlorpyrifos ethyl and cypermethrin), 0.05-1 mg/L for deltamethrin, 0.01-0.2 mg/L endosulfan (α and β), 0.1-1 mg/L DDT metabolites (p,p'-DDE, p,p'-DDD, o,p'-DDT and p,p'-DDT) shows a correlation coefficient (r2) > 0.995. This indicates the standard curves for all the pesticides are clearly linear. The average recoveries of DDT and its metabolites, permethrin and chlorpyrifos ethyl were in the acceptable analytical range (70-120%). However, the average recoveries of cypermethrin were above 120% in all food matrices of the replicate tests, while, the most lipophilic pesticide endosulfan both α and β isomers shows consistently low recoveries (< 60%) but with good repeatability (% RSD < 10). This may be due to the physico-chemical properties of these two pesticides such as their solubility in water and other organic solvents. LOD and LOQ respectively varied from 0.001 to 0.092 µg/g and 0.002 to 0.307 µg/g for the pesticides evaluated. The percent relative standard deviations (% RSD) are less than 11% for all the pesticides. These results indicate that the method is accurate as most of the pesticides recovered in the required analytical range (70-120%) and the method is also precise as the % RSD < 20.

Table 3.2. Method validation results of the evaluated of food matrices (maize, teff, red pepper and coffee beans)

Pesticides	Spiked conc. (µg/g)	Maize			Coffee beans			Red pepper			Teff		
		% Recovery	LOD (µg/g)	LOQ (µg/g)	% Recovery	LOD (µg/g)	LOQ (µg/g)	% Recovery	LOD (µg/g)	LOQ (µg/g)	% Recovery	LOD (µg/g)	LOQ (µg/g)
chlorpyrifos ethyl	0.2	120.4 (1.4)	0.009	0.031	101.4 (1.0)	0.006	0.021	98.9 (1.6)	0.012	0.041	99.3 (1.6)	0.009	0.031
cypermethrin	0.2	143.2 (9.5)	0.059	0.197	121.7 (9.9)	0.032	0.107	132.2 (9.8)	0.061	0.202	135.1 (10.3)	0.062	0.207
permethrin	0.5	113.5 (4.9)	0.065	0.218	106.3 (8.8)	0.092	0.307	102.9 (4.7)	0.063	0.209	87.0 (5.7)	0.063	0.209
deltamethrin	0.5	123.3 (1.3)	0.022	0.075	135 (0.8)	0.016	0.055	108.9 (1.3)	0.018	0.06	96.9 (1.0)	0.015	0.05
p,p’-DDE	0.5	77.2 (1.0)	0.012	0.041	73 (1.4)	0.01	0.033	80.3 (1.2)	0.016	0.053	78.4 (1.1)	0.016	0.053
p,p’-DDD	0.5	102 (2)	0.028	0.092	80.1(1.7)	0.022	0.072	93.5 (1.5)	0.023	0.076	102.6 (1.5)	0.023	0.076
o,p’-DDT	0.5	86 (1.4)	0.015	0.051	86.7 (1.3)	0.015	0.051	89.4 (3.3)	0.045	0.151	94.1 (2.4)	0.036	0.121
p,p’-DDT	0.5	94.2 (1.6)	0.019	0.064	99 (1.8)	0.02	0.066	104.4 (2.5)	0.03	0.1	101.5 (2.2)	0.03	0.1
endosulfan α	0.05	57.4 (1.2)	0.001	0.002	50.1 (1.1)	0.001	0.004	50.6 (3.3)	0.004	0.014	52.7 (4.3)	0.004	0.014
endosulfan β	0.05	46.5 (1.7)	0.001	0.003	34.3(2.7)	0.001	0.004	44.1 (3.4)	0.005	0.016	38.1 (9)	0.004	0.014

Values in parenthesis indicate % RSD, LOD = limit of detection, LOQ = limit of quantification, % RSD = percent relative standard deviation

3.3.2. Pesticide residues in tested food samples

After validation of the QuEChERS method, the pesticide residues in maize, teff, coffee beans, coffee beans with pulp and coffee beans after removal of pulp were determined. The results reveal that each food item analysed had one or more pesticides **figure 3.3**. This indicates that the environment in which these commonly consumed food items grown or stored are contaminated by pesticides. Specifically, DDT, endosulfan, cypermethrin and permethrin, were the most abundantly detected pesticides. The chlorinated pesticides such as DDT and endusulfan were mostly used for malaria control in the study area. Their presence in the food items may be due to, cross contamination or may be from their persistent nature in the environment. From the staple food items, the red pepper and green coffee beans contained all the pesticides evaluated at varying concentrations. Pesticide residues varied from 0.0321 to 1.148 mg/kg in red pepper, 0.011 to 0.301 mg/kg in maize, 0.014 to 0.351 mg/kg in teff, 0.016 to 1.115 mg/kg in green coffee beans, 0.077 to 1.519 mg/kg in coffee pulp and 0.037 to 0.619 mg/kg in coffee bean after removal of pulp. DDT and its metabolites; p,p'-DDE (19.04%), p,p'-DDD (92.9%), o,p'-DDT (66.67%) and p,p'-DDT (100%) were detected in the food samples. The p,p'-DDT was detected in all food samples compared to the metabolite p,p'-DDE, which indicates the recent use of the pesticide DDT in the study area.

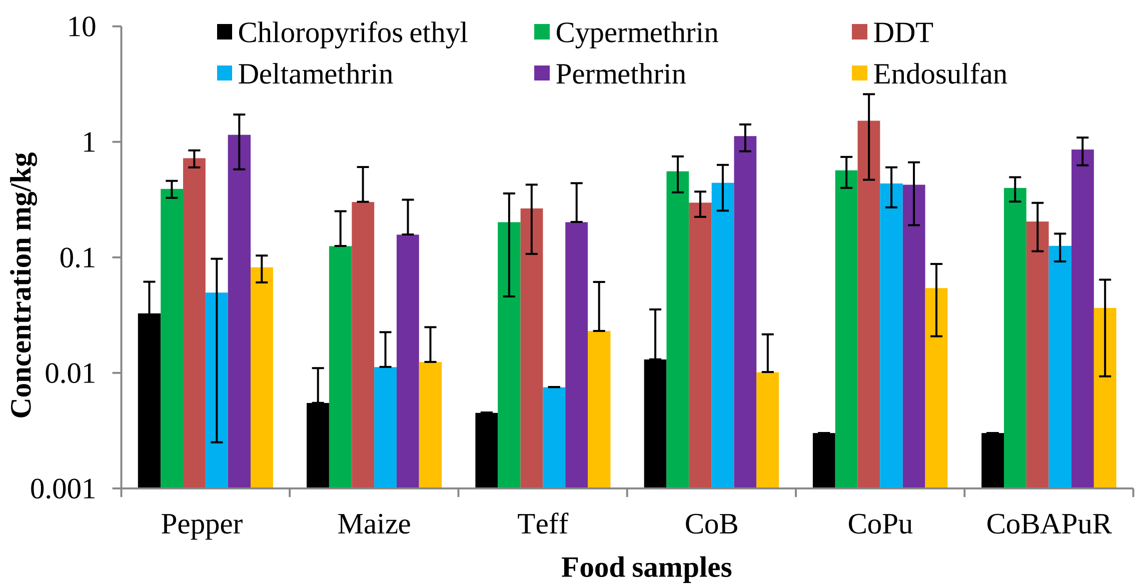


Figure 3.3. Average concentration (mg/kg) of pesticides in evaluated foods: Error bars indicate the standard deviation; CoB = coffee beans; Copu = coffee pulp; CoBAPuR coffee beans after removal of the pulp

Post hoc analyses compared the distribution of pesticides residues between crop samples and illustrated by box and whisker plots **figure 3.4**. This comparison is important to identify which food item is highly contaminated by specific pesticide and need food safety concern. For this statistical comparison, the non-detects were included as being half the limit of detection (1/2 LOD) (Hornung and Reed, 1990). From the box and whiskers plot, DDT is highly distributed in coffee pulp, deltamethrin in coffee beans and coffee pulp, chloropyrifos ethyl in red pepper and coffee beans, cypermethrin and permethrin in red pepper, while endosulfan is highly distributed in red pepper and coffee pulp. All food items contained one or more pesticides at varying concentrations among which red pepper and coffee pulp were highly contaminated food items. This raises the need for safety concerns for the general public consuming red pepper as major food spices and rural Ethiopian community who mostly consume coffee pulp because it is cheaper than the coffee beans (personal observation).

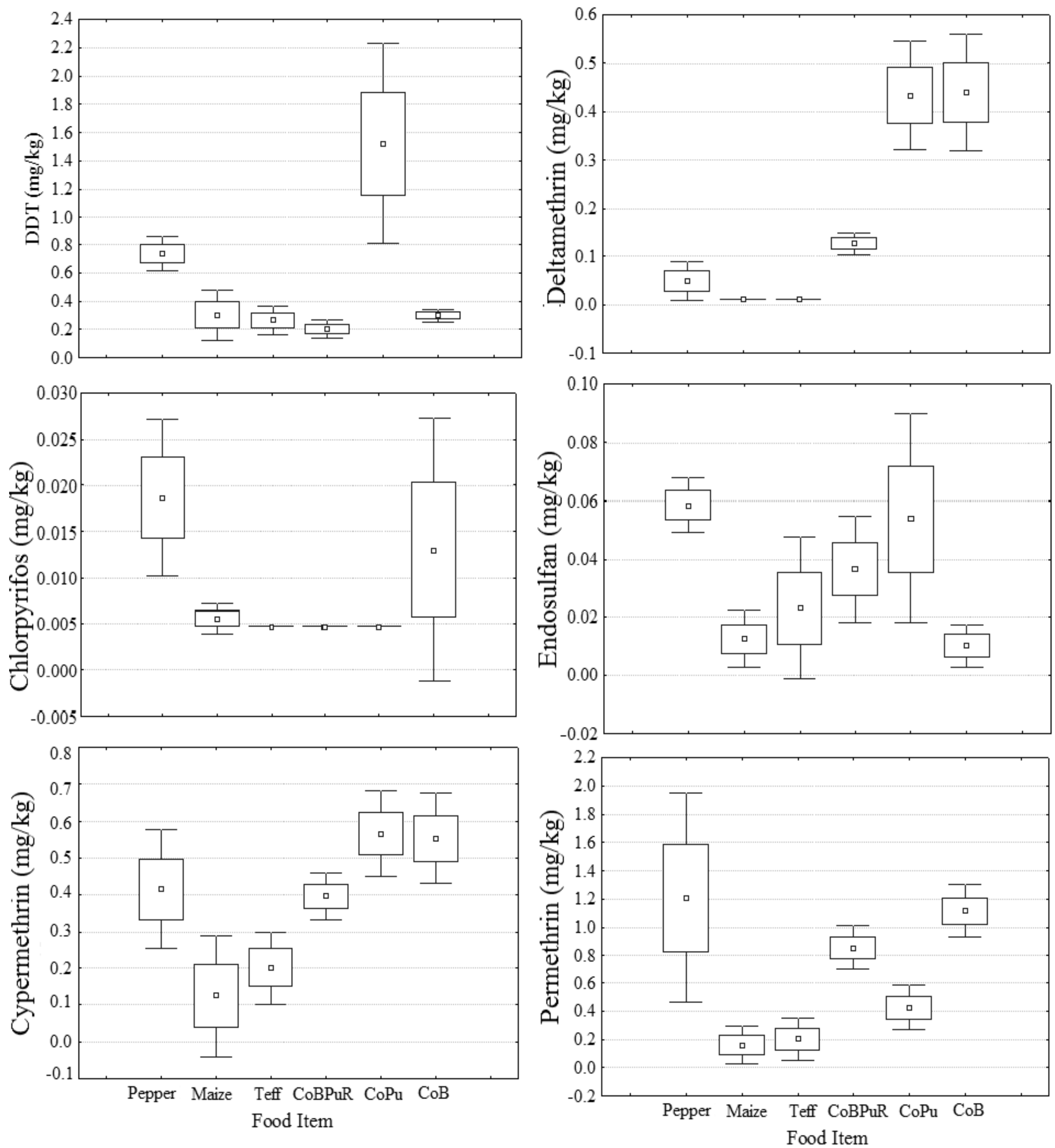


Figure 3.4. The concentration distribution of pesticide residues in evaluated food items. The small square within each box represents the mean; the box represents the mean ± standard error; and upper and lower whiskers represent 95% confidence intervals. Copu = coffee pulp; CoB = coffee beans; CoBAPuR = coffee bean after removal of the pulp

3.3.3. Comparison of pesticide residue with the maximum residue limit (MRL) set by Codex Alimentarius

Pesticide residues in red pepper, maize, green coffee beans, and teff were compared with corresponding MRLs of each pesticide and indicated in **table 3.4**. Hence, there is no national MRL for any pesticide used in Ethiopia, we consider the MRL set by Codex Alimentarius. From all samples analysed, about two third had residues bellow the maximum residue limits (MRLs), while about one third had a residue above the MRL set by the Codex Alimentarius (FAO/WHO, 2013). Cypermethrin exceeded the corresponding MRL (11.9%) most frequently, followed by permethrin (9.52%), DDT (4.76%), and endosulfan, chlorpyrifos ethyl, and deltamethrin (2.38%). The exceedance of MRL by the pesticide residues in the food items indicates that there is illegal use of these pesticides in the study area.

Table 3.4. Comparison of pesticide residues (mg/kg) in food items with the maximum residue limits (MRL)

Pesticides	Red Pepper	MRL	Maize	MRL	Teff	MRL*	CoB	MRL
chloropyrifos ethyl	0.032	2	0.011	0.05	ND	0.05	**0.063**	0.05
cypermethrin	**0.390**	0.1	**0.156**	0.05	**0.351**	0.03	**0.553**	0.05
DDT	0.734	NA	**0.301**	0.1	**0.296**	0.1	0.308	2.0
deltamethrin	**0.069**	0.03	ND	2.0	ND	2	0.440	1.0
permethrin	**1.148**	0.01	0.157	2.0	**0.282**	0.05	**1.115**	0.05
endosulfan	0.0599	5.0	0.012	NA	**0.014**	0.01	0.016	0.2

Values above the MRL are indicated in bold, *The MRL of grains was adapted for teff, MRLs = maximum residue limits; NA = not available; ND = not detected; CoB = coffee beans; CoPu = coffee pulp; CoBPuR = coffee beans after removal of the pulp

3.3.4. Pesticide concentration (µg/kg) in lentil (*Lens culinaris*) and wheat (*Triticum spp.*) flour

To see whether the semi-processed food items such as dehulled lentil and ground wheat powder contain the residues of some pesticides, these two food items were collected from the market and analysed. During screening of these two food samples ten pesticides (DDT, deltamethrin, cypermethrin, permethrin, chlorpyrifos ethyl, pirimiphos methyl, diazinon, malathion, fenpropimorph and 2,4-D) were checked. Seven of the screened pesticides were not detected except malathion, diazinon and chlorpyrifos ethyl. The type of pesticides detected in dehulled lentil and wheat flour were different from other food items such as teff, maize, coffee beans and red pepper.This may be due to the source of the food items for the market. The retailers for teff, red pepper, coffee and maize is brought these food items from the farmers in surrounding study areas, while for dehulled lentil and wheat flour were coming to the market from different factories. Therefore, the pesticides applied may be differed from areas to areas.

The organophosphate pesticides malathion, diazinon and chlorpyrifos ethyl were detected in both lentil and wheat flour even though at low concentrations **table 3.5**. Chlorpyrifos ethyl is detected in all lentil samples and in 86.7% of the wheat flour. Diazinon is detected in 50% and 83.3% of the lentil and wheat flour samples, respectively. While, malathion was detected in all wheat flour and 90.6% of lentil samples. The mean concentration of malathion and chlorpirifos ethyl were far below the MRL in wheat flour and common beans set by the Codex Alimentarius (FAO/WHO, 2013). As there is no maximum residue limit for semi-processed lentil MRL of common beans was considered for comparison. The results indicate that the use of these pesticides for the production of these two crops was with respect to the legal limit of the specific pesticide. Or this can be explained as the semi-processing steps may decrease the pesticide residue. Additionally, the organophosphate pesticides are not persistent in the environment, so that the concentration in the food items may be decreased by degradations.

Table 3.5. Concentration of pesticides (µg/kg) of semi-processed lentil and wheat

Food items	pesticides	mean	Min	Max	StDv	MRL
Lentil	diazinon	0.0012	0.0006	0.0142	0.0032	200*
	malathion	0.0011	0.0002	0.0041	0.0009	2000*
	chlorpyrifos	0.0019	0.0002	0.0121	0.0021	10 *
Wheat flour	diazinon	0.0045	0.0006	0.0443	0.0085	N/A
	malathion	0.0008	0.00001	0.0047	0.0012	200
	chlorpyrifos	0.0009	0.0003	0.0049	0.0011	100

N/A = Not available, * = MRL of common beans

3.4. Discussion

The validation of the QuEChERS method indicates that the % recoveries for most of the pesticides under study, the % RSD, LOD and LOQ were in agreement with the European requirements for residue trials (Document N° SANCO/12495/2011, 2011). This points out that the QuEChERS method is appropriate to determine pesticides in the corresponding food matrices. The average recoveries of cypermethrin were consistently above 120% in all food matrices of the replicate tests. If the recoveries of pesticides are repeatedly high or low in the replicate tests, this outcome is acceptable according Document N° SANCO/12495/2011, (2011). Among the food matrices, the recovery of endosulfan was lower in green coffee bean which was 50.1% and 34.3% for the α and β isomers of endosulfan, respectively. According to Pizzutti et al. (2012) the modified QuEChERS method is known to obtain lower recoveries for lipophilic pesticides such as endosulfan extracted from more fatty matrices like green coffee beans having a 7-17% fat content. Both LOD and LOQ were below the lowest standard concentration which indicates that the analytical method is able to detect and quantify lower concentrations from the food matrices. The % RSD is the measure of agreement or the consistency of the analyte concentrations to each other when the analyses are performed using identical conditions, i.e. same method, same sample, same operator, and same laboratory conditions over a short period of time (Geletu et al., 2009). The % RSDs for all the pesticides studied were below 11%, which is in line with the commonly accepted analytical level (% RSD < 20) (Lehotay and Mastovska, 2005). The result of the present study is consistent with a study done in Portugal on multi-pesticides residue analysis using acetonitril extraction, with a % RSD < 20 for all the pesticides detected (Cunha and Fernandes, 2011).

From the box and whisker plot, a significantly higher concentration of cypermethrin was observed in green coffee bean and coffee pulp relative to maize and teff (p-value < 0.01). This indicates a high variation in contamination among food items. No significant differences were observed for the concentration of chlorpyrifos ethyl between the evaluated food items. This indicates that there may be a similar usage level of chlorpyrifos ethyl by farmers in the study areas. The concentration of DDT in coffee pulp is significantly different (p-value < 0.01) from other food items except for red pepper. This might be due to illegal use of DDT in coffee and red pepper. As indicated in **chapter two**, DDT was used for malaria control in Ethiopia as indoor residual spraying (IRS) (Yewhalaw et al., 2011; Hamusse et al., 2012). There may be cross contamination in the food items in particular for those food items stored in the IRS spraying areas. According to Bempah et al. (2012), DDT is not degraded easily in the environment due to its persistent nature, which may be also the reason for its presence in high concentrations in the food items. In general, lower concentrations of pesticides were observed in samples of maize and teff except for DDT which was above the MRL of grains set by Codex Alimentarious (FAO/WHO, 2013). This may be due to a restricted use of the pesticides evaluated in these food items except for DDT. The presence of DDT in the food items may also come from environmental contaminations or there may also be illegally applied in agriculture. From the coffee samples analysed, the maximum concentration of DDT was found in coffee pulp (1.52 mg/kg); while relatively lower concentration was found in coffee beans (0.30 mg/kg). The concentration in coffee beans were lower compared to the coffee pulp, this might be due to the rich nutrient content of the coffee beans, which may promote the growth of microorganisms that are able to degrade the pesticides (Barragán-Huerta et al., 2007). Additionally, the maximum concentration in coffee pulp may be due to the deposition of these contact pesticides (DDT and its metabolites) on the surface of the crop as the pesticide is used not only during the growing season but also during storage and transportation of the food items.

Compared to recent studies (from 2009 to 2012) in some African countries (**table 3.6)**, the commonly consumed food items in Jimma zone, southwest Ethiopia shows, higher concentrations for all pesticide residues except the residues detected in food items in Nigeria for DDT. This might be explained by the extensive application of pesticides in Ethiopia to obtain a higher production, insufficient knowledge to apply the correct dose/rate and due to poor equipment available to spray the pesticides (Amera and Abate, 2008). Additionally, higher residues may result from historical use and previous environmental contamination, particularly from those compounds demonstrating environmental persistence and accumulation of obsolete pesticides near the study area (Amera and Abate, 2008).

The findings of our study in the analysed food items indicate a lower concentration of the organochlorine pesticides than the study results observed in Nigeria. According to Tijani and Nurudeen (2012), there is excessive use of organochlorine pesticides for the control of insects in Nigeria. Although, DDT is officially banned for agricultural application, contamination of food items and the environment still occurs. This contamination might be explained by drift from indoor residual spraying (IRS) of DDT for malaria control or by leakage from dumped obsolete pesticide stocks (Amera and Abate, 2008). Records indicate that the most persistent and dangerous pesticides such as DDT, aldrin, heptachlor, pirimifos methyl and fenitrothion are the main components in obsolete pesticide stocks which are dumped over 1000 sites in Ethiopia

(Hussien, 2007). Endosulfan was detected in 61.9% of the food items but in lower concentration than the other pesticides studied. Compared to a study done in Togo, the residue in the present study was three times and two times lower for endosulfan α and β, respectively. This might be due to a lower contamination of the food items by endosulfan in our study area or the lower recovery of this pesticide from the evaluated matrices.

Table 3.6. Comparison of pesticide residue in different food items of the present study with findings in other African countries

Country	Pesticides	Concentration (mg/kg)	Food items analysed	Authors
Ghana	p,p'-DDE	0.004-0.041	watermelon, cabbage, mango and banana	Bempah et al, 2012
	p,p'-DDT	0.004-0.038	carrot, watermelon, onion and banana	
	chlorpyrifos	0.003-0.055	watermelon, tomato, pineapple or onion	
	cypermethrin	0.008-0.035	mango, cucumber, papaya or tomato	
	Permethrin	0.004-0.041	cucumber, pear, cabbage and pineapple	
	deltamethrin	0.004-0.044	carrot, pear, onion and pineapple	
Nigeria	p,p'-DDE	0.001-0.175	chili pepper, tomato	Benson et al, 2011
	p,p'-DDD	< LOD-0.172	chili pepper, tomato	
	p,p'-DDT	0.036-0.099	tomato, chili pepper	
Togo	p,p'-DDE	0.005-0.007	cowpea, maize	Mawussi et al, 2009
	p,p'-DDD	< LOD-0.005	maize, cowpea	
	o,p'-DDT	0.002-0.055	maize, cowpea	
	p,p'-DDT	0.011-0.023	cowpea, maize	
	endosulfan α	0.034-0.100	maize, cowpea	
	endosulfan β	0.065-0.090	maize, cowpea	
South Africa	chlorpyrifos	0.01–0.050	Wheat	Dalvie and London, 2009
	cypermethrin	0.008–0.019	Wheat	
	Permethrin	0.014–0.448	Wheat	
Ethiopia	p,p'-DDE	< LOD-0.086	CB,CP, CBAPR, teff, red pepper and maize	This study
	p,p'-DDD	0.049 -0.128	maize, CBAPR, teff, CB, red pepper and Cp	
	o,p'-DDT	0.085-0.193	CB, CBAPR, CP, teff, maize and red pepper	
	p,p'-DDT	0.099 -0.461	CBAPR,teff, maize, CB, red pepper and Cp	
	chlorpyrifos	0.011 - 0.063	CB or CP, teff, maize, red pepper and CB	
	endosulfanα	0.004-0.033	CBAPR, teff, maize, CB, CP and red pepper	
	endosulfanβ	< LOD-0.063	CBAPR, maize, CB, teff, red pepper and CP	
	cypermethrin	0.156 -0.553	maize, CBAPR, teff, CP, red pepper and CB	
	permethrin	0.157 -1.150	maize, teff, CP,CBAPR, red pepper and CB	
	deltamethrin	0.069 -0.157	maize or teff, red pepper, CBAPR, CP and CB	

The food items listed according to the increasing order of pesticide residue, < LOD = less than limit of detection, CB = Coffee Beans; CP = Coffee Pulp; CBAPR = Coffee Beans After Pulp removal.

Comparing the detected residues with the MRL it was found that DDT which was expressed as the total sum of its metabolites: p,p'-DDE, p,p'-DDD, o,p'-DDT and p,p'-DDT, was above the MRL set by Codex Alimentarius (FAO/WHO, 2013) in the two commonly consumed cereals such as maize and teff. This indicates that there is illegal use of this pesticide in the production of these two crops in study area and/or there may be contamination from the environment. Among the metabolites, p,p'-DDE contributed less to the total DDT residue load. On the contrary, p,p'-DDT which was found in all the food samples analysed contributed more to the total DDT residue load. According to the European Food Safety Authority (Alexander et al., 2012), this indicates that there is recent use of DDT in the study area. According to (Bempah et al., 2012), even low exposure to pesticide residues of DDT accumulate and pose a health risk to consumers. Consequently, under the framework of chronic and acute food safety risk assessments, pesticides residue data below MRL should also be considered when assessing food safety (The 2010 European Union Report on Pesticide Residues in Food, European Food Safety Authority, 2013).

Semi-processed food items such as dehulled lentil and wheat flour also contain pesticide residues such as malathion, diazinon and chlorpyrifos ethyl at a concentration range from 0.001 to 0.002 µg/kg and from 0.001 to 0.045 µg/kg in dehulled lentil and wheat flour, respectively. From a study done in Ethiopia, diazinon and malathion were also detected in unprocessed wheat grain collected from different regions of Ethiopia (Daba et al., 2011). The present study shows that all the pesticides detected were below the MRL set by Codex Alimentarius (FAO/WHO, 2013). This may be due to the semi-processing or may be due to the farmers may follow good agricultural practice (GAP) during the production of wheat and lentil.

There were no MRLs found for the screened pesticides in teff and coffee pulp. This is perhaps due to the unfamiliarity of these food items worldwide. For this study, the MRLs for grain were considered to evaluate the residue in teff. As teff is the major grain consumed in Ethiopia and a principal staple diet for almost all Ethiopians (Hrušková, 2012), setting an own MRL for the different pesticides used on the crop is important. As the consumption of coffee pulp becomes more and more important in rural population of Ethiopia (Kefale et al., 2012) monitoring of this coffee component should also be a focus for consumer safety. Except for permethrin, the concentration of all pesticides decreased in coffee beans after removal of the pulp. This might be explained by the deposition of most pesticides on the surface of the crop. Pesticide application can be undertaken at the pre-harvest season (during the growing season) or at post-harvest. Hence, the pesticides may appear at the surface of the crop as a result of the time of application or it may come from environmental contamination.

3.5. Conclusions

The QuEChERS method with dispersive solid Phase Extraction (d-SPE) clean-up was accurate and precise for the analysis of the pesticides evaluated in commonly consumed food items. All samples collected from the local market in Jimma zone, Ethiopia contained one or more pesticides. This indicates there is contamination of the general environment, in particular to the areas where the studied food items grown. Approximately, one third of the samples had pesticide residues above the MRLs set by Codex Alimentarius, except for semi-processed lentil and wheat flour. This indicates over use of pesticides in the southwestern Ethiopia. Hence, a great concern has to be taken for the safety of consumers. Red pepper and coffee bean samples contained all pesticides that were looked for. Some banned pesticides and those not authorized for use in cereals, vegetables and coffee production such as organochlorines (e.g. DDT and endosulfan) were also detected. This might be due to cross contamination during indoor residual spraying (IRS) or due to the persistent nature of these pesticides, which make them to remain for a long time in the environment. Recent use of DDT was also observed in the study area. This indicates that farmers in southwestern Ethiopia use DDT illegally for the control of agricultural pests. An establishment of national MRLs for pesticides used in the cultivation of these crops is necessary to safeguard consumer health. More monitoring of pesticide residues in other food items will result in a better risk characterization and assurance of food safety.

Chapter Four: Risk of DDT to infants consuming maize as complementary diet in southwestern Ethiopia

Redrafted from: Mekonen S., Lachat C., Ambelu A., Steurbaut W., Kolsteren P., Jacxsens L., Wondafrash M., Houbraken M., and Spanoghe P. (2015). Risk of DDT residue in maize consumed by infants as complementary diet in southwest Ethiopia. Science of The Total Environment, Vol.551, 554-560, http://dx.doi.org/10.1016/j.scitotenv.2014.12.087

Abstract

Infants in Ethiopia are consuming food items such as maize (*Zea mays*) in their complementary diet. However, this may expose infants to toxic pesticide residues like DDT. A consumption survey was undertaken using the 24 hour recall method for two non-consecutive days. Maize samples were collected from the households visited during the consumption survey and from markets in Jimma zone, southwestern Ethiopia. The residues of total DDT and its metabolites were analysed using similar analytical method in **chapter three**. Deterministic and probabilistic methods of analysis were applied to determine the consumer exposure of infants to total DDT. The results from the exposure assessment were compared with the health based reference value in this case the provisional tolerable daily intake (PTDI) set by the joint meeting of food and agricultural organization and world health organization (JMPR of FAO/WHO). DDT was above the detection limit in all maize samples, with a mean concentration of 1.77 mg/kg maize. The mean concentration was far above the maximum residue limit (MRL) set by the Codex Alimentarius. The mean and 97.5 percentile (p97.5) estimated daily intake (EDI) of total DDT for consumer infants were respectively, 0.011 and 0.309 mg/kg bw/day from the deterministic and 0.011 and 0.083 mg/kg bw/day from the probabilistic exposure assessments. For the total infant population (consumers and non-consumers), the p97.5 EDI were 0.265 and 0.032 mg/kg bw/day from the deterministic and probabilistic exposure assessment, respectively. Health risk estimation reveals that the mean and p97.5 EDI of total DDT for actual consumers and p97.5 for total population were above the PTDI set by FAO/WHO. Therefore, in Ethiopia, the use of maize as complementary food for infants may pose a health risk due to the presence of DDT residue.

4.1. Introduction

Dichlorodiphenyltrichloroethane (DDT) was an effective insecticide until it was banned in most industrialized countries since the late 1970's. Even though it was banned, DDT is still used in some African countries where malaria vector control is important (Bouwman and Kylin, 2009). Although the use of DDT resulted in a successful elimination of the malaria vector (Beard, 2006), there is a great concern on its risks to human health (Eskenazi et al., 2009). DDT is a persistent chemical in both biotic and abiotic environment, as a result its residue can be detected in almost every human body (Vall et al., 2014).

Human beings can be exposed to DDT in utero, during breast feeding and through consumption of contaminated food (Jusko et al., 2012). From all these routes of uptake, dietary intake is the main route of human exposure because, 90% of the residue stored in a human body is due to consumption of different food items (Center for Food Safety, 2006). Studies in different countries indicate that food commodities are often contaminated with DDT. For example, a study done in Turkey indicated that 83-96% of the wheat samples were contaminated with DDT and its metabolites (Guler et al., 2010). Toteja et al. (2006) also report that residues of total DDT and its metabolites were detected in 59% of wheat grains and in 78% of wheat flour in different geographic regions of India. Additionally, a study done in Nigeria reports the presence of DDT and its metabolites in maize (Tijani, and Nurudeen, 2012). Moreover, a recent study in Ethiopia, also indicates that staple food items such as teff, maize, coffee beans and red pepper are contaminated with DDT (Mekonen et al. 2014). In addition to dietary exposure, indoor residual spraying (IRS) of pesticides for disease vector control may be another source of human exposure (Yewhalaw et al., 2011). From a study done in South Africa, in indoor residual spraying (IRS) villages, the level of DDT concentration in breast milk is higher than the MRL and the intake of DDT by infants was above the PTDI (Bouwman et al., 2012). From the dietary exposure, the actual intake may depend on the concentration of DDT and its metabolites in the food people eat and the amount of food consumed (Agency for Toxic Substances and Disease Registry, 2002).

Compared to adults, infants are more susceptible to toxic pesticides like DDT when they are exposed. This is because of their metabolic mechanism to detoxify these chemicals is not well matured (Casals-Casas and Desvergne, 2011). Additionally, the infants food intake per body weight is higher compared to adults (Daston et al., 2003). In utero-exposure to organochlorine pesticides like DDT has also a potential risk on the infants neurodevelopmental growth at the age of 6-12 month (Eskenazi et al., 2006). Infants can also be exposed to DDT during breast milk feeding. A study done in India indicated that the concentration of total DDT in breast milk for some infants was above the tolerable daily intake (Bedi et al., 2013). Additionally, infants are exposed to DDT when taking complementary homemade baby foods (Jeong et al., 2014).

In Ethiopia, maize is the dominant cereal used in complementary food administered to infants after the age of 6 month (Akalu et al., 2010). However, maize can easily be affected by insects in the field and during storage (Sori and Ayana, 2012). To tackle these insects, farmers use pesticides including non-authorized ones, such as DDT which is actually banned for agricultural use. A recent study done in Southwest Ethiopia indicates that DDT was found above the MRL set by Codex Alimentarius in maize samples collected from Jimma zone, southwest Ethiopia (Mekonen et al. 2014). If this crop is contaminated with DDT and its metabolites, infants are exposed and face health risks. Pesticide application often results in residues in food which may cause a health risk for humans (Akoto et al., 2013). So consumer risk assessment is an important step for the regulation of pesticide use on food crops (Hamilton et al., 2004). Despite these problems, there is no study that has estimated the exposure and risks of pesticides to infants, particularly with DDT, in Ethiopia. The present study contemplated to investigate the risk of total DDT for infants associated with consumption of maize in their complementary diets.

4.2. Materials and Methods

4.2.1. Study area

The study was conducted in southwest Ethiopia, around Jimma zone in the catchment area of Gilgel Gibe hydroelectric reservoir, one of the biggest artificial lakes in Ethiopia (**figure 4.1**). The study area includes four districts (Tiro Afeta, Sekoru, Omo-Nada and Kersa) around Gilgel Gibe reservoir, which is located at 265 km southwest of Addis Ababa and 65 km north east of Jimma town. Maize (*Zea mays*), sorghum (*Sorghum bicolor)* and teff (*Eragrostis tef*) are the commonly grown food crops in the area. Among these cereals, maize production ranks first in

Jimma zone (Gebremedhin et al., 2007) and used as commonly consumed food item in particular to southwest Ethiopia (Sori and Ayana, 2012).

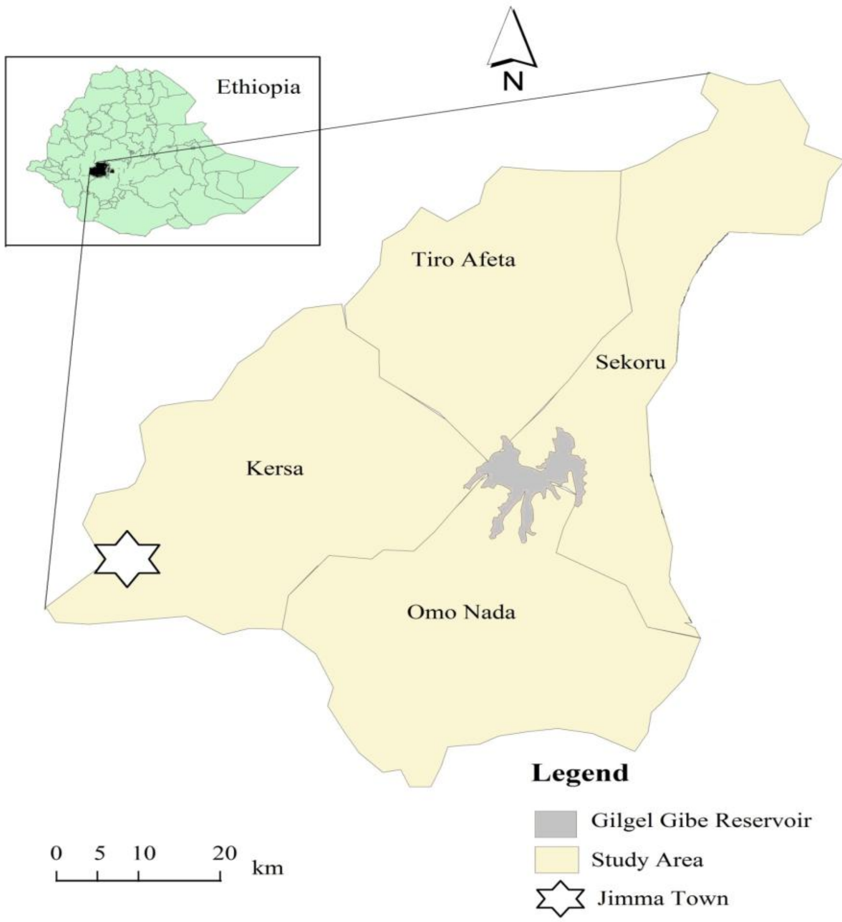


Figure 4.1. Map of the study area in southwestern Ethiopia

4.2.2. Dietary survey

To assess the food intake of 6-12 month old infants' consumption data were collected for 130 infants from randomly selected households during the months of June to September. Socio-demographic (age, sex and weight) data of the infants were collected during this household survey. Dietary data was collected in a repeated 24 h recall interview administered by trained interviewers using a face-to-face interview. This recall was done for two non-consecutive days separated by 15 days. Food composition data, portion sizes, and recipes were entered and processed in an online application to get dietary intake data (Lucille, Belgium, www.foodintakesoftware.ugent.be). The amount of different ingredients of complementary foods consumed by an infant per day was calculated. The average of both recall days was used to estimate food consumption on a daily basis. The mean daily consumption (g/kg bw/day) for each infant was calculated by dividing the infant's average daily consumption (g/day) by his/her body weight (kg). Weight of the infants was measured naked or with light clothes using mother-child digital scales and recorded to the nearest 0.1 kg (SECA Uniscale, Hamburg, and Germany).

4.2.3. Maize sampling

A first batch of maize samples came from households interviewed during the dietary assessment. In these households, the maize sampling was done randomly from different depths in the traditional thatched basket stores. Assuming that the people, who may not have maize in their stores, can buy from markets around the study area, a second batch of maize samples were collected from four different markets (Sekoru, Omo-Nada, Kersa and Tiro Afeta). The maize sampling from the market was done in similar way with Mekonen et al. (2014). Maize samples (250 g each) were packed in polyethylene plastic bags, labelled accordingly and transported to the laboratory. The samples were stored at -20°C until analysis.

4.2.4. Reagents and materials, analytical equipments, extraction and clean-up of the maize samples, method validation procedures

The reagents and materials, the analytical equipment with the set chromatographic conditions, extraction and clean-up of the maize samples and method validations were done according to Mekonen et al. (2014).

4.2.5. Statistical and exposure analysis

The difference of the residue of total DDT between the market and the household maize samples were evaluated based on the Mann-Whitney U test. As there was no statistical difference (p-value > 0.05), all DDT residue data were consequently pooled and used for the dietary exposure assessment of the infants. A Kruskal-Wallis test was performed to compare the residue of DDT metabolites in the maize samples and a graphical representation was made using box and whisker plot.

To evaluate the safety of consumers regarding pesticide residues, exposure should be assessed and compared to toxicological limits or reference values (Claeys et al., 2011). For the present study, the exposure assessment was done by assuming that the food processing (baking of maize powder as a traditional Ethiopian flat bread (injera) or cooking in the form of porridge, before administration to the infants, may not have an effect on the concentration of DDT (worst case scenario). As recommended by the European Food Safety Authority, (2011a), dietary exposure has to be done using deterministic and probabilistic approaches. The exposure assessment in the present study was done for the actual maize consumer infants and for the total population (both consumers and non-consumers). Including non-consumers was used to assess the chronic exposure. The Mann-Whitney U test was applied to compare the estimated daily intake (EDI) of total DDT by the infants from deterministic and probabilistic exposure analysis.

4.2.6. Deterministic exposure assessment

The dietary exposure to total DDT (mg/kg bw/day) was calculated deterministically based on the maize consumption data (g/kg bw/day) and DDT residue (mg/kg maize). In this approach, dietary exposure was computed by multiplying a single value of consumption and DDT concentration (point estimate). The mean, p50, p75, p90, and p97.5 consumption was combined with the mean, p50, p75, p90 and p97.5 total DDT concentration as explained by McKinlay et al. (2008).

4.2.7. Probabilistic exposure assessment

A probabilistic exposure assessment was conducted using @Risk® 5.7 software program for Microsoft excel 2010 (Palisade corporation, USA), in which the consumption (g/kg bw/day) and residue distributions (mg/kg) are combined into an exposure distribution (mg/kg bw/day). First-order Monte-Carlo simulation was undertaken considering 100,000 iterations. Estimated total DDT intake (mean, standard deviation, maximum, minimum and percentiles) were determined from the output of the simulation model.

The results from the deterministic and probabilistic exposure assessment were compared with the corresponding PTDI of DDT (0.01 mg/kg bw/day) set by JMPR of Food and Agricultural Organization and World Health Organization (FAO and WHO, 2013).

4.3. Results

4.3.1. Socio demographic results

The percentage of male and female infants (6-12 month of age) was 61% and 39%, respectively. The result indicates that more male infants are considered for the study, while in most of the cases male: female ratio at birth is 1:1. This may be due to the fact that the consumption survey was undertaken through a voluntary bases and mothers or care givers who have male infants may be more volunteer than those who have female infants. The average weight of the infants was 7.6 ± 1.25 kg. Regarding the maize consumption there is no significant difference observed among the male and female infants (p-value = 0.354). This indicates that there is similar feeding pattern for infants.

4.3.2. Concentration of total DDT and its metabolites in household and market samples

The distribution of total DDT from the two sample sources (households and markets) was not significantly different (p-value = 0.321) as indicated in **figure 4.2**. This result revealed that the contamination of maize samples with DDT was similar whether the infants consumed either from household or from market maize sources. Additionally, the maize samples in the markets may come from those households used for consumption survey because; the people use maize as consumption commodity as well as income source.

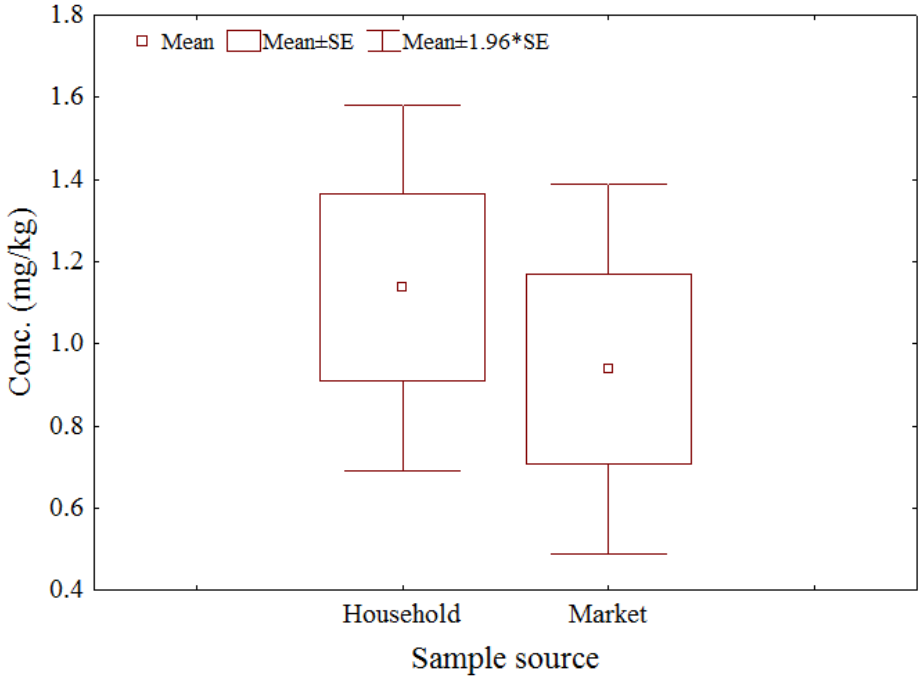


Figure 4.2. The concentration distribution of total DDT (mg/kg) between household and market maize sources. SE = Standard Error

DDT concentration in an increasing order for all maize samples is presented in **figure 4.3**. Total DDT was above the LOD for all maize samples. This indicates that the study area is highly contaminated by this very persistent pesticide in the environment. Most of the residue of DDT in maize samples was above the MRL set by Codex Alimentarius (FAO/WHO, 2013). This indicates that there is illegal use of DDT in the study area or the contamination might come from the historical and/or the use for malaria vector control. The farmers or pesticide applicators in Ethiopia usually stored the pesticides together with food commodities in the house or near to the storage areas of the crops (personal observation). Due to these, cross contamination of the food items may occur.

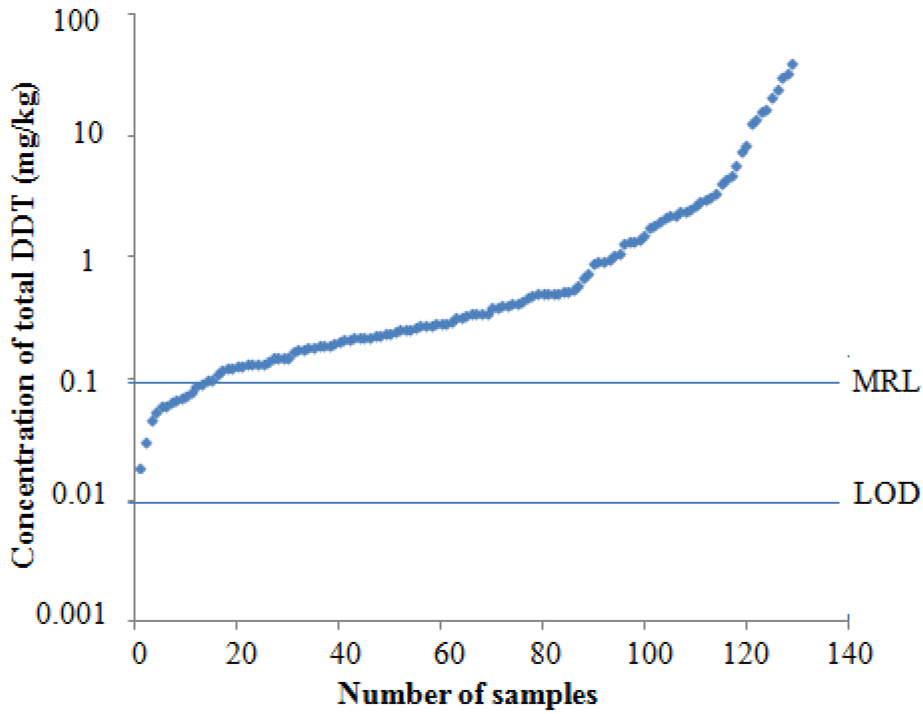


Figure 4.3. Concentration of total DDT (mg/kg) in an increasing order of the maize samples. LOD = limit of detection, MRL = maximum residue limit

The distribution of DDT metabolites in the maize samples is presented in **figure 4.4**. The result of the present study revealed that p,p'-DDE, p,p'-DDD, o,p'-DDT and p,p'-DDT were detected in 78%, 76%, 94% and 88% of the maize samples, respectively. From the box and whisker plot, p,p'-DDT present in most of the samples followed by o,p'-DDT, while a very low concentration of p,p'-DDE was observed. If the concentration of parent compound p,p'-DDT is higher than the metabolite p,p'-DDE, then the result indicates recent use of DDT in the study area as has been mentioned by European Food Safety Authority (Alexander et al., 2012).

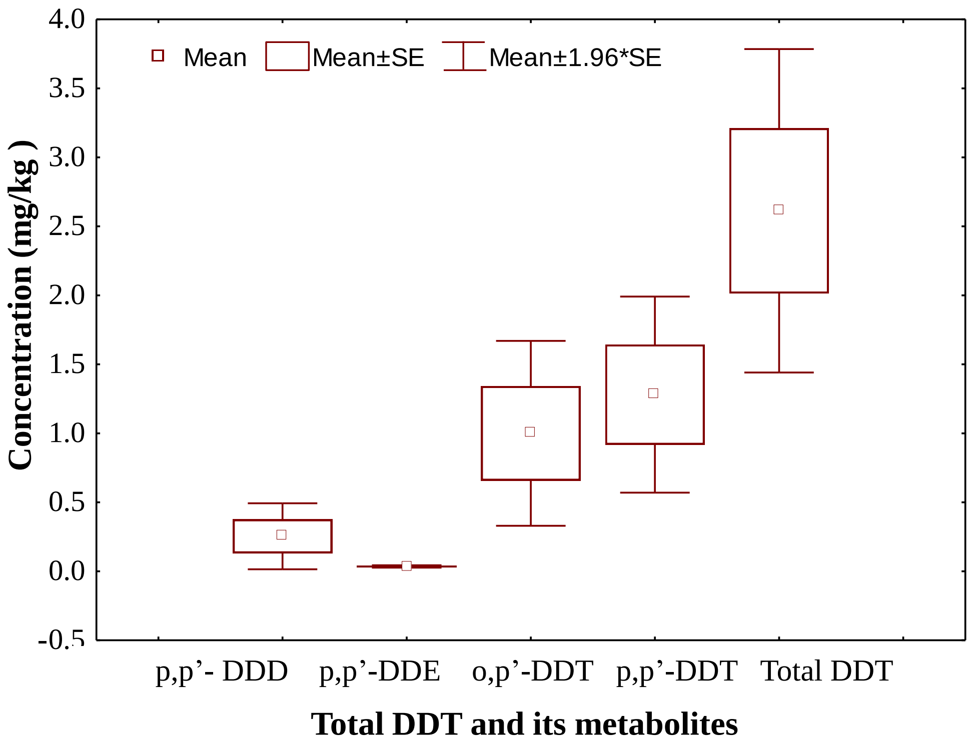


Figure 4.4. Concentration distribution of total DDT and its metabolites (mg/kg) in maize samples. SE = Standard error

4.3.3. Exposure assessment

Deterministic and probabilistic analyses were worked out to evaluate whether the level of exposure to infants to total DDT from maize consumption exceeds the PTDI set by FAO/WHO. The mean and p97.5 of estimated daily intake were used to represent the average and high consumer exposure scenario, respectively. A DDT intake above the PTDI indicates that there will be a chronic health risk for the exposed infants. DDT has no acute reference dose (ARfD), therefore, we did not consider the acute risk for this chemical.

4.3.3.1. Deterministic exposure assessment

The deterministic analysis was done based on the point estimate in which the mean maize consumption was multiplied with the mean DDT concentration and the same is true for the percentiles as indicated by McKinlay et al. (2008). **Table 4.1** presents, the results of deterministic exposure analysis to infants. The mean and p97.5 maize consumptions were 5.98

and 21.05 g/kg bw/day. The mean indicates the average consumption of maze by infants and p97.5 indicates the high consumer group of infants' i.e. if 97.5% of infants consume 21.05 g/kg bw/day, the rest (2.5% of the infants) consume above this value. The mean and p97.5 total DDT concentration were 1.770 and 14.700 mg/kg maize, respectively. Infants' exposure assessment indicates that the mean and p97.5 estimated daily intake (EDI) of total DDT were 0.011 and 0.309 mg/kg bw/day for actual consumers of maize, while 0.004 and 0.265 mg/kg bw/day for total infant population. The median estimated daily intakes were 0.001 and 0.0001 mg/kg bw/day for maize consumers and total population, respectively.

Table 4.1. DDT concentration, maize consumption and estimated daily intake (EDI) of total DDT from the deterministic exposure assessment

Deterministic analysis	Mean	p50	p75	p90	p95	p97.5
DDT concentration (mg/kg)	1.770	0.350	1.370	4.560	10.280	14.700
Consumption in consumer infants (g/kg bw/day)	5.985	4.160	7.500	13.930	19.770	21.050
Consumption in total population* (g/kg bw/day)	2.390	0.000	2.700	7.500	11.390	18.050
EDI of total DDT (mg/kg bw/day)						
Consumers only	**0.011**	0.001	**0.01**	**0.064**	**0.203**	**0.309**
Total population*	0.004	0.0001	0.004	**0.034**	**0.117**	**0.265**

*including non-consumer infants, values in bold indicates results above PTDI = 0.01 mg/kg bw/day

4.3.3.2. Probabilistic exposure assessment

In the probabilistic exposure analysis, both maize consumption and DDT residue data were used in the @risk software to identify the best fit distribution using the Monte-Carlo simulation model. From the fitting results, the P-P plot provided roughly a straight line joining the diagonals for both consumption and DDT concentration. This is considered as the best fit distribution and taken for simulation. During fitting of the distribution consumption for the total infant population, data deviations from the normal line were observed. This deviation may be due to the presence of zero consumption patterns. **Table 4.2** presents, the distribution of the consumption pattern, the distribution of total DDT concentration and EDI of total DDT by Ethiopian infants from the probabilistic exposure analysis. The results reveal that the mean and p97.5 consumption and DDT concentration were 6.484 and 30.153 g/kg bw/day; 1.770 and 13.155 mg/kg, respectively. The mean and p97.5 estimated daily intake of total DDT were 0.011 and 0.083 mg/kg bw/day for consumers only; 0.004 and 0.032 mg/kg bw/day for total population. These results are above the health based reference value of DDT (PTDI). This indicates that there will be a health risk for the infants. The median (p50) estimated daily intakes were 0.002 and 0.001 mg/kg bw/day for consumers only and total population, respectively, which is below PTDI similar to the deterministic analysis. This indicates 50% of the infants have no health risk from consumption of maize in their complementary diet.

Table 4.2. Distribution in DDT concentration, maize consumption, and estimated daily intake (EDI) of total DDT from the probabilistic exposure assessment

Probabilistic analysis	Mean	p50	p75	p90	p95	p97.5
DDT concentration (mg/kg)	1.77	0.415	1.29	4.015	7.771	13.155
Consumption among Consumers (g/kg bw/day)	6.484	3.591	7.405	14.337	21.339	30.153
Consumption among total population (g/kg bw/day) *	2.273	1.639	3.296	5.487	7.144	8.802
EDI of total DDT (mg/kg bw/day)						
Consumers only	**0.011**	0.002	0.006	**0.021**	**0.044**	**0.083**
Total population*	0.004	0.001	0.002	0.008	**0.017**	**0.032**

*including non-consumer infants, values in bold indicates results above PTDI = 0.01 mg/kg bw/day

4.3.4. Comparison of EDI of DDT for the total population in the deterministic and probabilistic exposure assessment

The estimated daily intake of total DDT by the Ethiopian infants from the deterministic and probabilistic exposure analysis was compared to see which method is appropriate to show the best exposure scenario. **Figure 4.5** revealed that the mean estimated daily intake of total DDT for total infants from both deterministic and probabilistic exposure assessment were the same and below the corresponding PTDI set by FAO/WHO. For the deterministic exposure assessment the p90, p95 and p97.5, while for probabilistic exposure assessment only the p95 and p97.5 estimated daily intake (EDI) of total DDT were above the PTDI. This indicates that the deterministic exposure analysis shows wider exposure scenarios. However, there is no statistical significant difference between the two methods (p-value = 0.42).

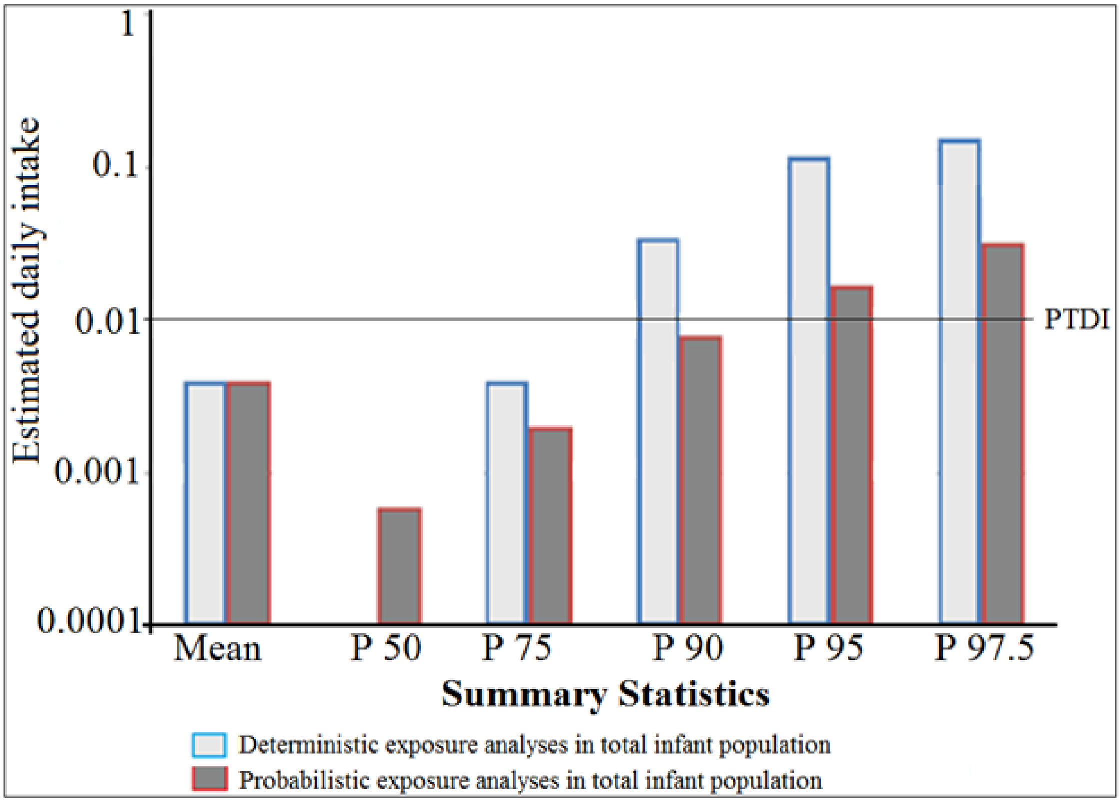


Figure 4.5. Comparison of estimated daily intake of DDT (mg/kg bw/day) from deterministic and probabilistic exposure assessment for total infants. Y-axis in logarithmic scale

4.4. Discussion

There is no significant difference (p-value > 0.05) in consumption pattern of maize between the male and female infant population. This may be due to the fact that maize is the commonly consumed food item and similar feeding habits occur in each family in Jimma zone southwestern Ethiopia. From the results it is found that there is no significant difference on the residue load of DDT between market and household samples. This may be explained because of the reason that the farmers who bring maize to the market are the same persons who have maize in their house. Additionally, DDT contamination in maize may come from the same surrounding environment. About three fourth of the maize samples had a total DDT residue above the MRL (0.1 mg/kg) (FAO/WHO, 2013). The residue load above the MRL indicates that there is over use of DDT in the study area for agricultural or household purpose. The detection of total DDT and its metabolites in maize may also come from the application for indoor residual sprayings to control malaria (Yewhalaw et al., 2011). Additionally, its detection may be, explained by the persistent or lipophilic nature of DDT as a contaminant in the environment (Bempah et al., 2012). Moreover, DDT may come from environmental contamination with dumped obsolete pesticides in different sites of Ethiopia (Amera and Abate, 2008). Among the metabolites of total DDT, the concentration of o,p'-DDT and p,p'-DDT were significantly higher (p-value < 0.0001) than the rest of the metabolites. However, no significant difference was observed in the concentration between o,p'-DDT and p,p'-DDT, and p,p'-DDD and p,p'-DDE (p-value = 0.125). A similar finding was observed in a previous study done in southwest Ethiopia (Mekonen et al., 2014). The high concentration of p,p'-DDT indicates that there was recent use of DDT in the study areas as has been explained by the European Food Safety Authority (Alexander et al., 2012). From the deterministic exposure assessment it can be derived that the mean and p97.5 estimated daily intake of total DDT for consumer infants were above the PTDI set by FAO/WHO, while for the total infant population they were below the PTDI. The p97.5 estimated daily intake of total DDT for the total infant population was also above the PTDI, while the mean estimated daily intake of total DDT was less than PTDI.

From the probabilistic risk analysis, the upper percentile (p97.5) indicates that the infants were exposed to high DDT concentrations. This result is consistent with the work of (Wason et al., 2013) which reports that the upper percentile exposed children are exposed to a high concentration of pesticides which influences their health more. Both exposure analyses indicate health problems from consumption of maize as complementary diet.

Different studies explain that infants chronic exposure to organochlorine pesticides such as DDT, develop health problems like attention deficient disorder, obesity and type two diabetics (Corin and Weaver, 2005; Polańska et al., 2013).

From the comparison of the two exposure assessment models (deterministic and probabilistic), the p90, p95 and p97.5 EDI of DDT for the total infant population from the deterministic exposure assessment were above the PTDI while, for the probabilistic assessment it was only the p95 and p97.5. According to the European Food Safety Authority (EFSA, 2012a) the deterministic exposure assessment shows a wide range of exposure values as it uses a point estimate for input and it generates a point estimate for exposure. However, the probabilistic exposure assessment uses distributions which take variability and uncertainties in the exposure assessment into account. To see whether there is a significant difference in estimating the daily exposure for the total infant population between deterministic and probabilistic exposure assessment the Mann-Whitney U test was applied. The results revealed that there is no significant difference (p-value = 0.42, U = 13, Z = 0.8) in estimating the daily intake of total DDT for the total infant population from maize consumption even though the deterministic analysis showed a wider range in exposure values. So both exposure assessment methods can be appropriate here for the determination of exposure and consequently to assess the risk of the infants to maize consumption.

4.5. Conclusions

This study estimated the exposure and risk of total DDT to infants from maize consumption in their complementary diets. DDT was above the detection limit in all maize samples, with no significant difference between market and household sample sources. About three fourth of the maize samples contain DDT above the MRL set by Codex Alimentarius. This indicates that there is illegal use of DDT in the production of maize or to protect storage pests and in addition to this, the recent use of DDT was observed. The deterministic and probabilistic risk assessment indicates that the average and high consumer estimated daily intake of total DDT for consumers and total population were above the health based reference value. This may result in chronic health problems for the infants. Monitoring of pesticides including DDT in other complementary food items of infants is desirable. Furthermore, consumer risk assessment for the general population in Ethiopia is important to contribute to the safety of human health.

Chapter Five: Effect of household processing on pesticide residues in coffee (*Coffea arabica*) and teff (*Eragrostis tef*)

Part of this chapter is redrafted from: Mekonen S., Ambelu A. and Spanoghe P. (2015). Effect of Household Coffee Processing on Pesticide Residues as a Means of Ensuring Consumers' Safety. *J. Agric. Food Chem.*, 2015, *63* (38), pp 8568–8573, DOI: 10.1021/acs.jafc.5b03327

Abstract

Coffee (*Coffea arabica*) is a highly consumed and popular beverage all over the world; however, coffee beans used for daily consumption may contain pesticide residues that may cause adverse health effects to consumers. Teff (*Eragrostis tef*) is an endogenous cereal crop in Ethiopia and also the most commonly consumed food item. Even though, the applications of pesticides such as insecticides are not common in the production of teff, it may be contaminated from the environment, leaking from storage areas and cross contamination during spraying for malaria control. In this monitoring study, the effect of household processing on pesticide residues in coffee and teff was investigated and the processing factor (PF) was calculated for each processing method. Twelve pesticides, including metabolites and isomers (endosulfan α, endosulfan β, cypermethrin, permethrin, deltamethrin, chlorpyrifos ethyl, heptachlor epoxide, hexachlorobenzene, p,p'-DDE, p,p'-DDD, o,p'-DDT, and p,p'-DDT) were spiked in coffee beans while, seven pesticides (permethrin, cypermetrin, chlorpyrifos ethyl, p,p'-DDE, p,p'-DDD, o,p'-DDT, and p,p'-DDT were spiked in teff flour collected from a local market in southwestern Ethiopia. The subsequent household coffee and teff processing methods such as, washing, roasting, brewing, doughing, and baking were established as close as possible with the traditional household processing methods in Ethiopia. From our findings, washing of coffee beans showes 14.6−57.7 percent reduction, while the roasting process reduced up to 99.8 percent. Chlorpyrifos ethyl, permethrin, cypermethrin, endosulfan α and β in roasting and all of the twelve pesticides in the coffee brewing processes were not detected. Kruskal−Wallis analysis indicates that the reduction of pesticide residues by washing is significantly different from roasting and brewing (p-value < 0.0001). However, there was no significant difference between coffee roasting and brewing (p-value > 0.05). This indicates that roasting and brewing of coffee are the most effective to decrease the residues of the pesticides under study. Dough preparation for teff flour decreases the residues up to 86.4%, while baking reduces up to 90.2%. The PF was less than one (PF < 1) for all the processing methods, which indicates reduction of pesticides under study during household processing. The cumulative effect of these household processing methods are important to evaluate the risks associated with ingestion of pesticide residues, particularly in coffee beans and teff.

5.1. Introduction

Wide applications of pesticides have different problems on human health and the environment as explained in previous chapters of this thesis. Therefore, it is important for consumers to know the possibilities to reduce pesticides intake from their food. As reported in literature, food processing such as baking, cooking, roasting, peeling, washing drying etc. may reduce pesticide residues from the raw food commodity (Kaushik et al., 2009).

Usually, pesticide residue analysis is undertaken on raw agricultural commodities to meet the purpose of marketing to consumers, import/export certification, regulatory monitoring and others. However, to estimate the level of exposure to pesticide residues in food, it is desirable to investigate the level of pesticides at the point of consumption, mainly after food processing which may lead to a reduction of pesticide residues (Satpathy et al., 2012).

Food processing is the action of transforming the food to a more edible form before the food is consumed. The processing can influence the pesticide residue present after the raw agricultural commodity is harvested (pranoy Pal and PG Sahah, 2008). Different household and industrial food processing; such as washing with water or with different chemicals, peeling of fruits and vegetables, frying, boiling, cooking and baking; may reduce the pesticide residue in food below the risk level (Ahmed et al., 2011).

Coffee is a highly consumed and a popular beverage all over the world as explained in chapter three**.** Coffee is the second most important commodity next to oil as a source of foreign exchange for most producing countries. In addition, it is considered as a primary food, due to its contents of compounds with an anti-oxidant effect and other beneficial biological properties. Its characteristic flavour and aroma makes it a unique beverage with more than thousands of volatile compounds present in roasted coffee (Yeretzian et al., 2003). As explained in chapter two, Ethiopia is the top producer and exporter of coffee in Africa. The country is believed to be the origin of *Coffea arabica*. On the other side, half of the coffee produced in Ethiopia is used for local consumption which makes the country the leading African continent in coffee consumption. Additionally, coffee is the most significant agricultural produce to develop the country’s economy (ICO, 2012).

Specifically, the beautiful traditional coffee ceremony makes the country very unique in consumption of coffee. The coffee ceremony and drinking of coffee is an important part of the Ethiopian cultures. Coffee is offered during holydays, when visitors or friends come to home and on a daily basis as a staple food item. Ethiopian coffee ceremony starts with washing the coffee beans with water followed by roasting until they turn to a dark brown colour. After roasting, sometimes the roasted coffee beans are brought to the family members or visitors to give them a closer breath of the aroma. Afterwards, the beans are grinded using traditional wooden mortar and pestle to obtain a fine powder. The powder is poured into boiled water in a special local coffee clay pot called “jebena”. Coffee is ready to be served when the stem starts to come out from the nozzle with an attractive flavour. After that the jebena is placed on the ground for about three minutes, to let the coffee sludge settle to the bottom. The brewed coffee is then poured into small cups and served (personal observation). However, the coffee beans used for this ceremony may contain some hazardous pesticides.

Due to the high consumption of coffee and its economic importance, people from the producer, exporter and consumer countries give more attention to its safety (Yang et al., 2011). Even though, pesticide contamination in green coffee beans is limited during agricultural treatments, there may be contamination during transportation and storage (Durand et al., 2006). According to the investigation of (Jacobs and Yess, 1993), the imported green coffee beans samples to US contained different pesticide residues such as chlorpyrifos at a concentration ranging from 0.01-0.04 mg/kg and pirimiphos methyl at 0.01 mg/kg. From a study done in Brazil, residues of different pesticides were detected in coffee beans either from registered or illegal use such as endosulfan, chlorpyrifos, cypermethrin and captafol (Pizzutti et al., 2012). Additionally, from the previous study done in Ethiopia, DDT, cypermethrin, permethrin, deltamethrin, chlorpyrifos ethyl and endosulfan (α and β) were detected in coffee beans collected from the market in southwest Ethiopia (Mekonen et al., 2014).

Teff (*Eragrostis tef*) is one of the major cereal crops, which is consumed by almost the whole population in Ethiopia. Teff is the most important staple food crop, used to make injera, a traditional fermented flat bread and for the preparation of porridge and kita (non-fermented bread) in Ethiopia. Although teff is the preferred grain for making the staple injera (Yetneberk et al., 2005), it gets little worldwide interest compared to other cereals such as rice, wheat and maize.

Nowadays, the recognition that teff is gluten-free has spurred global research interest by nutritionists and food scientists. Consequently, the number of studies on the nutritional composition of teff and its health benefits increase (Baye, 2014). Teff is also the second most important cash crop next to coffee, which generates about 500 million USD per year for local farmers in Ethiopia (Minten et al., 2013) and is in the meantime an export commodity to many European countries, Middle East and North America. It is a largely consumed crop especially in urban and semi-urban Ethiopia with a national per capita consumption of 25.9 kg (Berhane et al., 2011). Even though, this cereal is very important for human consumption and for generation of income, it may be contaminated by different pesticides from application or from historical use. As teff is not the common crop produced and consumed globally, there is almost no study done on the status of pesticides residues in this grain. However, a recent study done in Ethiopia detected cypermethrin, permetrin and deltamethrin, chlorpyrifos ethyl, and DDT and its metabolites, in teff grain (Mekonen et al., 2014).

Pesticide residues, which are to a variable extent present in the food materials after harvesting, are beyond the control of consumers. As Ethiopia is the largest producer, exporter and consumer of coffee and teff in Africa, a pragmatic solution should be developed to tackle the problem of coffee and teff safety. Different studies revealed that food processing such as washing, roasting, cooking, baking, peeling drying etc. can be an important solution to reduce pesticide residues in different food items (pranoy Pal and PG Sahah, 2008;Ahmed et al., 2011; Keikotlhaile et al., 2010;Satpathy et al., 2012).

Nowadays, there is an increasing need for information on the effect of various food processes on pesticide residues. Few studies indicate an effect of coffee roasting on reduction of pesticide residues (Cetinkaya et al., 1984; Sakamoto et al., 2012). However, no studies are available on the effect of the household processing of coffee and teff such as washing, roasting and brewing, doughing and baking methods on pesticide residues. Therefore, the main aim of the present study is to evaluate the effect of household processing on pesticide residues in coffee beans and teff, and to determine the processing factor (PF) for each of the processing steps.

5.2. Materials and Methods

5.2.1. Chemicals and reagents

Analytical grade acetonitrile (99.9% purity) were supplied by VWZ prolabo, HPLC grade n-hexane (98% purity) and acetone (98.9% purity) obtained from ALLthec were used to extract pesticide residues from raw and processed coffee beans and teff flour. Thermo Fisher Scientific supplied MgSO4 (98% purity), NaAc (99% purity), 50mL centrifuge tube, 15mL dispersive solid phase extraction clean-up tube packed with primary secondary amine (PSA) at 99% purity, magnesium sulphate (MgSO4) and octadecyl (C18) at 99% purity. The pesticides under study (p,p'-DDE (99.9%), p,p'-DDD (99.3%), o,p'-DDT (100%), p,p'-DDT (99%), endosulfan α (98.5%), endosulfan β (98%), permethrin (98%), cypermethrin (98%), deltamethrin (99%), chlorpyrifos ethyl (99.5%), heptachlor epoxide (99.5%) and hexachlorobenzene (98%)) with their highest analytical purity, were obtained from Supelco and delivered by Sigma-Aldrich Logistic Analytical. **Table 5.1** presents, the physico-chemical properties of the pesticides investigated in this study.

Table 5.1. The physico-chemical properties of the pesticides under study

Pesticides	Water solubility (mg/L)	$Logk_{ow}$	Vapour pressure (mmHg)	Mode of action
p,p'-DDE	0.025	6.91	$1.6*10^{-7}$	Contact
p,p'-DDD	0.120	6.51	$1.4*10^{-6}$	Contact
o,p'-DDT	0.090	6.02	$6.0*10^{-6}$	Contact
p,p'-DDT	0.085	6.79	$1.1*10^{-7}$	Contact
endosulfan α	0.530	3.83	$1*10^{-5}$	Contact
endosulfan β	0.280	3.62	$1*10^{-5}$	Contact
permethrin	0.006	6.10	$2.15*10^{-8}$	Contact
cypermethrin	0.009	5.40	2.3*10-7	Contact
deltamethrin	0.002	6.10	1.5*10-8	Contact
heptachlor epoxide	0.350	5.40	$3.0*10^{-4}$	Contact
hexachlorobenzene	0.006	5.73	$1.09*10^{-5}$	systemic
chlorpyrifos ethyl	1.400	4.70	$1.9*10^{-5}$	Contact

$Logk_{ow}$ = logarism of octanol-water partition coefficient

5.2.2. Sampling

A 3 kg of coffee beans (*Coffea arabica*) and 5 kg of teff (E*ragrostis tef*) from randomly selected shops (1kg sample/shop) were bought from the local market in Jimma zone, southwestern Ethiopia. These samples used as a blank and for spiking of the pesticides under study. Samples were packed in polyethylene plastic bags after which they were sealed and labelled properly. The samples were transported to the laboratory and stored at -20 °C until extraction was done.

5.2.3. Quality control

The quantitative determination of the pesticide residues in processed and unprocessed coffee beans and teff were done based on an external standard method. The calibration curves were obtained by injecting five different concentrations of the pesticide standards in the range of 0.005-1 mg/L. The regression coefficient (r^2) of the standard curve was greater than 0.995 for all the pesticides under study. Identification and quantification of the pesticides were done based on the retention time and peak area, respectively.

5.2.4. Treatment of raw coffee beans

A 10 g raw coffee beans was weighed on the analytical balance and spiked with 40 µL of 100 mg/L of each pesticide under study in three replicates to increase the reliability of results. Spiking was done for each household processing method for both coffee and teff (washing, roasting, brewing, doughing and baking). The pesticides selected for this study were those pesticides detected in the previous study on Ethiopian coffee beans and teff (Mekonen et al., 2014). The coffee beans were sprayed on petri dishes to get equal distribution of the pesticides for each bean. After spraying the petri dishes were covered with aluminum foil and placed in refrigerator for 24 hours to increase the contact time between the pesticides and the matrix. After one day, the processing methods (washing, roasting, brewing, doughing and baking) were undertaken.

5.2.4.1. Raw coffee beans

After one day the spiked samples of the raw coffee beans were grinded and homogenized using a coffee grinder with a knife in it. The extraction and clean up of the raw and processed samples were done using the same procedure in chapter three (Mekonen et al., 2014).

5.2.4.2. Washed coffee beans

The spiked coffee beans were washed thoroughly for 5 minutes under normal tap water (25-30°C) resembling coffee bean washing at household levels. Then all other procedures in unprocessed coffee beans were applied for the extraction, cleanup and analysis for the determination of pesticides using gas chromathography with electron capture detector (GC-ECD).

5.2.4.3. Roasted coffee beans

The spiked coffee beans were roasted at a temperature range of 230-240 °C (light to medium) roasting and an average time of 12-14 min until the characteristic aroma or flavor of coffee appeared. The roasting temperature and duration of the roasting was based on a study done by (Moon and Shibamoto, 2009). Then the other procedures for extraction and cleanup used for raw coffee beans were applied.

5.2.4.4. Brewed coffee

Following the roasting, the brewing process was undertaken. The roasted coffee beans were grinded to fine powder using coffee grinder (Type 4041, 220-230V/150w). The fine coffee powder was added in a coffee pot containing 100 mL boiled water and brewed for 10-12 min. After brewing, the coffee pot was picked up from the stove and put on the ground until the infusion of the coffee is cooled and the coffee sludge is settled down to the bottom of the coffee pot. Then the upper liquid layer was taken carefully to the cups for further analysis. This brewed coffee was extracted, cleaned up and analyzed in a similar way like that of the raw coffee beans.

5.2.4.5. Raw teff flour

A 5 g of teff flour was weighed on the analytical balance and spiked with 40µl of 100 mg/l of each pesticide under study. After waiting for one day, the samples were extracted in a similar procedure with the raw coffee beans.

5.2.4.6. Dough preparation

A 50 g of teff flour was weighed on an analytical balance and then spiked with 40µl of 100 mg/l of each pesticide under study. The spiked teff flour was combined with 100 ml of water (equivalent to 100 g water) and 0.5 g instant yeast, (together 150.5 g) and then mixed thoroughly to get equal distribution of the pesticide throughout the dough. Then the prepared dough stayed for 72 hours (three days). Then 15 g of the dough was weighed on an analytical balance using a 50 ml centrifuge tube. The extraction and clean-up was done in similar procedure as the raw teff flour.

5.2.4.7. Baking

After sampling 15 g fermented dough for extraction, all the remaining dough was used for baking of teff injera. The teff was backed using a pan in a similar condition with the traditional Ethiopian injera baking. The baked injera was grinded and homogenized. Then from the homogenous teff injera 10 g was taken and the extraction and cleanup of the samples was done in similar way with the raw teff flour. During baking, the food is cooked in a closed environment by heated air. Heat is supplied to the surface of the food by convention from the circulating air and by conduction through the pan containing the food as explained in the report of (Fellows, 2009).

5.2.5. Determination of processing factor (PF)

The effect of household processing on the level of pesticides often correlates with the physico-chemical properties of the pesticides under study, so it is important to adequately monitor the processing factor. The PF for all transformation steps was calculated by the ratio between the pesticide concentrations in the processed commodity (mg/kg) to the pesticide concentration in the raw commodity (mg/kg). According to Bonnechere et al. (2012), a PF less than one indicates that there is a reduction of pesticides by the processing method (PF < 1: reduction factor), while a PF greater than one indicates no reduction in weight or volume, (PF > 1: concentration factor). The processing factor is the proportional amount by which the residues changes when food is processed. For this study PF was calculated by the formula below:

$$\text{Processing factor (PF)} = \frac{\text{Concentration of pesticides in processed coffee beans or teff (mg/kg)}}{\text{Concentration of pesticides in raw coffee beans or teff (mg/kg)}}$$

5.2.6. Analysis of wash water and coffee sludge

Before the coffee bean was roasted, it was washed with water. Additionally, after the coffee was brewed there is sludge remaining in the bottom of the coffee pot. These two samples may contain some pesticide residues that can be able to pass the washing and roasting process. Traditionally, this wash water and coffee sludge is often disposed into the environment, which may contaminate the foods grown there. To check whether the wash water from the samples and the sludge of the coffee contain pesticide residues, these two samples were also analyzed for the presence of pesticide residues. The procedure was as follows: a 10 mL of the wash water (assuming 10 g of water) and 6- 8 g of coffee sludge were weighed on an analytical balance and taken with a 50 mL centrifuge tube. Then the extraction, clean-up and analysis were done in a similar way with the raw coffee beans.

5.2.7. Analytical equipment

Quantitative estimations and chromatographic separation of each pesticide were done by gas-chromatography with electron capture detector (GC-ECD, Agilent Technologies 6890N) with an auto sampler. The coffee bean processing was undertaken to see their effect on the pesticides previously detected in Ethiopian green coffee beans (Mekonen et al., 2014), so that the chromatographic conditions for this study were the same as in chapter three.

5.2.8. Statistical analysis

All the treatments of the processing methods (washing, roasting, brewing, doughing and baking) were done in three replicates. Statistical significance was checked using Kruskal-Wallis test to see if processing methods differed in reduction of pesticides under study. Differences at p-value < 0.05 were considered as significant.

5.3. Results

5.3.1. Effect of household coffee processing on pesticide residues

This study investigated the effect of washing, roasting and brewing on the stability of twelve pesticides. The concentration of the pesticides under study in raw and processed coffee beans is presented in **figure 5.1**. Among the household processes, washing decreased the pesticide residues in the range of 14.6- 57.7%. Maximum reduction was observed for endosulfan α and β where the residue decreased to the extent of 53.1 - 57.7%, respectively. The results indicate that the reduction of the pesticide residues after washing with tap water may be due to the removal of some pesticides together with the thin upper layer of the coffee beans.

In this study, roasting is found to be effective to affect the stability of pesticide residues in coffee beans. By this process, reduction of the pesticide residues was in the range of 72.5-99.8%. Maximum reduction was observed for hexachlorobenzene (99.8%), followed by heptachlor epoxide (97.9%), p,p’-DDE and p,p’-DDD (97.6%) and o,p’-DDT (96%). Five of the pesticides (chlorpyrifos ethyl, permethrin, cypermethrin, endosulfan α & β) were not detected after the roasting process. This may possibly due to instability of these pesticides due to the heat applied in the roasting process as reported by other studies (Bo and Zhao, 2010; Bajwa and Sandhu, 2014). After the coffee brewing process, none of the pesticides under study were detected. This process is found to be the most effective to mitigate the pesticides under study in coffee beans.

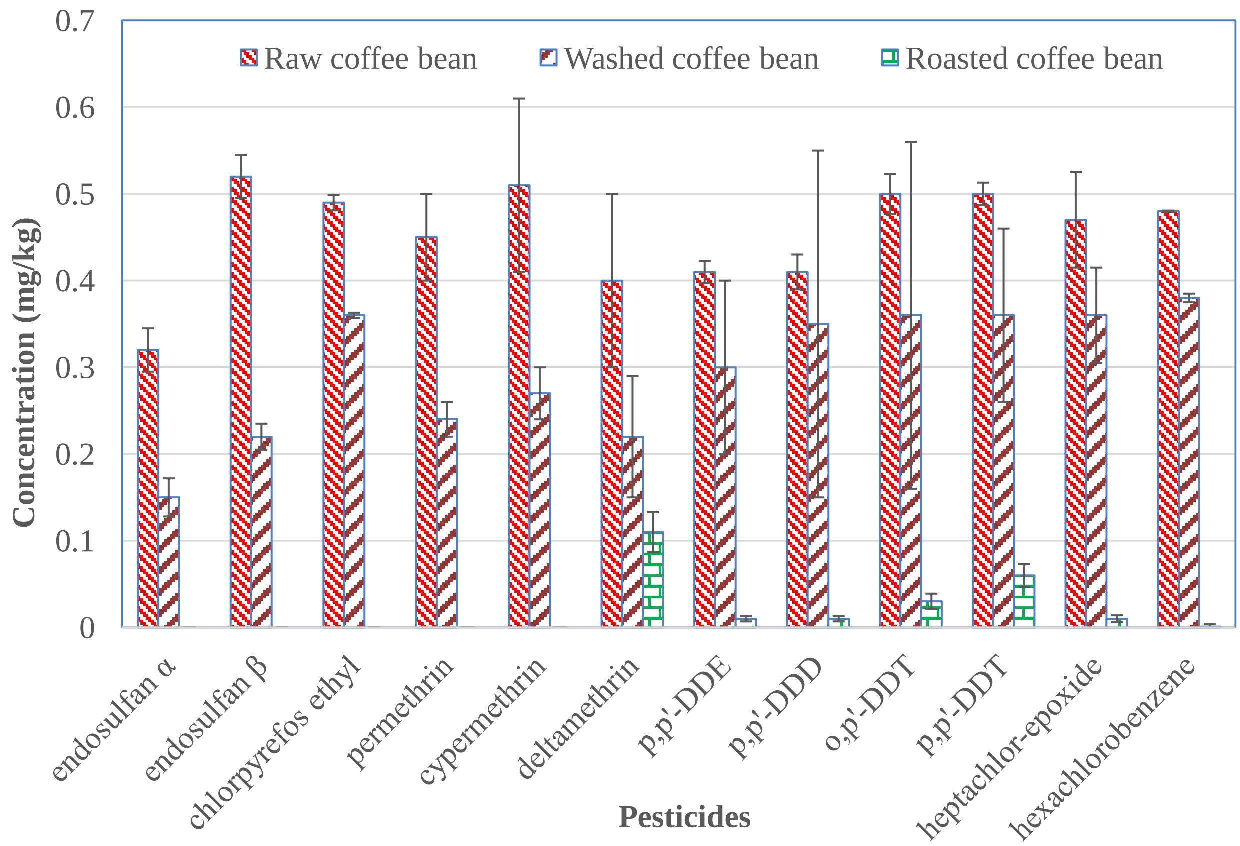


Figure 5.1. Mean pesticide concentration (mg/kg) in raw and processed coffee. The error bars indicated standard deviation

5.3.2. Determination of the processing factor (PF)

The PF is defined as the ratio of the residues in the processed food commodity to raw commodity before processing. From the findings of our study, PFs for household processing of coffee was less than one, which indicates that the processing methods have a reduction effect on the studied pesticide residues. The PFs for the detected pesticides in the roasting process were the lowest particularly for hexachlorobenzene, heptachlor epoxide, p,p’-DDE, p,p’-DDD, and o,p’-DDT ranged from 0.001-0.04. This indicates that the roasting process played the most important role in removing the pesticides from coffee beans compared to the washing process. The results presented in t**able 5.2**

Table 5.2. The processing factor for each household processing method in coffee

Pesticide	PF Washing	PF Roasting	PF Brewing
endosulfan α	0.47	*	*
endosulfan β	0.42	*	*
chlorpyrifos ethyl	0.73	*	*
permethrin	0.53	*	*
cypermethrin	0.53	*	*
deltamethrin	0.55	0.28	*
p,p'-DDE	0.73	0.02	*
p,p'-DDD	0.85	0.02	*
o,p'-DDT	0.72	0.04	*
p,p'-DDT	0.72	0.12	*
heptachlor epoxide	0.77	0.02	*
hexachlorobenzene	0.79	0.001	*

*****for non-detected pesticides processing factor is not calculated

5.3.3. Pesticide residue in wash water and coffee sludge

Wash water after washing process and coffee sludge which were settled to the bottom of the coffee pot after coffee brewing were analysed for the presence of those pesticides in raw coffee beans. The result of the analyses reveales that all DDT metabolites (p,p'-DDE, p,p'-DDD, o,p'-DDT and p,p'-DDT) and deltamethrin were detected in the wash water f**igure 5.2**. This may be due to the fact that these pesticides may be removed with the thin layer of the coffee beans during the washing process. Additionally, the pesticide may come to the coffee beans from environmental contamination and may appear on the surface of the beans can easily be removed. Most of the pesticides disappeared from coffee beans during the roasting process. In the meantime, pesticides were not detected in coffee sludge except deltamethrin and p,p'-DDT. The detection of these two pesticides may be due to the lower percent reductions of deltamethrin (72.5%) and p,p'-DDT (88%) during roasting process of coffee beans as a result, some part end up to coffee sludge.

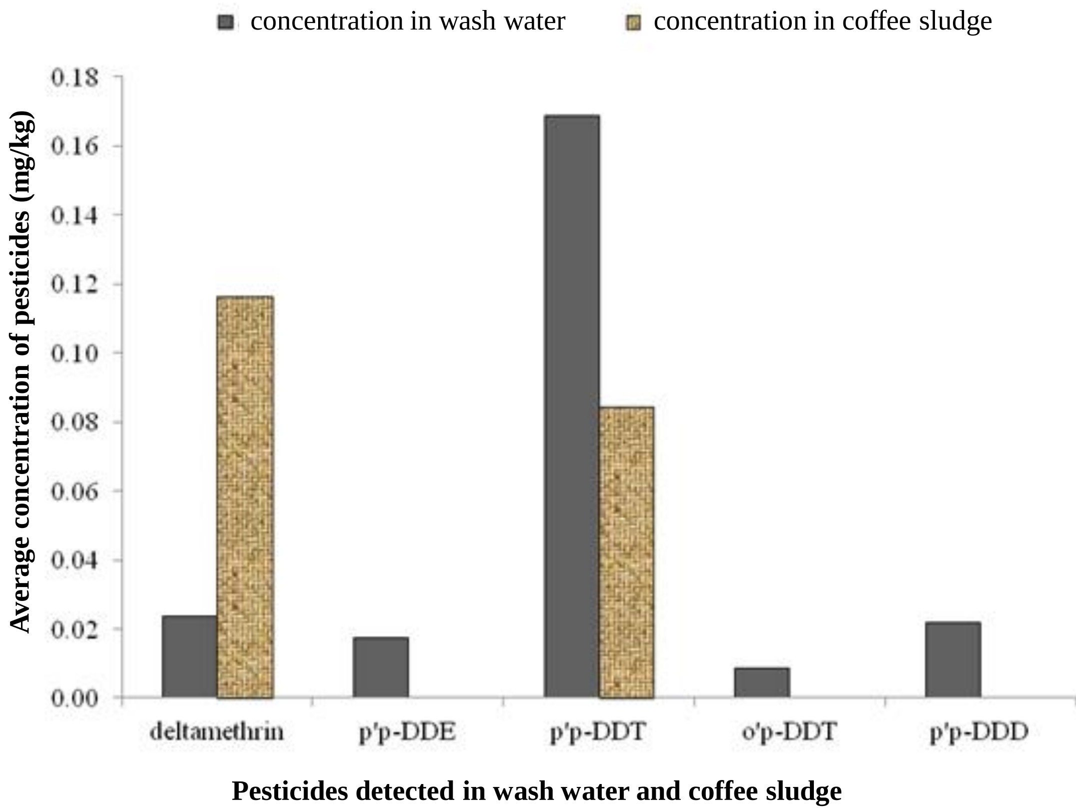


Figure 5.2. Average concentrations of pesticides in wash water and coffee sludge

5.3.4. Effect of household processing on pesticide residues in teff

Figure 5.3 shows the effect of doughing and baking on pesticides residue in teff. The preparation of dough decreases the pesticide residues in teff in the range of 59.9-86.3%, while baking decreases in the range of 63.2-90.2%. This indicates that household processing has an effect on reduction of the level of pesticides in teff and this will be helpful for the safety of consumers' health.

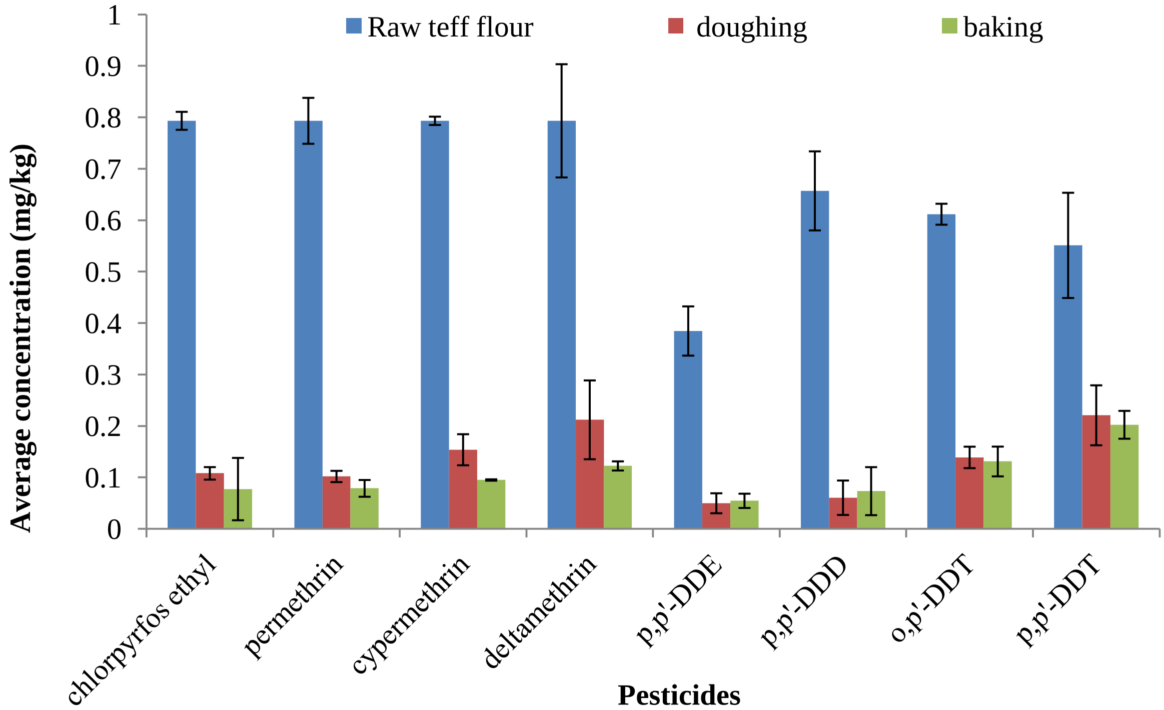


Figure 5.3. The average concentration of pesticides in raw and processed teff, Error bar indicates standard deviation

The processing factors for both doughing and baking were less than one, for all the pesticides under study (**table 5.3**). These indicate that there is a reduction of the pesticide residue due to the household processing of the teff flour. As reported in different literatures, PF < 1 indicates there is reduction of pesticide residues due to processing (pranoy Pal and PG Sahah, 2008; Hasmukh et al., 2012; Bajwa and Sandhu, 2014).

Table 5.3.The processing factor for making dough and baking teff

Pesticide	Raw teff flour	PF Doughing	PF Baking
chlorpyrifos ethyl	0.793	0.136	0.098
permethrin	0.793	0.129	0.099
cypermethrin	0.793	0.194	0.120
deltamethrin	0.793	0.267	0.154
p,p’-DDE	0.385	0.130	0.142
p,p’-DDD	0.657	0.092	0.112
o,p’-DDT	0.612	0.227	0.214
p,p’-DDT	0.551	0.401	0.367

PF = Processing factor

5.4. Discussion

Washing of coffee beans decreases pesticide residues up to 57.7%. This effect may be due to the fact that most of the pesticides are found on the surface of the coffee beans. Surface residues are removed by washing processes with the very thin layer of coffee beans located just above the main coffee beans or with the removal of dust or soil on the coffee beans. Pesticides are applied during the pre- and post-harvest stage of the crop. Contamination due to the presence of pesticides takes place in both cases. In addition to this, pesticide contamination may come from the environment due to previous persistent applications so that they may appear on the surface at any stage of the crop. Indeed, (Ahmed et al., 2011) confirm that surface residues are removed by washing processes with water. The majority of the pesticides applied on agricultural crops are confined to the surface (contact action). Few pesticides really penetrate into the plant system (Toker and Bayındırlı, 2003). As a result, they are removed by washing, trimming or peeling. During the washing process, a maximum reduction was observed for α- and β-endosulfan for which the residue decreased to an extent of 53.1 to 57.7%, respectively. A similar result was observed from a study done in India. Washing of the vegetable brinjal (an eggplant) with tap water decreased endosulfan residues up to 55%. The researcher concluded in that rinseability of some pesticides by washing with water not always correlates with their water solubility (Cengiz et al., 2007). As indicated in **table 5.1**, most of the pesticides in this study have low water solubility with a high octanol-water partition co-efficient ($\log K_{ow} > 3$). As a result washing was comparatively found less effective in reducing the residues of the lipophilic pesticides such as p,p'-DDD (14.6%), hexachlorobenzene (20.8%), heptachlor epoxide (23.4%), chlorpyrifos ethyl (26.5%), o,p'-DDT (28%) and p,p'-DDT (28%). (Yoshida et al., 1992), reports that heptachlor epoxide in cucumber was not well removed during the washing process.

In this study, roasting is found to be effective to affect the pesticide residues in coffee beans. This process reduces the residue in the range of 72.5 - 99.8%. Thermal processing treatments like cooking, baking, blanching, steaming, roasting and boiling have been found effective for the dissipation of various pesticides (Bajwa and Sandhu, 2014). Additionally, there is a loss of pesticides due to the heat treatment in related to the physico-chemical properties of the pesticides (Sharma et al., 2005). A study done in Japan on the behaviour of pesticides in coffee beans reports that during the roasting process the pesticide residues decrease to a significantly lower concentration (Sakamoto et al., 2012).

After the coffee brewing process, none of the pesticides under study were detected reflecting a 100% removal. This process is found to be the most effective method to mitigate the problem related to pesticides under study. As it has been explained by (Abou-Arab and Abou Donia, 2001) the elimination of pesticide residues from the boiled extract of coffee may be due to the decomposition of the pesticide residues by the application of heat. The pesticide residues were significantly influenced by the roasting and brewing processes. In general, the three household coffee processing methods have a cumulative effect in the reduction of pesticides as a result the safety of consumers is assured. **Figure 5.4** illustrates the cumulative reduction of the pesticides by the household processing methods.

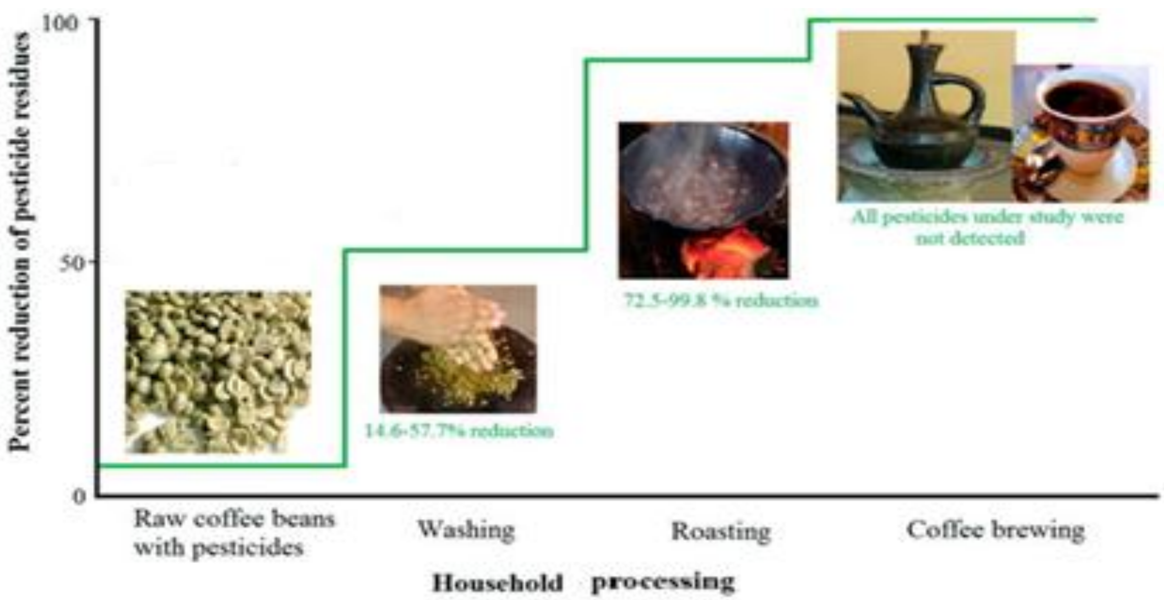


Figure 5.4. The percent reduction of pesticide residues during household processing of coffee

The Kruskal-Wallis test showed that there is strong variation in the stability of pesticide residues in coffee beans among the processing methods (p-value = 0.00001). The reduction of pesticide residues due to washing is significantly lower compared to roasting (p-value = 0.0001) and brewing processes (p-value = 0.00001). According to Hasmukh et al. (2012) the washing process depends on different factors like location, age of residue, water solubility and temperature which explains the variation. However, there is no significant difference between the roasting and brewing process (p-value > 0.05). As indicated in the **figure 5.4**, the roasting process decreases the residues of the pesticides in this study up to 99.8% ~ 100% just like coffee brewing. This may be due to the application of heat in both roasting and brewing. Heat affects the stability of the pesticides (Bajwa and Sandhu, 2014). Pesticide residues were also determined in wash water and coffee sludge. The results indicate that all DDT metabolites and deltamethrin were detected in the wash water while only p,p'-DDT and deltamethrin were detected in coffee sludge. The detection of p,p'-DDT and deltamethrin in coffee sludge may be due to the lower percent reduction of these compounds during the roasting process compared to the other pesticides. The analysis of the residue of these two pesticides in the coffee sludge is important because, in Ethiopia, the wash water from the coffee beans and the sludge after brewing the coffee are discarded into the open environment which may indirectly contaminate the food. So, care should be taken during the disposal of both the liquid (wash water) and semi solid (coffee sludge) waste. In addition, during pouring of the coffee solution to drink or serve coffee, only the upper layer of the coffee infusion should be drunk for the safety of consumers.

The household processing of teff to dough and baking of teff injera also decreases the pesticide residues under study. Dough preparation decreases the residue of the pesticides up to 86.4%. This may be due to the fermentation process for three days. Fermentation is a process which leads to a large reduction in pesticide residues from the original amount (Regueiro et al., 2015). Sharma et al. (2005) also reports that fermentation facilitates the reduction of pesticides in food products.

The baking process decreases the residues in particular chlorpyrifos ethyl up to 90.2%. This may be due to the application of heat during the baking process. According to Byrne and Pinkerton, (2004), baking decreases the residue of chlorpyrifos up to 98% in winter squash and up to 70% in sweet potato.

The preparation of dough and baking has a lower removal effect on p,p'-DDT (parent compound DDT). The residue of p,p'-DDT reduced only up to 59.9% and 63.3% during doughing and baking, respectively. This may be due to the persistent nature of DDT for degradation by bacteria during fermentation or resistance to heat application during baking. The processing factor (PF) for both coffee beans and teff flour were below one for each processing steps and indicates a reduction which is also reported by (Bonnechere et al., 2012).

5.5. Conclusions

Food is a basic need to human beings more than shelters and/or clothing. However, food may be contaminated by different chemical, physical or biological hazards. Pesticides are one of the major chemical hazards in food. Different scientific investigations are required to understand the real dietary intake of pesticides to setup regulatory standards in commonly consumed food commodities. To support these processing studies, it is important to understand the real dietary intake of pesticides. The present study demonstrates the dissipation of the pesticides under study during household processing of coffee and teff. Roasting and brewing of coffee were the most effective methods to reduce pesticide residues. Pesticides were detected in coffee sludge. Hence, the mix up of the coffee with the residue should be avoided and the brewed coffee infusion should be mandatory to drink pesticide free coffee. The household teff processing has also an effect on the reduction of pesticide residues. Good reduction of the residue was observed during the baking process. This may be due to the application of heat. The PF for both coffee and teff were less than one ($PF < 1$) which means that there is a reduction of pesticides due to the applied household processing methods. Such reduction is important in evaluating the risks associated with ingestion of pesticides, specifically in coffee which is a widely consumed beverage in the world and teff which is the most commonly consumed cereal in Ethiopia. The result of this study assured that household processing of coffee and teff have a paramount effect on the reduction of pesticide residues and this initiates to work on the effect of different household processing methods on other pesticides.

Chapter Six: Pesticide residues in drinking water and associated risks to consumers in Ethiopia

Redrafted from: Mekonen S., Argaw R., Simanesew A., Houbraken M., Senaeve D., Ambelu A. and Spanoghe P (2016). Pesticide residues in drinking water and associated risk to consumers in Ethiopia. Chemosphere, 162, 252-260, doi.org/10.1016/j.chemosphere.2016.07.096

Abstract

Access to safe and reliable drinking water is vital for a healthy population. However, water sources may become largely contaminated with pesticides because most of them are located nearby agricultural areas. Water samples were collected from different water sources (river, spring, well, pond, and community taps) in Jimma zone and Addis Ababa, Ethiopia. The extraction and clean-up of the samples were undertaken using liquid-solid and liquid-liquid extraction methods. Human exposure to the detected pesticides was assessed by calculating the estimated daily intake (EDI) of pesticides from the water. To evaluate the risk, the EDI was compared with the acceptable daily intake (ADI) for the long term exposure and the acute reference dose (ARfD) for the short term exposure. The findings of our study indicate that 2,4-D, malathion, diazinon and fenpropimorph were present in all water sources while pirimiphos methyl was detected only in well and spring water. The mean concentration of 2,4-D, diazinon, malathion and fenpropimorph from Jimma water sources, were in the range of 2.27 to 2.94, 1.59 to 5.65, 1.71 to 5.03 and 7.25 to 13.90 µg/l, respectively. Only 2,4-D, diazinon and fenpropimorph were detected in the water source of Addis Ababa with a mean concentration range of 0.60 to 69.10, non-detected to 32.10 and 0.11 to 138 µg/l. From both sample sources (Jimma and Addis Ababa), the residues of some of the pesticides were above the European drinking water guide line set by European Commission (EU) and World Health Organization (WHO), which indicates that there is overuse of pesticides by farmers in the study area. Concerning human health risk estimation, all the pesticide detected have no acute risk for consumers (EDI < ARfD). However, chronic risks to human health were observed from diazinon and fenpropimorph (EDI > ADI), for Jimma and Addis Ababa population. A comprehensive monitoring and source oriented remediation action is required to reduce the level of pesticide residues in water and to minimize particularly the long term health risks on human health.

6.1 Introduction

Access to clean water is a fundamental human right and vital to sustain healthy life. Reports, however, indicate that residues of pesticides occur in different water sources (Ntow, 2001; Sankararamakrishnan et al., 2005; Flores-García et al., 2011; Chowdhury et al., 2012; Varca, 2012). Environmental contamination of natural water by pesticide residues during and after field applications is of a great concern. Water is an important component of public health and failure to supply safe drinking water causes a heavy health burden to humanity (Kooij, 2014).

The contamination of surface and ground water by pesticides is discussed around the world (Donald et al., 2007; Varca, 2012). A study done by Teklu et al. (2015), detected 2,4-D, malathion, deltamethrin, atrazine, chlorothalonil and endosulfan in surface water in Ethiopia.

Pesticides may enter into the aquatic system by diffusion to the surface or subsurface hydrological pathways. Pesticide transport to surface water, is mainly caused by spray drift, runoff water and drainage water (Ikehata and Gamal El-Din, 2005). This may happen, due to improper operations such as filling of sprayers, washing of measuring utilities, disposing of packing materials and cleaning of spraying equipment. The pesticide sprayers in Ethiopia can do mixing/loading or dilution of pesticides near to water sources, as illustrated in **figure 6.1**, which contaminates the water as well as the irrigated crops. In turn, such practices affect the health of the communities living around these areas. Additionally, applications on lawns and runoff during rain events are prone to be flushed into the sewerage channels and end up in the receiving water bodies (Gerecke et al., 2002).

Due to the persistence of some pesticides in the environment and potential adverse acute or chronic health effects for consumers, contamination of surface and ground water has long been recognized as an important issue in many countries. Understanding the fate and quantity of pesticides before and after treatment of drinking water is crucial (Benotti et al., 2009). In developing countries, the need to ensure local agricultural production and food security while simultaneously protecting the population against health effects from pesticide exposure remains a major public health challenge. According to Kesavachandran et al. (2009), developing countries use only a small amount of the world's agrochemicals, but they suffer from 99% of deaths due to unsafe application of the pesticides and poor handling due to illiteracy and poverty status of the users.

Figure 6.1. A farmer preparing pesticides not far from a water source which is used by the communities living around (Environment and Social Assessment International, 2006)

Although Ethiopia has a huge potential of surface and ground water sources, the country utilizes a small portion of these resources. Nowadays, the access to safe drinking water is a bit improved. According to central statistics agency of Ethiopia, more than half of the households in Ethiopia (57%) have access to an improved source of drinking water, with a much higher proportion among urban households (Central Statistical Agency [Ethiopia], 2014).

Some of the water sources in the rural community around Jimma zone, southwest Ethiopia are springs, well water, and ponds. The dwellers of Jimma town use water from different sources such as tap water, springs, wells and rivers for drinking and other domestic purposes. There is one conventional water treatment plant in the town which supplies purified water for the population of the town (Kifle and Gadisa, 2006). Other parts of the communities in Jimma zone do not have a conventional water treatment plant but rely on the different water sources such as springs, well and river water.

Addis Ababa, the capital of Ethiopia and the diplomatic centre of Africa, is one of the fastest growing cities in the continent. Its population has nearly doubled every decade. The city is subdivided into ten sub-cities with a total population of 3,048,631. The main water supply sources in Addis Ababa are generated from the three ponds (Geferesa, Legedadi, and Dire) and ground water source (Akaki ground water). Water coverage of the city is about 94% in 2012 with a daily supply of 374,000 cubic meters (City Government of Addis Ababa, 2013).

The Legedadi pond is the largest water supply source which contributes 40% of the town water. The water treatment capacity of the plant is 150,000 cubic meters per day (UNEP/UNESCO/UN-HABITAT/ECA, 2003). The catchment area of Legedadi is about 206 square kilometres and is surrounded by farm lands. These farm lands are owned by smallholder farmers who use different crop protection products. During application these pesticides may contaminate the environment in particular the water sources which are used for drinking by the communities living around. The other possible sources of pesticide contamination in the drinking water sources may be attributed to leaks of pesticides from obsolete stockpiles near the study area ("Sireguyo pesticides store") (personal observation). Inappropriate storage, poor handing practice, inappropriate labelling (labels are in English rather than in local language), and illegal use of pesticides due to poor knowledge of farmers may aggravate the contamination of the water sources (Haylamicheal and Dalvie, 2009).

The Jimma and Legedadi water treatment plants use conventional water cleaning processes, such as coagulation/flocculation, filtration and chlorination which have been shown to be fairly ineffective to completely remove pesticides (Saifuddin et al., 2011). Recent studies indicate that reverse osmosis, nano-filtration and adsorption on granular and powdered activated carbon are more effective to remove pesticides (Benotti et al., 2009). However, these treatment processes are not applied in the water treatment processes in Ethiopia.

A study conducted in the rift valley region of Ethiopia detected organochlorine pesticides such as endosulfan and DDTs soil samples at a concentration of 56 mg/kg and 0.00023 mg/kg, respectively. Such contamination is known to pose a risk of contamination to the surface and surrounding water bodies (Westbom et al., 2008). In Ethiopia, the health hazards associated with pesticide handling are not well understood by both the sprayers and consumers. Misuse of pesticides, lack of awareness towards the proper handling of pesticides and poor monitoring systems and the presence of high amounts of pesticides are the main contributors of water source contamination (Mekonnen and Agonafir, 2002). Historically, pesticide use by small holder farmers has been low. Recent developments, however, increased the use of pesticides to diversify food production and to enhance the international floriculture industry (Amera and Abate, 2008). In the rift valley regions of Ethiopia, water samples taken from surface waters around agricultural fields, effluent waters from floriculture and intake of the Zeway water supply system show that concentrations of pesticides exceeded the European Union drinking water standards (Jansen and Harmsen, 2011).

To date, there are no comprehensive assessments of pesticide residues from different drinking water sources upon which consumer risk could be predicted. Therefore, the present study aims at investigating pesticide residues from different water sources (river, spring, well, and community taps) that are used for human consumption and to undertake consumers risk assessment.

6.2 Materials and Methods

6.2.1. Study area

One of the sampling areas is the Addis Ababa water supply system. Addis Ababa lies 9°1′48″N latitude and 38°44′24″E longitude. The city is located at the heart of the country at an altitude of 2100 meters above sea level. The city occupies a total area of 540 km^2 and has a complex mix of highland climate regions with annual average temperature of 22.2°C. The study area encompasses the Legedadi pond which is the main water supply source for Addis Ababa population. During data collection, geographical positioning system (GPS) coordinates were recorded on sites where water samples are collected to construct a map of study area for both Jimma zone and Addis Ababa. The catchment area of Legedadi pond is about 206 square kilometres, which has similar rainfall and temperature conditions compared to Addis Ababa **figure 6.2**.

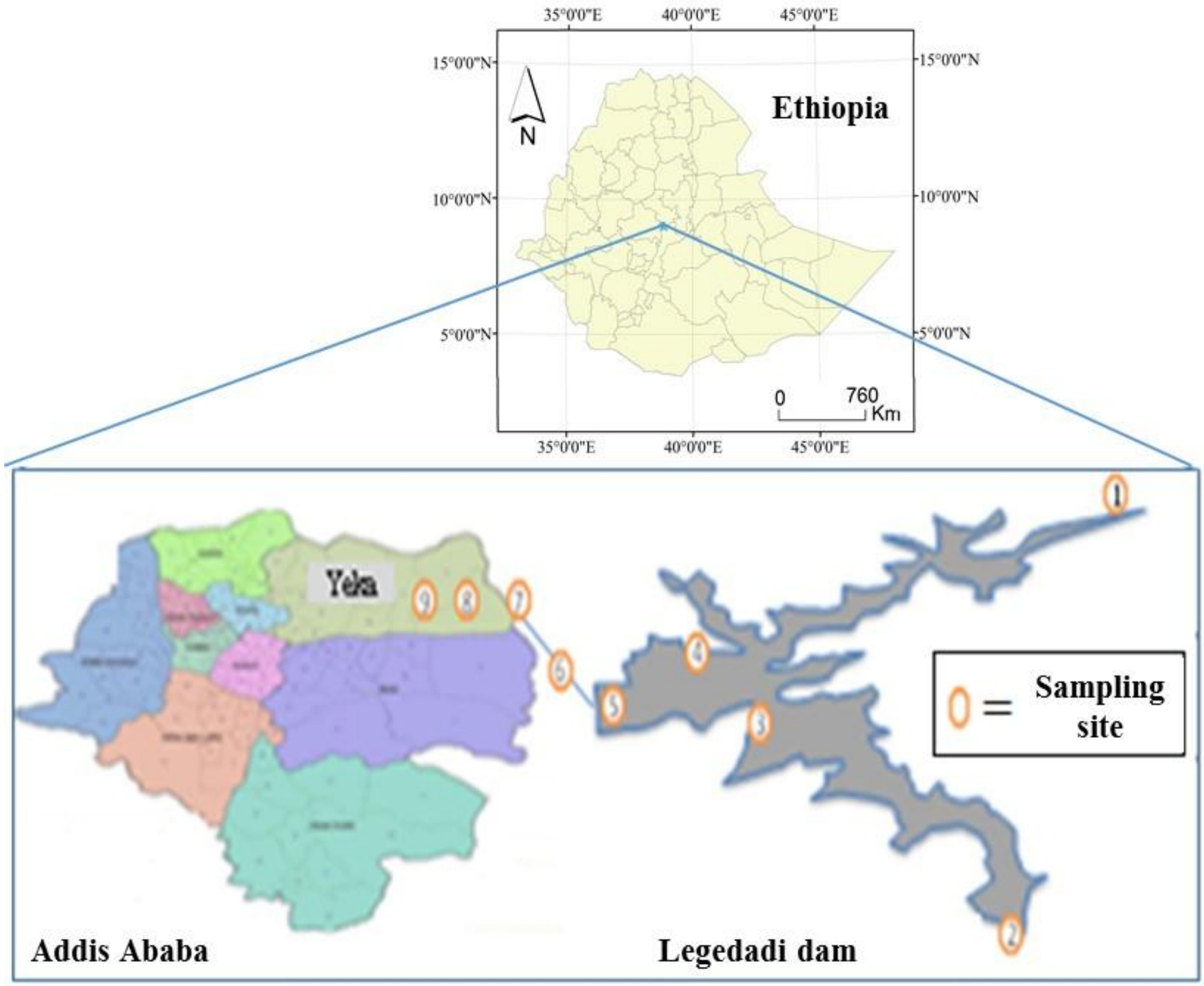


Figure 6.2. Map of the study area in Addis Ababa water supply system

The other sampling area is Jimma zone Southwestern Ethiopia water sources (spring, well, river, and community taps). The study area includes ten districts located in Jimma zone in which water samples were collected. Jimma zone is located in Oromia region, 1744m above sea level, at latitude of 7°40’0.01” and longitude of 37°0’0” **figure 6.3**.

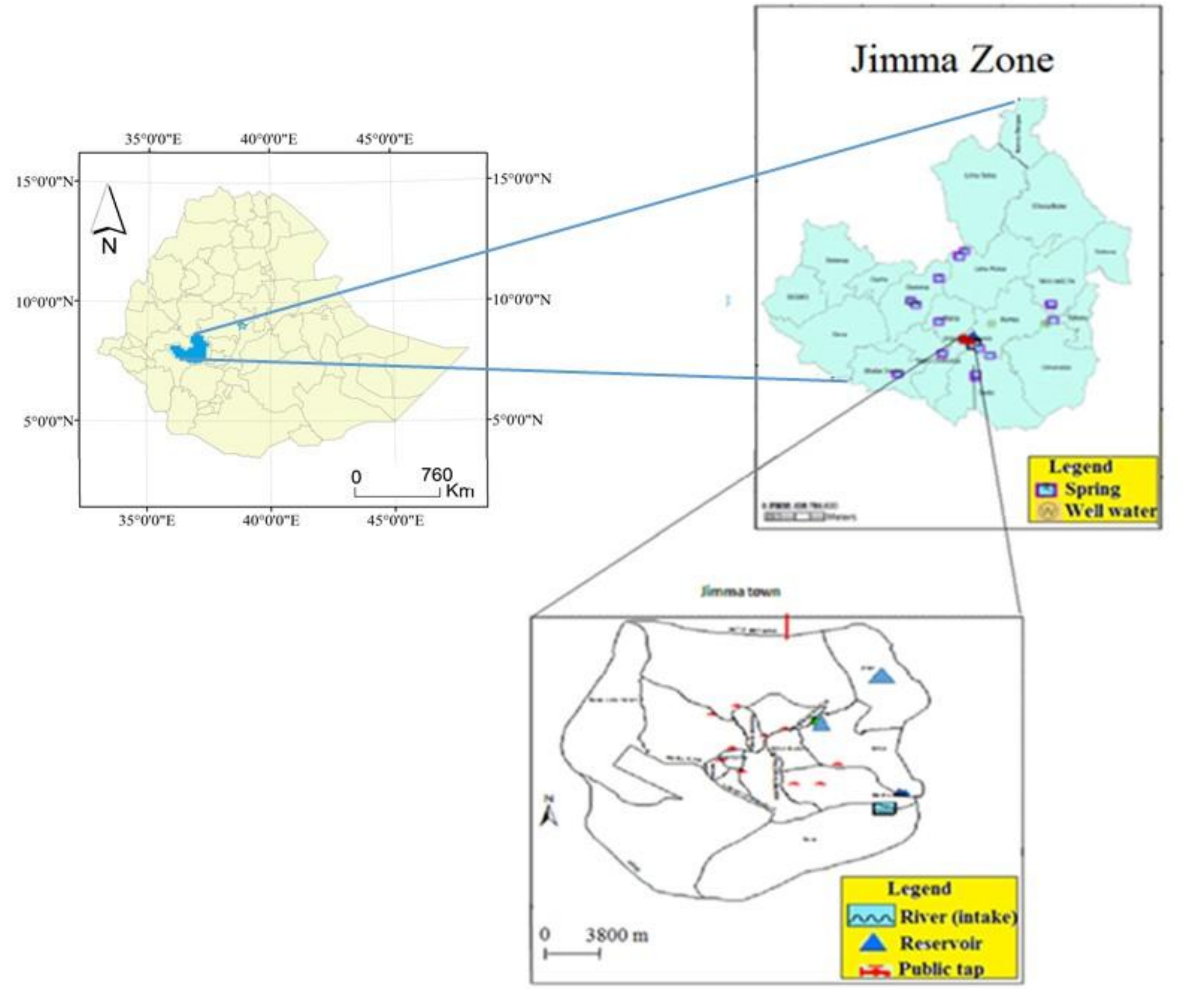


Figure 6.3. Map of the study areas in Jimma zone, southwestern Ethiopia

6.2.2. Sampling

Water samples were collected from different water sources (springs, river, well) using a grab sampling method, in which the sampling bottle is submerged at a certain depth in water (15-20 m) and take the required amount of water. Additionally, water samples also taken from treatment plants before and immediately after treatment including the community taps and distribution reservoirs. Most of the water sources are surrounded by agricultural fields where chemical pesticides were applied (personal observation). The water samples were classified in to treated (samples taken immediately after treatment, community taps and reservoir) and untreated water (samples taken from river water, spring and well).

The water samples were also collected from Addis Ababa main water supply source that is the Legedadi pond and from two small rivers that pass through agricultural fields and join the Legedadi pond. In the pond just before the water treatment plant three different sampling points were considered. In the distribution system four sampling sites were considered (one from just after the treatment, one from central reservoir and two from community taps). A total of nine sampling points were considered from the source up to the consumption points. The samples were collected two weeks after the pesticide application. After collection the water samples were transported using cold box to the laboratory and stored at 4°C until the extraction was done.

6.2.3. Chemicals and reagents

Acetonitrile (99.9%), n-hexane (99%), methanol (100%) and anhydrous sodium sulphate, saturated sodium chloride and HPLC (high-performance liquid chromatography) water were used for the extraction and clean-up of the pesticides. Anhydrous sodium sulphate was used to remove water from the hexane solution and saturated sodium chloride was used to facilitate the separation of water and hexane solution. Methanol and HPLC water were used to activate the Sep-Pak cartridges (C18) before extraction of the water samples. Five point calibration curves were obtained from solutions containing the standard mixture of the pesticide under study (2,4-D, malathion, diazinon, fenpropimorph and pirimiphos methyl). The standard curves were prepared using a concentration range of 0.001– 0.1 mg/l.

6.2.4. Extraction and clean-up of the samples

Based on the collected samples, extractions were made in two different ways: liquid- solid and liquid-liquid extractions. This is important to select a method which gives good recovery for the pesticides under study.

6.2.4.1. Liquid- solid extraction procedure

Sep-Pak C18 classic cartridge with 360 mg sorbet per cartridge, 55-105 µm particle size and 125A° pore size column was used to extract the samples. The Sep-Pak is silica- based bonded phase with strong hydrophobicity which is used to adsorb the analyte of even weak hydrophobicity from the aqueous solution. For our study, the Sep-Pak was first activated with 1 ml methanol passing through the bonded phase and rinsing with 1 ml HPLC water, sequentially. Then, one litre of water sample was passed through the cartridge. The Sep-Pak samples were rolled with Teflon, well labelled and stored at -20°C until the extraction with solvents was done. The extraction procedures were as follows: the Sep-Pack cartridges, in which a 1000 ml of water sample passed through, were opened. Then, 10 ml of n-hexane as extraction solvent was pushed through the Sep-Pak using 10 ml syringe into a flask. Five to six grams of sodium sulphate (Na_2SO_4) was added to the flask which is to separate water from the solvent. Then n-hexane was evaporated to dryness using a rota-vapour at 40°C and reconstituted with 10 ml of acetonitrile to make the samples amenable for LC-MS/MS injection. Then the extract was put into vials for the determination of pesticides using LC-MS/MS instrument.

6.2.4.2. Liquid-liquid extraction procedure

In addition to the Sep-Pak samples, raw water samples were collected from each of the sampling sites. A 250 ml plastic bottle (two bottles for each site) was used to collect raw water samples, which were rinsed well with distilled water and dried in the air before sample collection. The bottles were carefully filled with the water samples to overflow without trapping air bubbles in the bottle. The samples were transported to the laboratory and stored in similar condition with the Sep-Pak samples.

The liquid-liquid extraction procedure was undertaken as follows: a 500 ml of water sample was added into a one litre separator funnel and 100 ml of n-hexane was added followed by 10 ml of saturated sodium chloride (NaCl). Samples were agitated by hand to enhance the contact of the solvent with the pesticides. After 10 minutes, the hexane and water medium were separated and the water phase was removed at the bottom of the separator funnel. The hexane solution passed through 15 grams of anhydrous sodium sulphate into a 250 ml flask in order to absorb any remaining water from the hexane solution. For the completeness of the extraction, this procedure was repeated once; again the hexane solution was diluted up to 250 ml n-hexane and then evaporated to dryness using rota-vapour at 40°C and reconstituted with 10 ml of acetonitrile. The extract was then transferred to LC-MS/MS vials for analysis.

6.2.5. Analytical equipment and chromatographic separation

For the determination of the pesticides under study, liquid chromatography with double mass spectrometer (LC-MS/MS), with water acuity UPLC instrument was used. The respective chromatographic conditions were as follows: The column was water HSS T3 (1.8µm) and an injection volume of 5µl. The oven temperature was 40°C and a flow rate of 0.4 ml min^{-1}. The detector of triple quadruple mass spectrometer with Electrons Spray Ionization (ESI), and with a potential of 500 volt and a temperature of 500°C. The scan type was multiple reactions monitoring mode (MRM). The respective ESI, Ions (parent and daughter ions) of the pesticides in this study were indicated in **table 6.1.**

Table 6.1. The parent and daughter ions for each pesticide analysed in water samples using LC-MS/MS

Pesticide	Parent ions m/z	Daughter ion m/z	ESI charge	Cone voltage (eV)	Collision energy (eV)
2,4-D	218.9/220.9	160.7/162.7	Negative	20/20	11/11
diazinon	305	95/169	Negative	31/31	35/22
malathion	331	99/127	Positive	20/20	24/12
fenpropimorph	304.2	57/147	Positive	50/50	30/28
pirimiphos methyl	306	100.1/164.1	Positive	30/30	32/22

m/z = mass to charge, eV = electron volt, ESI = Electron Spray Ionization

During the analysis, analyte concentration below the limit of detection (LOD), but giving signals were reported as less than the LOD. Moreover, the observed concentrations from the instrument were compared with the norm value of 0.1 µg/l set for single pesticides by the European Commission (EU) and World Health Organization (WHO) guidelines (World Health Organization,2008, World Health Organization, 2011, Northern Ireland Environment Agency, 2011).

6.2.6. Screening

Screening was carried out before the actual water samples were analysed to determine which pesticide was presented in the samples. During the screening, pesticides such as DDT, deltamethrin, permethrin, cypermethrin were also included, while only fenpropimorph, diazinon, malathion and 2,4-D were detected. The pesticides that are not detected may be due to their low water solubility. The screening was performed by mixing a small portion of sample from each site and passing it through the Sep-Pak cartridge. Subsequently, the quantitative determination and identifications of each pesticide was worked out for all the samples using peak area and retention time, respectively.

6.2.7. Quality control

Representative samples were collected in clean materials for the extraction and analysis. Sep-Pak C18-column extraction was made within 48 hours of sample collection, which helped to avoid degradation of the pesticides and to determine the accurate quantity of the residues. Both the Sep-Pak cartridges and the raw water samples were transported in a cold box with ice pack to the laboratory and were stored at refrigerator temperature until the extraction was made. The glass-ware materials used were kept clean to avoid cross contamination and all the reagents used were analytical grade.

Before the analysis of the samples, the method validation was undertaken by determining the recovery of pesticides, limit of detection and quantification and the regression coefficient. To determine the accuracy of the method, recovery studies were performed by spiking a known amount of the standard mixture of pesticides in ultra-pure water. The percent recovery was calculated by dividing the recovered concentration by the spiked concentration. The limit of detection (LOD) and the limit of quantification (LOQ) were also determined by taking the signal to noise ratio (S/N) into account. For LOD the lowest concentration when S/N ratio is over 3 was considered, while for LOQ the lowest concentration when S/N ratio is over 10, was considered.

6.2.8. Statistical analysis

Statistical analysis was performed using SPSS (Version 20). Before the actual data analyses, normality of the data was checked using histogram. The concentration of all the four pesticides (2,4-D, malathion, diazinon and fenpropimorph) showed skewness. As the result, a non-parametric statistical analysis was followed. Kruskal-Wallis test was applied to compare the distribution of pesticides residues among the water sources (spring, well, river, pond, and community taps). The level of significance was set at p-value = 0.05. The results were presented using a box and whisker plot.

6.2.9. Risk assessment

To understand the intake of pesticides under study from consumption of water, risk (both chronic and acute) was assessed for human population with different groups (Adult, Children and Infants).

6.2.9.1. Chronic risk assessment

The estimated daily intake (EDI) was calculated from the residue of pesticides in water and the consumption of water. Then to characterize the risk EDI was compared with acceptable daily intake (ADI) for each pesticide. The ADI is an estimate of the daily maximum intake of a substance over a lifetime that will not result in adverse effects at any stage in human life span. It is expressed on a body weight bases (Food and Agriculture Organization of the United Nations and World Health Organization, 2009).

The EDI (mg/kg bw/day) is found by multiplying the residual pesticide concentration in drinking water (µg/l) with the daily intake of water and dividing it with the average body weight of consumers based on the equation below (Mahmood et al., 2014). The average daily intake of drinking water for a 60 kg adult, 10 kg child and 5 kg infant were 2 l, 1 l and 0.75 l per day, respectively (Younes and Galal-Gorchev, 2000).

EDI is calculated by the following formula.

$$EDI = C_w * I_w / B_w$$

Where:

C_w = concentration of the pesticide in water (µg/l)

I_w = daily intake of water (l/day)

B_w = Body weight (kg)

For our study, the maximum concentration in water was considered (worst case scenario) to calculate the EDI. From our findings, EDI greater than ADI indicates a potential chronic human health risk for consumers as reported by (Darko and Akoto, 2008).

6.2.9.2. Acute risk assessment

The short term exposure to pesticide residues was assessed via oral intake of drinking water containing the pesticides under study. The acute reference dose (ARfD) is an estimate of an oral exposure of a chemical for short term duration (usually 24 hours). The ARfD is used as a measure of acute toxicity of pesticides. The presence of pesticides in water sources may not be uniform or under average expected level. The non-uniform distribution of residues within water may result in some individuals being exposed to above average levels for short periods of time resulting in an acute risk (Renwick, 2002). The EDI for short term exposure of the pesticides under study was calculated by the same equation of the chronic exposure and compared with ARfD. EDI greater than ARfD is considered as, there is a potential acute human health risk for consumers.

6.3 Results

6.3.1 Method validation results

The method validation results are given in **table 6.2**. The regression coefficient (r^2) was greater than 0.995 for all the pesticides under study which indicates that there is a perfect linearity of the standard curves. The mean percent recovery (% recovery) was in the range of 72.2-92.0%. The method validation results met the requirements of European commission (EU) (SANCO/12571/2013, 2013). This revealed that the method was accurate to determine the pesticides under study. The LOD and LOQ were in the range of 0.003 to 0.0150 and 0.01 to 0.050 µg/l, respectively. This indicates that the method is able to detect up to a minimum concentration of 0.0003µg/l.

Table 6.2. Method validation results

Pesticides	r^2	% recovery	LOD (µg/l)	LOQ (µg/l)
2,4-D	0.9998	85.400	0.0030	0.010
diazinon	0.9995	83.000	0.0003	0.001
malathion	0.9997	92.000	0.0003	0.001
pirimiphos methyl	0.9999	82.000	0.0150	0.050
fenpropimorph	0.9998	77.200	0.0003	0.001

LOD = limit of detection, LOQ = limit of quantification

6.3.2 Pesticide residue in drinking water sources in Jimma zone, southwest Ethiopia

Water samples collected from all water sources were analysed for the presence of pesticides. From the results, 2,4-D, diazinon, malathion, fenpropimorph and pirimiphos methyl were detected. From these pesticides pirimiphos methyl was detected in only two water sources such as well and spring water with a concentrations of 0.33 and 168.20 µg/L, respectively. A summary of the residues of the pesticide in the water samples is given in **table 6.3**. The average concentrations of 2,4-D ranged from 2.27 to 2.94 µg/l among the different water sources, where the highest concentration was found in well water. The highest concentration of diazinon (~5.65 µg/L) was registered in the river water sample of which Jimma town receives water for its municipal water treatment.

Table 6.3. Summary statistics for the pesticide residue (µg/l) in drinking water sources of Jimma zone

Pesticides	Water source	Min	Max	Mean	StDv
2,4-D	River water	1.590	3.971	2.666	0.796
	Spring	1.574	5.703	2.690	0.931
	Tap water	1.412	3.472	2.270	0.517
	Well water	1.559	5.797	2.939	1.361
	Distribution reservoir	1.368	3.365	2.354	0.583
diazinon	River water	3.000	7.477	5.646	1.321
	Spring	0.0001	15.633	1.592	3.229
	Tap water	0.0001	9.644	2.023	2.187
	Well water	0.0001	11.491	2.197	3.863
	Distribution reservoir	0.0001	24.734	3.280	6.997
fenpropimorph	River water	1.854	9.042	5.028	1.940
	Spring	0.008	7.036	1.711	1.622
	Tap water	0.008	7.773	2.681	2.279
	Well water	0.008	11.491	2.408	3.290
	Distribution reservoir	0.008	8.085	3.300	2.088
malathion	River water	0.0001	50.162	7.658	11.945
	Spring	0.0001	67.439	7.252	12.707
	Tap water	5.1000	11.948	7.713	2.745
	Well water	0.0001	105.031	13.947	27.839
	Distribution reservoir	0.0001	33.844	12.165	13.302

StDv = Standard deviation, river water = intake for treatment

The mean concentration of malathion ranged from 7.25 to 13.95 µg/l, in which the highest concentration was observed in the well water. Fenpropimorph was mainly observed in the river water. The detection of the pesticides at different concentration indicates the contamination of the water environment and which will result in exposure of the communities who consume water from these sources. These pesticides may come to the water bodies from the surrounding agricultural fields. Therefore, building up of buffer zones and strict control of the pesticides applied near to the water sources is important.

Kruskal-Wallis test indicates that the distribution of diazinon and fenpropimorph among the water sources were significantly different (p-value < 0.004) (**figure 6.4)**. But other pesticides had no statistical difference between the water sources. On the other side, water samples taken from the Gilgel Gibe River before treatment, contaminated by high concentrations of diazinon and fenpropimorph compared to the well and spring water sources. This indicates that the environment is highly contaminated by fenpropimorph and diazinon or these two chemicals may be applied more in the surrounding agricultural areas.

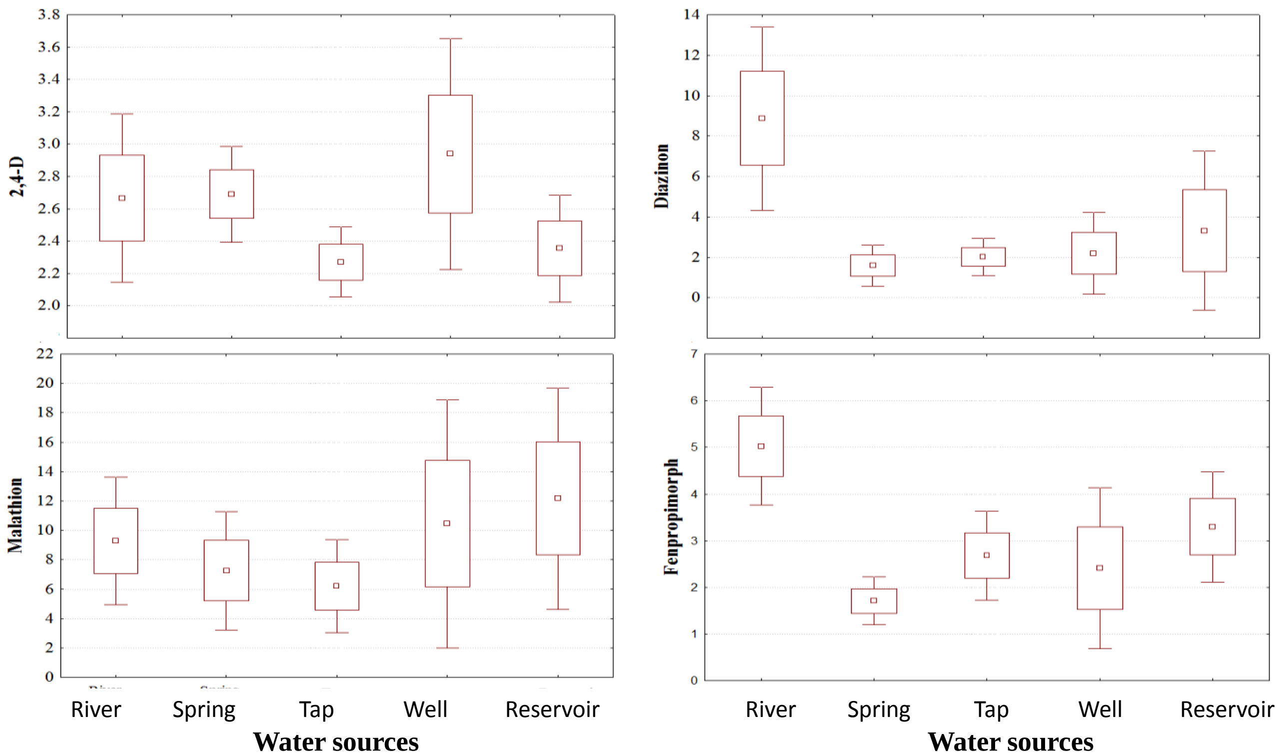


Figure 6.4.Concentration distribution of the pesticides under study (µg/L) among different water sources. The inner box indicates the mean, the outer box indicates mean ± standard error and the whiskers indicate the 95% confidence interval

As it can be depicted from the box and whisker plot, 2,4-D and malathion residues were much higher in the well water compared to other pesticides under study. This may be due to, long residence time of the water in well.

6.3.3 Exposure assessment of population in Jimma zone to pesticide residues in water

Human exposure was assessed based on the residue of each pesticide in the water sources and the average water consumption **table 6.4**. From the findings, the estimated daily intake (EDI) for adults, children and infants were below the ARfD for all the pesticides under study. The EDI for diazinon was above ADI for adults, children and infants, while all other pesticides under study were below the ADI. The ADI and ARfD for each pesticides were taken from (FOOTPRINT-IUPAC-PPDB, 2015).

Table 6.4. The estimated daily intake of pesticides by population Jimma zone from consumption of water

Pesticides	Water sources	Max. Conc. (µg/l)	EDI (µg/kg bw/day)			ADI (µg/kg bw/day	ARFD (µg/kg bw/day
			Adult	Children	Infants		
fenpropimorph	River water	9.042	0.301	0.904	1.356	3	30
	Spring	7.036	0.235	0.704	1.055		
	Tap water	7.773	0.259	0.777	1.166		
	Well water	11.491	0.383	1.149	1.724		
2,4-D	River water	3.971	0.132	0.397	0.596	50	750
	Spring	5.703	0.190	0.570	0.856		
	Tap water	3.472	0.116	0.347	0.521		
	Well water	5.797	0.193	0.579	0.869		
diazinon	River water	7.477	**0.249**	**0.748**	**1.122**	0.2	25
	Spring	15.633	**0.521**	**1.563**	**2.345**		
	Tap water	9.644	**0.321**	**0.964**	**1.447**		
	Well water	11.491	**0.383**	**1.149**	**1.724**		
malathion	River water	50.162	1.672	5.016	7.524	20	300
	Spring	67.439	2.248	6.744	10.116		
	Tap water	11.948	0.398	1.195	1.792		
	Well water	105.031	3.501	10.503	15.755		

EDI = Estimated Daily Intake, ADI = Acceptable Daily Intake, ARfD = Acute Reference Dose, Values in bold indicate EDI above health based guidance values.

6.3.4 Pesticide residues in Addis Ababa water sources

The findings of the present study showed the detection of 2,4-D, diazinon and fenpropimorph consistently from the source (river, pond) up to the distribution points in the Addis Ababa water supply system **table 6.5.** The maximum concentration of fenpropimorph (164 µg/l) and 2, 4-D (127 µg/l), were observed in samples collected from the two small rivers while the maximum concentration of diazinon (96.23 µg/l) was observed in Legedadi pond. Regarding the community taps 2,4-D, diazinon and fenpropimorph were detected at concentration of 4.728 µg/l, 0.132 µg/l and 0.126 µg/l, respectively. The conventional treatment process decreases some amount of the pesticides however, complete removal was not observed. As a result the pesticides still detected up to point of consumption (community taps) which may cause a health problem for human health.

Table 6.5. Concentration (µg/l) of 2,4-D, diazinon and fenpropimorph for each sampling point

Pesticides	Water sources	Min	Max	Mean	StDv
2,4-D	River	11.200	127.000	69.100	81.800
	Pond	7.040	30.200	14.900	13.200
	Distribution	4.800	6.590	5.700	1.250
	Reservoirs	5.810	6.120	5.970	0.200
	Community taps	0.230	4.730	0.597	1.260
diazinon	River	ND	ND	ND	ND
	Pond	0.014	96.230	32.100	55.500
	Distribution	0.065	11.000	5.500	7.770
	Reservoirs	0.250	1.240	0.633	0.800
	Community taps	0.034	0.132	0.083	0.600
fenpropimorph	River	112.000	164.000	138.000	36.760
	Pond	0.430	76.900	26.000	44.000
	Distribution	0.173	0.335	0.254	1.100
	Reservoirs	0.107	0.110	0.109	0.020
	Community taps	0.105	0.126	0.116	0.140

ND = not detected, StDv = Standard Deviation

6.3.5 Exposure assessment of population in Addis Ababa to pesticide residues in water

The human exposure for adults, children and infants in Addis Ababa is expressed as the EDI and is presented in **table 6.6.** Adults, children, and infants consuming water from the two small rivers and children and infants consumed water from the Legedadi pond, were exposed above the health based guidance value (EDI > ADI), but less than the ARfD. The EDI for 2,4-D from source up to the community taps were below the ADI and ARfD. Regarding diazinon, the EDI was above the ADI but below the ARfD for adults; children and infants consuming water from the pond. For the exposure assessment the results of the two rivers were combined assuming that the farmers applied similar pesticides as they produced similar crops (personal observation). This hypothesis was confirmed by the obtained study results. The EDI above the health based reference values indicates that there will be health problems for people drinking water from these sources.

Table 6.6. The estimated daily intake of the detected pesticides in water sources

Pesticides	Water sources	Max. Conc. (µg/l)	EDI (µg/kg bw/day			ADI (µg/kg bw/day	ARfD (µg/kg bw/day
			Adult	Children	Infants		
fenpropimorph	River	164	**5.467**	**16.400**	**24.600**	3	30
	Pond	76.868	2.562	**7.687**	**11.530**		
	Community Taps	0.126	0.004	0.013	0.019		
2,4-D	River	126.597	4.220	12.660	18.990	50	750
	Pond	30.247	1.008	3.025	4.537		
	Community Taps	4.728	0.158	0.473	0.709		
diazinon	River	*	*	*	*	0.2	25
	Pond	96.198	**3.207**	**9.620**	**14.430**		
	Community Taps	0.132	0.004	0.013	0.020		

* = exposure was not calculated for non- detected, NA = Not Applicable, ADI = acceptable daily intake, ARfD = acute reference dose, values in bold indicate EDI > ADI

6.4 Discussion

The result of the present study confirmed the presence of 2,4-D, malathion, diazinon, pirimiphos methyl and fenpropimorph, in drinking water sources from Jimma zone southwestern Ethiopia. The herbicide 2,4-D was detected in all water samples and this may be due to the time of sample collection (July-August), in which weed control pesticides are applied in agriculture. Higher concentration of 2,4-D and malathion were detected in well water, while diazinon and fenpropimorph occurred more in river water. This may be due to the rainy season in which erosion may bring pesticides to the water sources from the surrounding agricultural fields.

The results from Addis Ababa drinking water sources (rivers, pond, reservoir and community taps) indicate the presence of 2,4-D, diazinon, and fenpropimorph from source up to the community taps. The highest concentration of 2,4-D and fenpropimorph were found in the river water passing through the agricultural fields. This may be attributed to the high amount of rainfall that occurred during the months preceding pesticide application and the lack of buffer zones around the Legedadi pond, which may help to prevent the contamination of the water body. The herbicide 2,4-D is used mainly for the control of dicotyle weeds in crops such as teff and wheat, which are mostly grown around the studied water basin (Kippie Kanshie, 2002).

In addition to this, the presence 2,4-D is explained by its high water solubility (900 mg/l, at 25°C), its low potential to move from the water into the air. Concerning 2,4-D, the result of this study was not consistent with earlier results obtained in Canada (Donald et al., 2007). This discrepancy may be attributed to the difference in the size of the water body, the pesticide handling systems, the scale of application, the climatic conditions and the amount and duration of the application.

Diazinon and malathion are important organophosphate insecticides in agricultural crops and mainly applied for the control of cockroaches, ants and fleas. Their presence in the water bodies from Jimma and Addis Ababa may be explained by transport from the point of application by drifting to the water and its stability to photolysis. According to (Fadaei et al., 2012), degradation of organophosphate pesticides by photons from the sun is very low in natural water. From a study done in Iran, diazinon and malathion were also detected in drinking water sources (Shayegehi et al., 2007). Chloripyrifos, malathion, alachlor, dimethoate and metribuzin were detected at varying concentration in water samples collected from India (Sinha, 2011). The residue of diazinon in the present study was lower than what is detected in surface water of Iran (Fadaei et al., 2012) and Venezuela (Flores-García et al., 2011). However, the concentration was higher than what is observed in Lebanon (Kouzayha et al., 2013).

Fenpropimorph is a fungicide which is mainly used for the control of fungal diseases like rust in cereals and in barley leaf. Its presence in drinking water may be explained due to leakage from obsolete pesticide stocks, biological desorption of fenpropimorph residues and its stability in the water. The residue of fenpropimorph was present in all Jimma water sources and in rivers and ponds in Addis Ababa water sources. The residues from Addis Ababa were considerably higher than the results obtained in Demark drinking water sources (Schriever et al., 2007). This may be attributed to the lack of awareness of Ethiopian farmers to follow good agricultural practices and to handle pesticide in addition to application conditions and local geological, hydrological and meteorological conditions.

The mean concentration of the pesticides from our study areas exceeded the European drinking water standard of 0.1 μg/l for individual pesticides and 0.5 μg/l for the sum of all the detected pesticides except for the mean concentration of diazinon in rivers. Based on WHO guidelines, the maximum allowable concentration for 2,4-D, diazinon and fenpropimorph in the drinking water is 30 μg/l, 1 μg/l and 0.1 μg/l, respectively (World Health Organization, 2008). The concentrations of 2,4-D and diazinon from Jimma water sources appear to be below the WHO standard, while diazinon and fenpropimorph in the Addis Ababa water sources were above the guide line. The results above the guidelines indicate that there is a high contamination of the water sources by the pesticides under study. The contamination may come from different agricultural fields surrounding the water sources and by poor application and storage of pesticides by users.

From the results in the Jimma zone, high concentrations of the pesticides were detected in untreated water sources (well and river water). This may be attributed to the fact that, the residence time for water is long in the well, which limites the pollutant flow. While for river water the eroded soil from the surrounding agricultural areas may directly enter into the water sources. Regarding the Addis Ababa water sources, there is a continuous reduction of the residues of each pesticide from the source (Legedadi pond and rivers) up to the community taps. This may be due to the longer distance of Addis Ababa from the agricultural area and degradation of the pesticides may happen in between. Additionally, the results may be attributed to the larger volume of water in the pond and the deposition of the pesticides with the sediment. The treatment facilities in the Legedadi treatment plant, may also contribute to further reduction of the pesticide residue in the water.

The detection of these chemical pesticides in the water sources may affect the health of consumers in Ethiopia who uses these water sources for drinking. From the results in the present study, the risk estimation of the pesticides in water from Jimma zone indicates that the EDIs for 2,4-D, malathion and fenpropimorph, were below the ADI and ARfD for all population groups.

This means that there is no acute and chronic health problems expected for consumers. However, the EDI of diazinon for adults, children and infants consuming water from the river, well, spring and even in community taps, were above the ADI, but below the ARfD. This indicates that there is a chronic health problem for the population in Jimma zone. A similar chronic risk of diazinon and malathion from drinking water is also reported for the Iranian population (Shayegehi et al., 2007). Organophosphate pesticides such as diazinon, malathion and others are known as neurotoxic substances inhibiting acetylcholine esterase enzyme and they have genotoxic effects causing DNA damage and also have cytotoxic effects on human health (Muranli et al., 2015).

From the results of Addis Ababa water sources, the EDIs of fenpropimorph for adults, children and infants consuming water from the rivers and for children and infants consuming water from the Legedadi pond were above the ADI, but below the ARfD. The EDI for 2, 4-D for the population who consume water from sources up to the community taps were below the ADI and ARfD. Regarding diazinon, the EDI was above ADI but less than the ARfD for adults, children and infants consuming water from the pond. As is described, the EDI for all the pesticides under study were below the ARfD. This indicates that there is no short term risk for consumers. However, this does not grantee safety for the chronic exposure of these pesticides as confirmed by the results of our study.

Due to cumulative effects of pesticides, adverse human health effects may occur through chronic exposure. This raises the need for a greater attention to create awareness about the use of pesticide by farmers across the water resources. Care should be taken to decrease the risk of these pesticides by establishment of buffer zones (15 to 30 m around the water sources) which minimize pesticide reliance to water sources. Moreover, maintain the application equipment in good working condition and avoidance of poorly timed application of pesticides is important for decreasing contamination of water as is explained by Waskom, (2010).

6.5 Conclusions

The result of this survey depicts that the drinking water sources are contaminated with diazinon, 2,4-D, fenpropimorph and malathion from the source up to the community taps. Some of the detected concentrations of pesticides exceed the European drinking water standard. This indicates that there is intensive use of these pesticides around the study area. The rural communities in Ethiopia, who are using untreated water sources for drinking (well, spring, rivers and ponds), may suffer more than urban communities from health risks. The Jimma and Legedadi water treatment systems reduce pesticides in the water however, complete removal was not observed. This indicates that the conventional water treatment is less effective for the removal of pesticides from water. Based on WHO and EU drinking water guidelines, high amounts of residues still remain in the distribution systems, reservoirs and community taps. Concerning human health risk estimation, there is no acute risk for people drinking water from all water sources as the estimated daily intake is less than the acute reference dose (EDI < ARfD). However, long term risks were observed especially for diazinon and fenpropimorph for which, the EDI > ADI for the Jimma and Addis Ababa population. A comprehensive monitoring and source oriented remediation action is required to reduce the level of pesticide residues and to minimize in particular the long term health risks on human health.

Chapter seven: Exposure to DDT from khat (*Catha edulis*) consumption: a double burden to consumers in southwestern Ethiopia

Redrafted from: Mekonen S., Ambelu A., Negassa B. and Spanoghe P. Exposure to DDT and Its metabolites from Khat (*Catha edulis*) consumption: a double burden to consumers in Southwestern Ethiopia

Submitted to: PLOS ONE and it is under review

Abstract

Khat (*Catha edulis*) is a highly consumed crop for countries located in the horn of Africa. Naturally, khat is known to have different adverse health effects on regular consumers. On top of that khat used for human consumption may contain pesticide residues which may also impose adverse health effects. The present study aimed to investigate DDT and its metabolite (p,p'-DDE, p,p'-DDD, o,p'-DDT and p,p'-DDT) residues in khat and human exposure of khat consumers. A khat consumption survey was undertaken using the 24 hour recall method. Khat leaves with tender stems samples were collected from the local market located in different districts of the Jimma zone in southwestern Ethiopia. Human exposure to this pesticide was determined using the probabilistic exposure assessment method. From the results, eighty percent (80%) of the khat samples contained total DDT. Some of the residues were above the maximum residue limit (MRL) set by Codex Alimentarius and most of the samples contained DDT residues above the European Commission MRL set by European Food Safety Authority (EFSA). The concentration of p,p'-DDE and p,p'-DDT in khat were in the range of 0.033 to 0.113 and 0.010 to 0.026 mg/kg, respectively. The metabolite (p,p'-DDE) was detected at high concentration compared to the parent compound (p,p'-DDT). This indicates that there was historical use of DDT in the study area. From probabilistic exposure analysis, the mean and the 97.5 percentile (p97.5), estimated daily intakes (EDIs) of total DDT were 0.002 and 0.006 mg/kg bw/day, which were below the provisional tolerable daily intake (PTDI). However, this does not guarantee full safety, as there may be contamination of other commonly consumed food items resulting in a cumulative DDT exposure. Khat leaves used for chewing had considerable concentrations of DDT and its metabolites which impose an additional health risk. Stewardship and education of farmers growing khat and continuous monitoring of pesticides including DDT in khat and other commonly used agricultural produce is important to minimize particularly the chronic health risk to consumers.

7.1 Introduction

The use of pesticides is considered to be a vital practice since they increase crop outputs, improve the quality of products, and decrease the incidence of illnesses propagated by insects such as malaria and typhus (Beceiro-González et al., 2012). However, the residue of the applied pesticides remains in the environment. The contamination of food items by hazardous substances, in particular the presence of residues of persistent organochlorine pollutants (POPs) is a worldwide public health concern (Kathpal and Kumari, 2009). Since certain pesticides are hazardous and toxic to human health, any residue remaining in or on food can pose danger to humans and may cause side effects (Aktar et al., 2009). Despite the ban of DDT since the 1970s, the use of this chemical has continued in certain parts of the world and in particular, in developing countries for the control of malaria due to its effectiveness and cheap price.

In the past decades, the intensive use of DDT for agricultural and anti-malarial purposes in developed and developing countries has resulted in significant contamination of food products (Nakata et al., 2002). The residue of DDT in different foodstuffs has been investigated in various countries to understand the status of contamination and to evaluate the possible impact on human beings. Due to its persistence and bioaccumulation properties, DDT can be transferred and magnified to higher trophic levels through the food chain (Man et al., 2013).

Because of their persistent nature, the residues of DDT and its metabolites still appear as a contaminant in both plant and animal based foods such as medicinal plants (Rodrigues et al., 2005), tea leaves (Amirahmadi et al., 2013), tobacco (Cai et al., 2005; Ghosh et al., 2014; Rahman et al., 2012), grapes (Turgut et al., 2011), wheat (Daba et al., 2011), and maize (Mekonen et al., 2015). Additionally, DDT can be detected in fruits and vegetables such as papaya, banana, mango, pineapple, tomato, lettuce, cabbage, green pepper, onion, cucumber (Bempah et al., 2012). Moreover, from a study done in Tunisia and Ethiopia, DDT and its metabolites are detected in cow and human milk (Ennaceur et al., 2007; Gebremichael et al., 2013).

Exposure to pesticide residues from food consumption is assumed to be five orders of magnitude higher compared to exposure through air or drinking water (Claeys et al., 2011). Pesticide residues in foods have received great attention as one of the most important food safety issues considered for consumers (Zhang et al., 2011). Pesticide residues can affect consumers more specifically, when food items are freshly consumed without any treatments or preparation processes which may reduce the pesticide residues (Daba et al., 2011). Khat chewers consume the fresh leaf without any processing.

Khat (*Catha edulis*), is a flowering evergreen tree or large shrub of the Celastracea family. It is a well-known natural stimulant and is chewed as a refreshment, excitement and euphoria. Even though, khat is consumed for different purposes, there is no data on the exact number of Khat chewers on a worldwide scale. However; the number of consumers is increasing in time (Dessie, 2013). Khat is known to have cathinone, cathine and norephedrine in which cathinone is the principal psychoactive component. For this reason khat is blamed for different adverse health effects (Damena et al., 2011; Douglas et al., 2011; Dessie, 2013). This is a reason that khat is illegal in most European and North American countries (Armstrong, 2008). The health effects due to the natural content of khat is a primary burden to regular khat users in the worldwide, especially in countries located in the horn of Africa, such as Ethiopia, Somalia, Kenya, Eritrea, Djibouti and Uganda, as well as across the Arabian Sea such as Yemen and Saudi Arabia (Al-Mugahed, 2008).

Additionally, khat leaf is expected to contain higher pesticide residues compared to other processed food groups of plant origin since it is consumed raw without any processing steps like washing or cooking. High residues of DDT were reported in khat leaves collected from specific farm lands in Ethiopia (Daba et al., 2011; Ligani and Hussen, 2014). According to El-Zaemey and his colleagues (El-Zaemey et al., 2015), Ethiopian khat cultivators use DDT and other pesticides as khat growing plant protection products. Despite the problems related to khat use, only a few studies are published which present very high concentrations of p,p'-DDT, ranging from 999.0 to 141.2 µg/kg in khat samples collected in the eastern part of Ethiopia (Daba et al., 2011). Another study done in the southern part of Ethiopia also indicates up to 44.8 µg/kg of p,p'-DDT in khat samples (Ligani and Hussen, 2014). Jimma zone is one of the khat growing areas in the southwestern part of Ethiopia where khat chewing is a deep-rooted tradition in the population (Damena et al., 2011). Therefore, the main aim of the present study is to determine the exposure of khat chewers to DDT and its metabolites.

7.2 Materials and Methods

7.2.1 Study area

The present study was conducted in Jimma zone, Southwestern Ethiopia. Jimma zone is one of the khat growing areas and khat is commonly intercropped with other agricultural crops such as maize and teff (Dessie, 2013). The study area includes six districts of Jimma which are considered as a source of Khat for local markets **figure 7.1.** Jimma town was selected to conduct the khat consumption survey. The town is located at 350 km to the southwestern part of the capital Addis Ababa. It is found on approximately 7°41′ N latitude, 36° 50′ E longitude and an average altitude of 1,780 meters above sea level. According to 2014-2015 report of Central Statistical Agency (CSA), the projected total population of the town is 170,955 (Male = 85,695 and Female = 85,260) with 85,260 as the number of households.

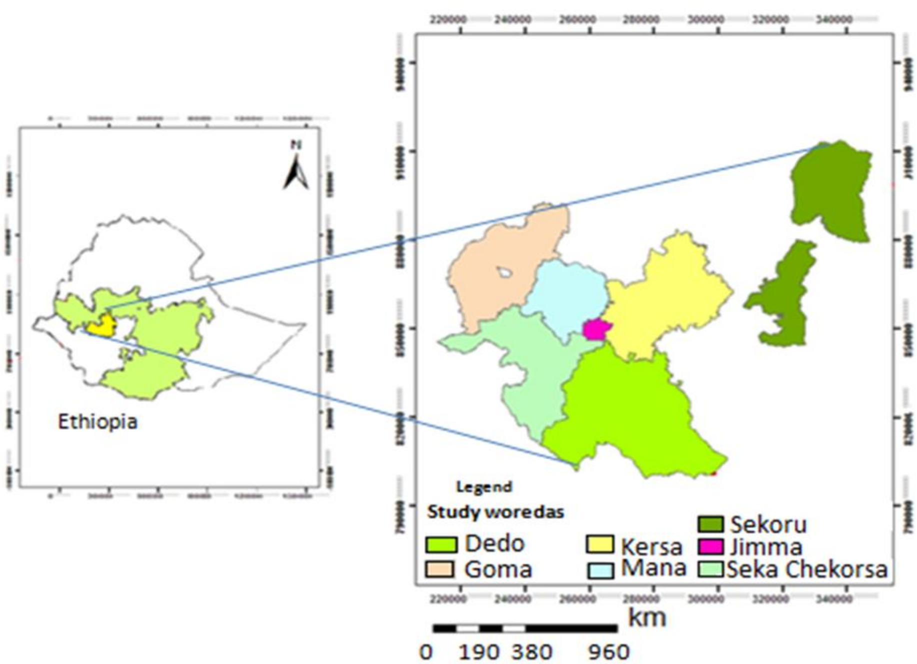


Figure 7.1 Map of the study area in Jimma zone, Southwestern Ethiopia

7.2.2 Sample size determination for consumption survey

The source population included all the people living in Jimma town. The study population were khat chewers that are selected from source population. Total sample sizes of 423 khat chewers were selected using a single population proportion formula. To maximize the sample size, prevalence of khat chewers was considered as 50%. Ninety five percent certainty (95%) confidence interval and 5% margin of error was taken. Ten percent non-response rate was added to the sample size as a contingency.

$$n = \frac{Z^2\, p(1-p)}{e^2}$$

Where:

Z = coefficient for 95% level of confidence, Z = 1.96

P = proportion of the population = 50%, because similar studies were difficult to find and it was assumed that 50% of khat chewers were exposed to DDT and its metabolites residue,

E = margin of error (5%) = 0.05.

n = 384.16**,**

10% of non-response rate was then added to get the final sample size (n_f).

Hence, n_f = 384.16 + (384.16X0.1) = 384.16 + 38.416 = 423

7.2.3 Consumption survey

After determination of the sample size, a consumption survey was undertaken to assess the daily intake of khat by 423 consumers. The survey was done in randomly selected households. Socio-demographic (age, sex, educational status, marital status and body weight) data of the chewers were collected during this household survey. The khat consumption data were collected using a repeated 24 hour recall method. An interview administered by trained interviewers using a face-to-face interview was undertaken for two non-consecutive days separated by fifteen days. The amount of chewable khat leaves with tender stems consumed per day was calculated. The average of both recall days was used to estimate khat consumption on a daily basis. The mean daily consumption (g/kg bw/day) for each khat chewer was calculated by dividing the average daily consumption of khat (g/day) by his/her body weight (kg). The body weight of the khat chewers was obtained by interviewing the study participants.

7.2.4 Data handling and analysis

Data analysis was performed using the Statistical Package for Social Sciences (SPSS for Windows version 20). The significant level was set at p-value < 0.05. Exposure analysis of the khat chewers was conducted using probabilistic exposure analysis method; in the @risk statistical software for Microsoft excels 2010 and Monte Carlo simulation model.

7.2.5 Khat sampling

Khat leaves with tender stem samples were collected from local markets in Jimma zone southwestern Ethiopia by interviewing the sellers regarding the source of the khat to the market. Chewable parts of khat samples (50-100 g each) were packed in polyethylene plastic bags and labelled accordingly to indicate the market source and transport the samples to the laboratory. Then the samples were dried under shade, grinded using mortar-pestle and stored at 4 °C until analysis.

7.2.6 Reagents and materials

Analytical grade acetone (99.5%), ethyl acetate (99.5%) and n-hexane (95%) were obtained from BDH limited Poole (BDH AnalaR®)), and were used as solvent for extraction of the khat samples. Sodium chloride (NaCl) and anhydrous sodium sulphate (Na_2SO_4) were used for removal of water from the sample. The analytical method for the extraction of the khat samples was Solid Phase Extraction (SPE) with column chromatography clean-up, for the removal of organic acids and polar pigments among other compounds. Standards of DDT metabolites of the highest analytical purity (p,p'-DDE (99.9%), p,p'-DDD (99.3%), o,p'-DDT (100%), and p,p'-

DDT (99%), were obtained from Supelco and delivered by Sigma-Aldrich Logistics and used for the preparation of the calibration curves.

7.2.7 Quality control

For the analysis of the khat samples, we adopted the method for pesticide residue analysis in khat from Daba et al. (2011), with slight modification. The quantitative determination of the pesticide residue in khat was done based on the external standard method. The chemicals and reagents were pure and analytical grade. The calibration curves were obtained by injecting five different concentrations of the pesticide standards in a range of 0.004-0.08 mg/l. The regression coefficient (r^2) was > 0.995 for all DDT metabolites. Identification and quantification of the pesticides were done based on the retention time and peak area, respectively.

7.2.8 Extraction and clean-up of khat samples

The modification of the method from Daba et al. (2011) were; 1) at the end of the extraction procedure we used 2 ml n-hexane for solvent exchange to make the samples amenable for GC-ECD injection. 2) We used the highly sensitive instrument (GC-ECD) for the determination of organochlorine pesticides like DDT as explained by (Oliveira et al., 2012) instead of GC-MS. The analytical procedure is explained in **figure 7.2**.

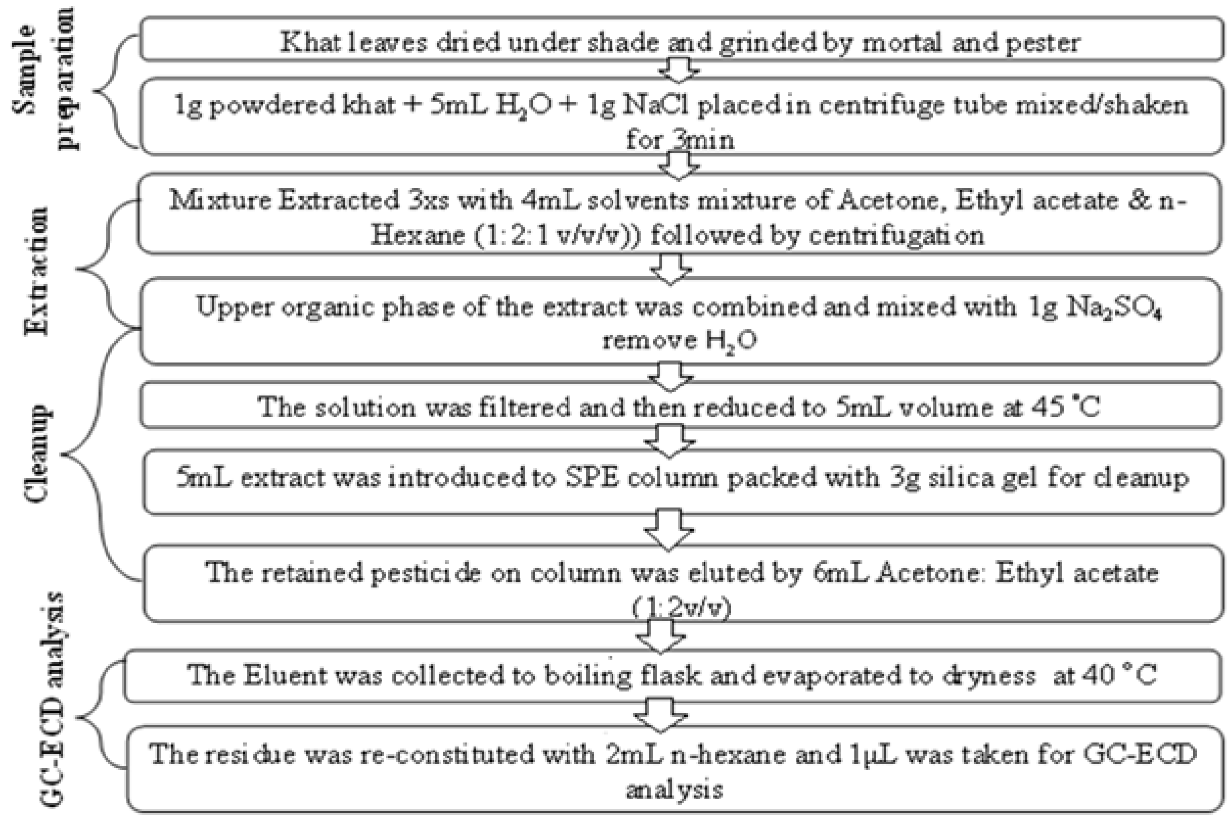


Figure 7.2. Flow-diagram for the sample preparation, extraction, clean-up and analysis of khat samples

7.2.9 Analytical equipment

Total DDT and its metabolites were determined by capillary gas–liquid chromatography with electron capture detector (GC–ECD; Agilent Technologies 6890N) in similar chromatographic conditions with the previous study worked out by Mekonen et al. (2015). After the analysis, the concentration of total DDT was determined by summing up its metabolites (p-DDT, p,p'-DDE, p,p'-DDD and o,p'-DDT).

7.2.10 Exposure assessment of khat consumers

To evaluate the safety of khat consumers regarding pesticide residues, the exposure was assessed using the probabilistic exposure analysis method. The exposure assessment in the present study was done for the real khat consumers. Total DDT was not detected in 20% of the khat samples.

According to Medeiros Vinci et al. (2012), when there are non-detected (ND) pesticide residues in food items, dietary exposure has to be done according to three scenarios such as the upper bound considers non-detected sample values which equal the limit of detection (ND = LOD), medium bound for which non-detected values equal half of the limit of detection (ND = 1/2 LOD) and lower bound for which non-detected values equal zero (ND = zero). As there was no significant difference in the lower, medium and upper bound scenarios in the exposure analysis (p-value < 0.05), the results of the probabilistic exposure analysis were only done for the upper bound (worst case) scenarios.

7.2.11 Probabilistic exposure analysis

The exposure of consumers to a number of pesticides was evaluated in more detail by the probabilistic risk assessment approach. This technique involves random sampling of each probability distribution within the model to produce hundreds or even thousands of scenarios (iterations or trials) (Claeys et al., 2011). A probabilistic exposure analysis was conducted using @Risk® 5.5 software program for Microsoft Excel 2010 (Palisade Corporation, USA). As mentioned in chapter four, the consumption and residue distributions were combined to give an exposure distribution and Monte-Carlo simulation ran with 100,000 iterations.

The results from the probabilistic exposure analysis were compared with the provisional tolerable daily intake (PTDI = 0.01 mg/kg bw/day) of DDT for every agricultural crop set by Codex Alimentarius (FAO/WHO, 2013). The mean and p97.5 values were considered as the average and high consumer exposure. The results above the health based reference value were taken as an indication for human health risk.

7.3 Results

7.3.1 Socio demographic results of the khat consumers

A total of 423 (male = 70%) and female = 30%)) khat chewers were included in the study. All study participants responded to the questioners, giving a response rate of 100%. From the Mann-Whitney U test, there is no significant difference in consumption of khat between male and female chewers (p-value = 0.141). According to this study, 64.4% of the chewers were in the age group between 20-39 years and 53.0%, 33.3%, 9.5% and 4.2% were married, single, widowed and divorced, respectively. Concerning the educational status, out of the total respondents 84.7% had a higher grade completed (above elementary school), 5.4% of the participants were illiterate, and 9.9% were able to read and write. This indicated that most of the khat consumers were in the young age groups, married and educated.

According to the present study, 72% of khat consumers spent more than fifty Ethiopian birr per day to buy khat and 26.2% and 24.5% of the respondents spent respectively three up to seven days per week on chewing khat. These results indicate that the participants of the survey spent a lot of resources on khat especially time and money. Hence exposure to pesticides from this crop is likely to be the case.

7.3.2 Occurrence of DDT and its metabolites in khat

The results of DDT and its metabolites detected in khat samples are given in **table 7.1.** Based on the results, p,p'-DDE, p,p'-DDD, p,p'-DDT and o,p'-DDT were detected in 80%, 70%, 61.7% and 58.3%, of the khat samples, respectively. Total DDT which is the sum of its metabolites was detected in 80% of the khat samples. The primary metabolites p,p'-DDE and p,p'-DDD were detected in a large number of khat samples.

Table7.1. Occurrence of DDT and its metabolites in the khat samples.

Pesticides	No of sample ND	% < LOD	No of sample Detected	% Detected
p,p'-DDE	12	20.0	48	80.0
p,p'-DDD	18	30.0	42	70.0
o'p-DDT	25	41.7	35	58.3
p,p'-DDT	23	38.3	37	61.7
Total DDT	12	20.0	48	80.0

ND = None Detected, LOD = Limit of Detection

The concentration of the total DDT in an increasing order of khat samples are indicated in **figure 7.3.** As it is noticed from the figure, some of the samples contain total DDT residue above the MRL set by Codex Alimentarius (FAO/WHO, 2013), while about more than three fourth of the khat samples contained a total DDT above the European Commission (EC) MRL (EFSA, 2012b). This indicates that there was illegal use of DDT in the study area and/or contamination from use for indoor residual spraying (IRS) in the framework of malaria control. Jimma zone is indeed known as one of the malaria endemic areas of Ethiopia (Karunamoorthi and Hailu, 2014).

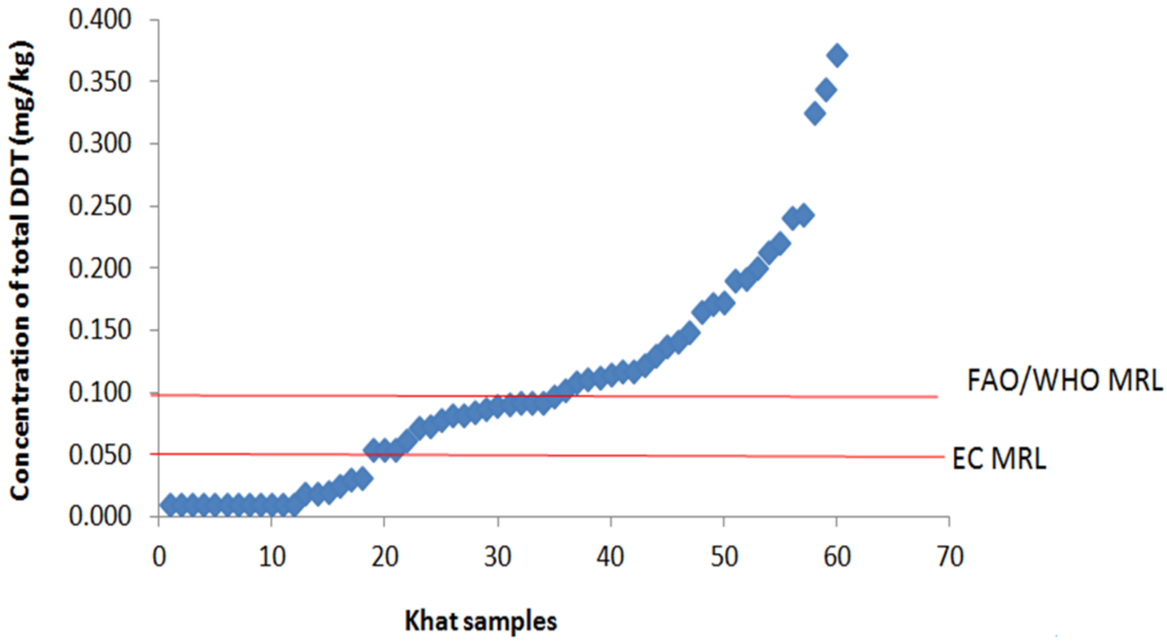


Figure 7.3. Concentration of total DDT in an increasing order of the khat samples. Lines = Maximum residue limit (MRL)

The mean concentration of total DDT and its metabolites for six different sample sources in southwestern Ethiopia are presented in **table 7.2.** From the results, the mean total DDT residue in khat was generally higher in samples from Sekoru (71.70 µg/kg), Dedo (149.00 µg/kg), Saka (137.00 µg/kg), Kersa (103.00 µg/kg) and Mana (73.00 µg/kg) districts except in the samples from Gomma district which was relatively low (41.20 µg/kg) compared to other five districts.

Table 7.2. Concentration of total DDT and its metabolites (µg/kg) in khat from six sources

Sample source	Number of samples	Mean concentration of DDT and its metabolites				
		p,p'-DDE	p,p'-DDD	o,p'-DDT	p,p'-DDT	Total DDT
Sekoru	6	61.65	7.53	7.18	13.96	71.67
Dedo	6	113.28	7.72	6.89	25.97	148.87
Seka	5	64.13	13.66	8.35	18.83	136.84
Kersa	30	68.94	21.155	7.595	16.71	102.89
Mana	5	60.12	12.07	8.49	10.31	72.98
Gomma	6	33.35	11.20	7.75	10.19	41.24

The concentration of DDT metabolites significantly (p-value < 0.0001) different in khat samples **figure 7.4**. The metabolite p,p'-DDE is distributed more in khat samples and is contributing more in the sum of the total DDT. The presence of more metabolite than the parent compound is due to the historical use of DDT in the study area.

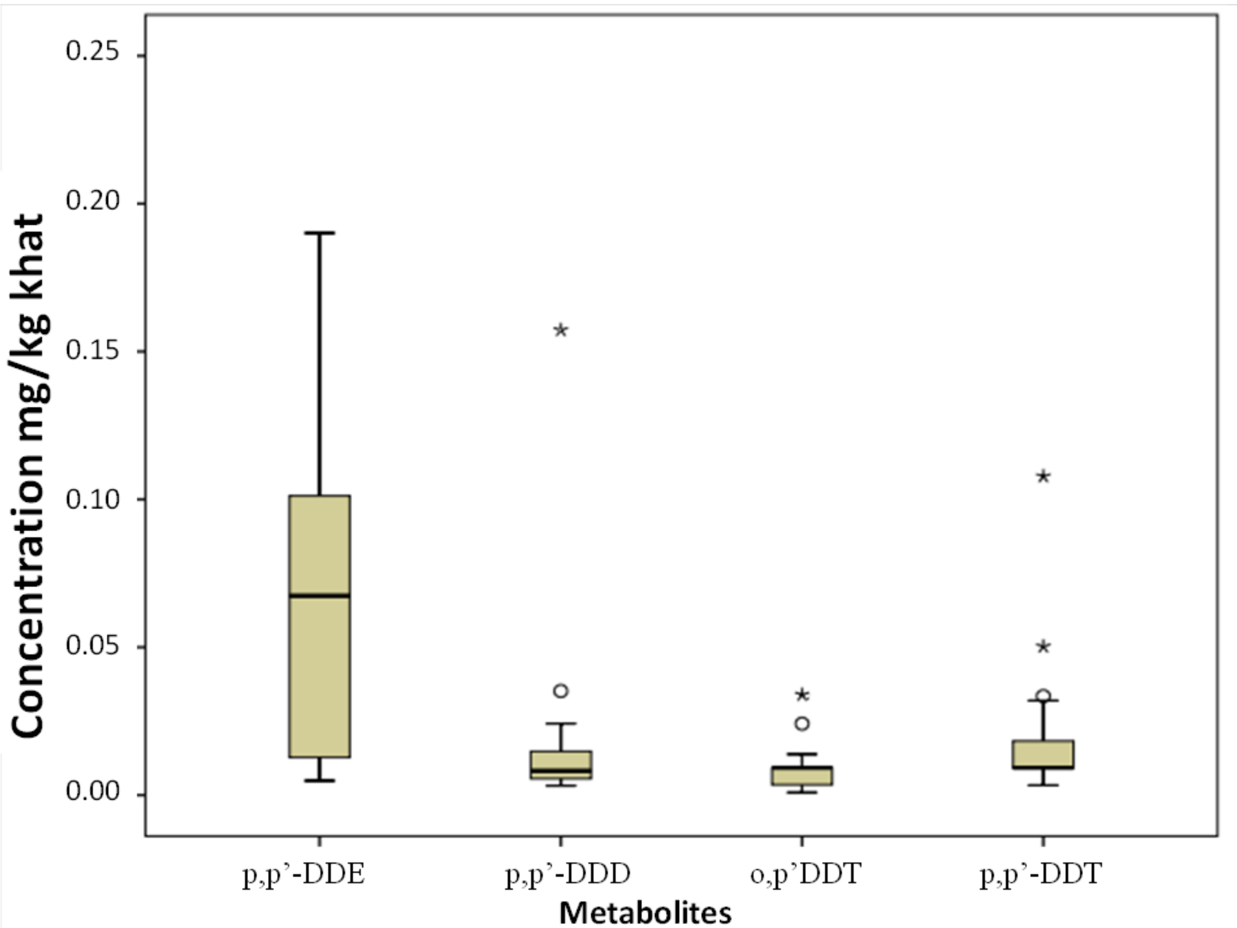


Figure 7.4. The concentration distribution of DDT metabolites in khat samples

7.3.3 Exposure assessment

Probabilistic exposure analyses were worked out to evaluate whether the level of exposure of khat consumers exceeded the provisional tolerable daily intake (PTDI) of DDT. A total DDT intake above the PTDI is considered as a health risk related to DDT for khat consumers.

7.3.4 Probabilistic exposure analysis

Table 7.3 presents the probabilistic estimates of total DDT intake from consumption of khat. The mean khat consumption for the total population was 19.590 ± 4.560 g/kg bw/day, while the mean DDT concentration detected in khat samples was 0.10 ± 0.080 mg/kg khat. The mean estimated daily intake (EDI) of total DDT from consumption of khat is 0.002 ± 0.003 mg/kg bw/day, while p97.5 is 0.006 mg/kg bw/day. Both values are below the PTDI and indicated no health risk.

Table 7.3. Results from probabilistic exposure analysis for khat consumer

Distributions	Mean	StDv	p50	p75	p90	p95	p97.5
khat consumption (g/kg bw/day)	19.585	4.561	19.206	22.024	25.147	27.461	29.846
DDT concentration (mg/kg)	0.100	0.080	0.085	0.142	0.208	0.255	0.301
EDI (mg/kg bw/day)	0.002	0.002	0.002	0.003	0.004	0.005	0.006

EDI = estimated daily intake

7.4 Discussion

From the results, 72% of khat chewers spent more than fifty Ethiopian birr per day to buy khat and 50.7% of respondents spent more than three days per week on chewing khat. This may be due to negligence of the participants, awareness of problems related to khat chewing and influence of peers who motivate them to chew khat. Additionally, the use of khat is resource intensive in terms of time, money and human power. Creating awareness about the benefits and risks of khat is important to protect consumers. From the Mann Whitney U Test, the distribution of average khat consumption (g/kg bw/day) is the same in male and female chewers (p-value > 0.05). This indicates that there may be similar exposure for both female and male khat chewers to DDT.

In the present monitoring study, results showed that a high incidence of DDT in/on the chewable parts of khat coming from all investigated areas. As reported in **figure 7.3**, more than three fourth of the khat samples contain total DDT concentrations above the EC MRL which is set for every agricultural crop worldwide by the European Food Safety Authority (EFSA, 2012b) and also other organochlorine pesticides in a variety of vegetables and fruits are set by default at 0.05 µg/kg (Zhang et al., 2011). The presence of DDT in khat above the MRL indicates that there was illegal use of DDT in the study areas and/or contamination of the khat samples from the use of DDT for indoor residual spraying (IRS) in the framework of malaria control. Jimma zone is indeed known as one of the malaria endemic areas from Ethiopia where DDT is applied to control the vector (Karunamoorthi and Hailu, 2014). The occurrence of DDT in khat can also be explained by its persistent nature as a pollutant in the environment as has been discussed in chapter three and four.

From Kruskal-Wallis test the difference in the concentration of total DDT between the khat sample sources (six districts of Jimma zone) was not significant (p-value = 0.423). Compared with khat samples collected from different parts of Ethiopia such as Hararge and Butajira (Daba et al., 2011), the mean residue of total DDT detected in the present study was lower. When compared with the study done in Sidama zone (Ligani and Hussen, 2014), higher total DDT residues were detected in the present study for all khat samples. This revealed that there was intensive application of DDT in our study area compared to Sidama zone. After application of DDT, it stays long in the environment with a half-life of 2 to 15 years due to its persistent nature (Mahdavian and Somashekar, 2013). From the post-hoc analysis, the concentration of the metabolite p,p'- DDE is highly distributed in the khat samples. Several literatures (EFSA, 2006; Kalantzi et al., 2001; Sifuentes Dos Santos et al., 2015) report that a high accumulation of p,p'-DDE and p,p'-DDD in environmental samples indicate that there was historical use of DDT. The parent DDT gets metabolized over time to p,p'-DDE and p,p'-DDD. From this it can be concluded that the contamination of khat in Jimma zone may be due to historical use of DDT. It may also originate from the dumped obsolete pesticides mostly containing organochlorine pesticides such as DDT in large amounts as reported by Daba et al. (2011). Due to this dumping, pesticides may leak from the storage areas to the different environmental compartments and contaminate agricultural products such as khat.

The probabilistic exposure analysis showed that the mean concentration of total DDT exceeded the maximum residue limit (MRL) set by the Codex Alimentarius for different agricultural food items which is 0.1 mg/kg (FAO/WHO, 2013). This indicates that there is a high contamination of the khat leaves by total DDT. From the results of the exposure analysis, the mean and p97.5 EDI was below the PTDI set by Codex Alimentarius. But this does not guarantee the safety of khat chewers. As stated in the socio-demographic results of the present study, around 25% of the participants chew khat seven days per week. A continuous exposure to DDT results in bioaccumulation of this pesticide in the body, which may result in chronic health problems. Additionally, the khat consumers may have cumulative exposure to DDT through consumption of other commonly consumed agricultural crops in Ethiopia like teff, maize, red pepper which also contain DDT and its metabolites as reported by previous study (Mekonen et al., 2014).

7.5 Conclusions

The present study investigated consumer exposure to DDT from khat consumption in southwestern Ethiopia. From the results of the study, most of the khat consumers were male. However, there is no significant difference in the pattern of the amount of khat consumed. This indicates that there is similar exposure to DDT between the male and female population. From the analysed khat samples, 80 percent contained DDT residues and some of the residues were above the MRL set by the Codex Alimentarius and most of the residues were above the EC MRL for DDT, indicating illegal use of DDT. Among the metabolites, p,p'-DDE was detected in large numbers of the khat samples compared to the parent compound p,p'-DDT. This indicates that the contamination of the khat samples comes from historical use of this pesticide. The presence of DDT and its metabolites in khat is posing an additional health risk to regular khat chewers. From the probabilistic exposure assessment, the mean and p97.5 EDI of DDT was below the health based reference value (PTDI) and indicated no risk for the khat consumers. However, this does not guarantee the safety of the khat consumers, because they may be exposed to DDT from other food sources. Above that the khat plant is consumed raw without any preceding processing. Therefore, continuous monitoring of pesticides including DDT in khat and other commonly used agricultural crops is recommended to sustain the safety of consumers.

Chapter Eight: General discussion, conclusions and future research prospects

8.1. General discussion

Despite the popularity of pesticides and their extensive use, serious concerns come up about health risks arising from the exposure of farmers, when mixing and applying pesticides, or working in treated fields and from exposure to residues in food and in drinking water (Maroni et al., 2006; Lopes Soares and Firpo de Souza Porto, 2009). This exposure of consumers may result in either long or short term health risks.

Environmental contamination of farmland and water in Ethiopia is mainly due to the use of pesticides in public health to control vector borne diseases and in agricultural sector to reduce pest problem and/or eliminate crop yield losses. For both agricultural and public health uses of pesticides, the targets are the pests and the diseases. However, human beings can be exposed to these chemical pesticides due to consumption of treated fruits, vegetables and cereals or due to pesticide contaminated drinking water. Problems related to pesticide residues are severe in developing countries, where there are no sufficient skills, no awareness, and very poor law enforcements (Yáñ ez et al., 2002).

8.1.1. Pesticide residues problems in foods

What is the problem related to pesticide use in developing countries, particularly in Ethiopia (RQ1)? Does the commonly consumed food commodities produced in southwestern Ethiopia contain pesticide residues (RQ2)?

The use of pesticides in Ethiopia is a common practice as explained in **chapter two**; however, this is at the expense of the environment and human health. Most of the farmers are illiterate. As a result they apply pesticides in violation with the recommendations because they do not understand the labels on pesticide containers, they use unsafe storage practice, they do not know the proper dose or concentration to apply, ignore risks and safety instructions, do not use protective devices during application of pesticides and dispose pesticide containers unsafely or they may use empty containers for drinking water or to store food in it (Mengistie et al., 2015). Such problems aggravate environmental contamination resulting in human exposure. To understand the risk of human exposure to pesticides, the residue at different sources, such as food, water and other consumer products should be known. Although there are a limited number of studies available dealing with pesticide residues in Ethiopia (Geletu et al., 2009; Daba et al., 2011; Gebremichael et al., 2013), no study evaluated the level of human exposure to pesticides from consumption of agricultural produce and drinking water. Additionally, in Ethiopia, pesticide monitoring programs for import products, about safe handling and human exposure to pesticides, in particular from food consumption, are not well coordinated by the government. The main focus of the government and people in Ethiopia is to secure food and is not dealing with risk/benefit analyses for pesticide use. Moreover, there was no study undertaken on market samples of agricultural produce which represent the majority of Ethiopian consumers.

Taking these gaps into account, this PhD study is the first in Ethiopia which addresses pesticide residues in different food items, khat, drinking water sources and human exposure based on the amount consumed. This study concentrated on evaluation of pesticide residues in major staple food items (commonly consumed foods) such as teff (*Eragrostis tef*), maize (*Zea mays*), red pepper (*Capsicum annuum*), coffee (*Coffea arabica*), lentil (*Lens culinaris*) and wheat (*Triticum ssp.*) (**chapter three**). The results of this study indicate that the commonly consumed food items were contaminated with different pesticides. This contamination may come from the use of pesticides in agriculture or public health and may be from the presence of dumped obsolete pesticides in different areas in Ethiopia. Pesticides which are no more in use for agriculture such as DDT and endosulfan were also detected in these food samples. Although, the use of DDT as an insecticide for agricultural use has been restricted since 1981, and was banned from the market since 1986 in Europe, DDT is still used in some developing countries until recent years. The results of our study confirmed that there was recent use of DDT, as the parent molecule p,p'-DDT was detected more than its metabolite p,p'-DDE in the staple foods.

The extended use of DDT as vector control for malaria is still recommended by WHO (EFSA, 2006), however this may also cause cross contamination to food, drinking water or other consumer products. The detection of DDT in the environment is a serious problem due to its persistent nature, the abilities to biomagnify in the food chain and bioaccumulate in the human body (Tomza-Marciniak and Witczak, 2009).

The concentrations of a couple of pesticides in the staple food items were above the MRL set by Codex Alimentarius (FAO/WHO, 2013). This may be due to the fact that most of the farmers in Ethiopia are not aware of and do not respect the legal requirements for the use of pesticides either for agricultural application or for other purposes. Moreover, the cause of higher pesticide residues detected in commonly consumed food items may be due the use of poor application techniques and equipment (Mekonnen and Agonafir, 2002).

8.1.2. Human exposure and associated risk to consumers

Are people exposed to different types of pesticides detected in the agricultural crops (RQ3)? How safe is the level of human exposure to pesticides from food, water and khat consumption (RQ4)? Do pesticides cause a health risk for short and long term exposure when residues are compared to health based reference values (RQ5)?

As has been explained in the introduction chapter human exposure to pesticides happens due to their occupation or from consumption of pesticide contamineted food. Infants are specific groups of the population who may suffer more from the risk of pesticide exposure. As an example, our study (**chapter four**), investigated infants exposure and health risks to DDT residue in maize used as major component of their food. From the risk estimation results, the mean and the 97.5 percentile estimated daily intake (EDI), were above the provisional tolerable daily intake (PTDI) of DDT set by Codex Alimentarius (FAO/WHO, 2013). This indicates a chronic health problem for the infants taking maize as a complementary diet ((EFSA, 2006). This looks problematic for most African countries, as they have a higher uptake of DDT compared to Europe and America where no exposure to these kind of compounds takes place (Jaga and Dharmani, 2003).

Does contamination of drinking water with pesticides cause a health problem to consumers (RQ6)?

Human beings are not only exposed to pesticides in their food but also from the water they drink. By the fact that most of the water bodies are at risk of pesticide contamination as they are mostly located near to the agricultural areas. Water contamination results from the release of pesticides from field run off or effluent discharges and water sources such as rivers, dams, ponds, springs, wells etc. are the immediate pesticide receivers (Chee et al., 1996). From our study (**chapter six**), water sources in Jimma zone and Addis Ababa were found to be contaminated by organophosphate pesticides (malathion, diazinon and pirimiphos methyl), a herbicide (2,4-D) and a fungicide (fenpropimorph). These pesticides were also detected in tap water and in distribution reservoirs. This indicates that the pesticides pass the conventional water treatment plant and appear until the water is ready for consumption.

Most water treatment facilities in the country are mainly focused on the removal of microorganisms, turbidity, and conductivity but do not consider chemical hazards such as pesticides. Conventional water treatment methods such as particle coagulation-flocculation, sedimentation and filtration are not 100% effective for the removal of pesticides unless there is a possibility to apply other advanced water treatment methods such as nano-filtration, reverse-osmosis (Karabelas and Plakas, 2011). As a result, urban communities are exposed to pesticides in drinking water. Moreover, the majority of the rural communities of Ethiopia who have no access to conventionally treated water sources are even exposed to higher concentrations of pesticides as they take water directly from the surface or ground water sources.

The frequent detection of pesticide residues in water indicate potential adverse effects to consumers even at very low concentrations (McKinlay et al., 2008; Sanborn et al., 2004). Pesticide exposure assessment in our study (**chapter six**) indicates that the estimated daily intake (EDI) from drinking water of some pesticides was above the health norm, particularly the chronic acceptable daily intake toxic parameter (EDI > ADI). This indicates that urban and rural communities of Ethiopia may face chronic health problems from pesticides in drinking water. While, all pesticides detected in drinking water sources were below the acute reference dose (EDI < ARfD). This may be due to the low concentration in the water which may not cause acute risk as it depends on the dose or concentration of the chemical. Pesticides found in water supplies were not usually present in high concentrations causing acute toxic effects (Trautmann et al., 2012).

The build up of buffer zones around the water sources and also stewardship and frequent monitoring of the pesticide application strategies, selecting the type of pesticides, and also advancing the water treatment technology in Ethiopia can help to minimize problems related to pesticide exposure from drinking water.

Is a human exposure to pesticides happen due to consumption of conventionally non-edible plant such as khat (RQ7?

The route of human exposure to pesticide is not only drinking water or conventionally edible foods, but also other consumer products such as khat (*Catha edulis*). Khat is a raw plant material consumed in many parts of East Africa. Even though, the use of khat is restricted in most European countries, it's common in countries located in the horn of Africa, such as Ethiopia. In Ethiopia, this plant is widely consumed regardless of man or women. Chewing khat increases the release of active constituents cathine and cathinone which causes loss of appetite (Murray et al., 2008). In some regions of Ethiopia where there is shortage of other crops, people believe that consuming khat is an important means to alleviate hunger and they consider it as food (personal observation). However, this plant may also be contaminated by pesticides which may cause a health risk to human. Our study (**chapter seven**) indicates that DDT and its metabolites were present in khat plant. The metabolite p,p'-DDE was detected at a high concentration and in a large number of samples, which indicates historical use of DDT. From the result of human exposure to DDT in khat, the EDI was less than the health based reference value (EDI < ADI). However, this does not guarantee 100% safety as the plant is consumed fresh without any preceding household processing steps. The presence of pesticides in khat should receive more attention from the government, community and also academia in order to be able to minimize the risk.

8.1.3. Cumulative risk assessment for multiple pesticides detected in commonly consumed foods

Is there a cumulative risk for consumers from mutual consumption of different food items (maize, teff, red pepper, coffee and khat) and drinking water in Ethiopia (RQ9)?

Ethiopian population may be exposed to multiple pesticides due to mutual consumption of food, drinking water and khat. Therefore, addressing the cumulative risk is interesting in this area.

Dietary risk assessment of the human exposure is traditionally performed for a single pesticide and a single crop. However, a food consumed daily may contain more than one pesticide or people may consume a combination of food items containing different pesticides (Quijano et al., 2016). If the detected pesticides have the same mechanism of action or the same toxicological endpoints, assessing the dietary risk for individual pesticides may underestimate the health risk (Boon et al., 2008). To address the combined effect of the compounds, undertaking cumulative risk assessment (CRA) is important. Cumulative risk assessment is defined as, the simultaneous exposures to various chemicals which contribute to a cumulative effect in the human body. The combined effects to humans can have additive, response addition or interaction either synergistic or antagonistic effects (European Food Safety Authority (EFSA), 2008). The combined hazard of the individual compounds in cumulative assessment groups (CAGs) can be determined using either the reference point (RPs also known as 'point of departure' (POD), or the 'reference values' (RVs) such us health based guidance values for example 'acceptable daily intake' (ADI) and the 'acute reference dose' (ARfD). Currently, there is no internationally agreed methodology to assess risks from multiple chemicals.

However, to assess the exposure to pesticides as a mixture, the first step is to identify the substance with a common mechanism of toxic action (CMGs) (Gallagher et al., 2015). For the assessment of the cumulative risk from exposure to CMGs, the 'relative potency factor' approach (RPF), based on dose addition is the appropriate method (Jensen et al., 2013; Kennedy et al., 2015).This approach assumes that doses of component chemicals, which act in a toxicologically similar way, can be added after scaling the doses by their potencies relative to an index chemical. The RPF approach is transparent and relatively easy to understand because potency correction is separated from consideration of exposure. As a consequence, it provides an effective means for standardizing the dose metrics for the toxicity of the different compounds of the cumulative assessment group (CAG) (Boobis et al., 2008). The risk is assessed after comparing the cumulative dietary exposure with a toxicological reference dose such as the ADI of the index compound in case of chronic exposure and the ARfD of the index compound for acute exposure (Quijano et al., 2016).

In our study (**chapter three**), pyrethroids such as deltamethrin, cypermethrin , permethrin and the organochlorine DDT were detected in commonly consumed food commodities such as maize (*Zea mays*), teff (*Eragrostis tef*), red pepper (*Capsicum annuum*) and coffee (*Coffea arabica*). DDT was also detected in khat (*Catha edulis*) (**chapter seven**). According to US environmental protection agency, pyrethroid and organochlorine pesticides have common mechanism of action (CMGs). They are neurotoxic chemicals acting on the axonic terminal of the central nervous system and causing behavioural changes for the exposed people (USEPA, 2011). Ethiopian people are exposed to these CMGs, through mutual consumption of the above food commodities, drinking water and khat containing multiple pesticides. Therefore, assessing the cumulative risk to these multiple pesticides is important to assure consumer safety. In dietary risk assessment, consumption and pesticide residue data's are always needed. For this study, only consumption data for maize (Mekonen et al., 2015) and khat (**chapter seven**) are available, while for other food commodities; the mean national annual per capita consumption is considered i.e., teff (25.9 kg) (Berhane et al., 2011), coffee (2.4 kg) (Zeru, 2006), and red pepper (0.62 kg) (CSA, 2001). From annual per capita consumption, the daily consumption of each food items was calculated (annual per capita consumption/365 days) and expressed in g/kg bw/day.

Food processing such as roasting, backing, peeling, cooking and others may have an effect on the concentration of pesticides and on level of human exposure as well. Therfore, for our cumulative dietary risk assessment, the processing factor (PF) was taken into account for teff and coffee. For red pepper and maize no data on the processing factor were available. The risk was assessed by assuming that processing had no effect on the residue of the pesticides under study (worst case scenario). Khat is consumed raw without any preceding processing and it is considered as it is. After assessing the cumulative exposure, the results were compared with the health based reference values such as the ADI and ARfD to assure food safety. For our study, the relative potency factor is determined based on the ADI of the three pyrethroids (cypermethrin, permethrin and deltamethrin) 0.02, 0.05 and 0.01 mg/kg bw/day, respectively and one organochlorine (DDT) with an ADI/PTDI of 0.01 mg/kg bw/day (FAO/WHO, 2013). From these pesticides, deltamethrin was chosen as an index compound due to the fact that its ADI value is five times lower compared to permethrin and two times lower compared to cypermethrin. Deltamethrin is also used as an index compound in another study (Quijano et al., 2016).

The deterministic calculation (low tier approach) was undertaken to assess the cumulative exposure from this multiple chemicals. The individual estimated daily intake (iEDI) for a pesticide in a food commodity was calculated as follows:

iEDI = Ci*Fi

Where:

Ci: residue level in food commodity

Fi: food consumption of food commodity

The cumulative exposure was expressed as deltamethrin equivalents by multiplying the iEDI of each pair pesticide-commodity by the relative potency factor (RPF) of deltamethrin and adding up to one cumulative intake i.e., ∑iEDI*RPF.

The results of the cumulative exposure are indicated in **table 8.1**. The result of this assessment indicates that the cumulative exposure to pyrethroid and organochlorine pesticides for each crop were above the ADI set by JMPR of FAO/WHO (FAO/WHO, 2013) except for those detected in coffee beans and khat. This reveals that the population in southwest Ethiopia will have chronic health problems from multiple pesticides due to mutual consumption of teff, red pepper and maize. The cumulative exposure from coffee is less than ADI, this may be due to the application of the processing factor as most of the pesticides disappear due to household processing (Mekonen et al., 2015). This result is consistent with the results obtained in China, on the long term cumulative risk for organochlorine and pyretroids from consumption of three types of nuts for which the results were above ADI (Liu et al., 2016). On the contrary, the study done in Spain indicates that the chronic cumulative intake of a pesticides through fruits and vegetables were below ADI and no cumulative chronic consumer risk is expected (Quijano et al., 2016). However, the pyrethroids and the organochlorine (DDT) were not detected in drinking water sources from both sampling points (Jimma and Addis Ababa). This may be due to the fact that these pesticides are not water soluble, while highly lipid soluble (Davies et al., 2007). For this assessment drinking water have no effect. Total cumulative exposure is 0.526 mg/kg bw/day, which is by far greater than the ADI and there will be a chronic health problem for consumers.

Table 8.1.Cumulative exposure to pyrethroid and organochlorine pesticides through consumption of staple food items, khat and drinking water

Food commodity	Pesticides	Upper bound concentration (mg/kg)	Mean consumption (g/kg bw/day)	iEDI (mg/kg bw/day)	Cumulative risk (iEDI*RPF) (mg/kg bw/day)	ADI (mg/kg bw/day)
Teff	cypermethrin	0.700	70.960	0.050	0.100	
	permethrin	0.481		0.034	0.171	
	DDT	0.444		0.031	0.031	
		∑iEDI*RPF			**0.302**	0.01
Red pepper	cypermethrin	0.618		0.001	0.006	
	permethrin	2.154		0.004	0.022	
	deltamethrin	0.139		0.001	0.001	
	DDT	0.868	2.000	0.002	0.002	
		∑iEDI*RPF			**0.031**	0.01
Coffee	deltamethrin	0.348	6.580	0.002	0.002	
	DDT	0.247		0.007	0.007	
	∑iEDI*RPF)				0.009	0.01
Maize	cypermethrin	0.428	40.540	0.017	0.087	
	permethrin	0.373		0.015	0.076	
	deltamethrin	0.075		0.003	0.003	
	DDT	0.416		0.017	0.017	
		∑iEDI*RPF			**0.182**	0.01
Khat	DDT	0.102	19.580	0.002	0.002	
		∑iEDI*RPF			0.002	0.01
Drinking water	-	-		-	-	
Total		∑iEDI*RPF			**0.526**	0.01

RPF = Relative Potency Factor, iEDI = individual Estimated Daily Intake, ADI = Acceptable Daily Intake, values above ADI indicated in bold

From the food commodities, teff contributed more to the cumulative exposure followed by maize (**figure 8.1**). This may be due to the high consumption level of these two cereals compared to others. In Ethiopia most of these food commodities are consumed together in one day. For example, one may eat injera (traditional flat bread) made from teff or maize with red pepper spiced sauce. Additionally, every day coffee is drunk and khat consumption is also common (personal observation). Therefore, people are highly exposed to multiple pesticides from mutual consumption of these food commodities.

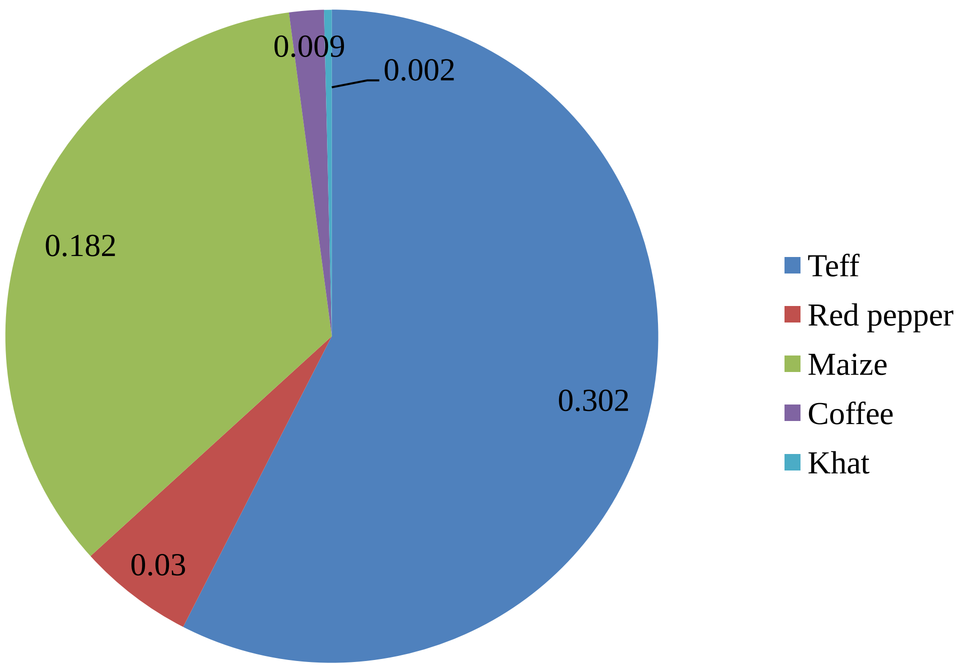


Figure 8.1. Cummulative risk (mg/kg bw/day) of multiple pesticides from mutual consumption of the above consumer products (mg/kg bw/day)

Therefore, creating awareness to pesticide users and application of integrated pest management (IPM) to minimize the use of pesticides should receive more attention in Ethiopia to curtail the cumulative risk.

Our findings in the cumulative risk assessment may have some limitations. The consumption data we used for the calculation of the cumulative exposure were from a single annual per capita consumption, due to the absence of enough consumption and pesticide residues monitoring data. This has hindered to work out a probabilistic cumulative risk assessment. Hence, deterministic (point estimate) evaluation was applied considering the mean of the estimated daily intake in the calculation. These may result in under or overestimation of the results as reported by different researchers (EFSA, 2012a; Nougadère et al., 2012; Quijano et al., 2016). In this regard, more effort has to be done for the collection of national consumption data and residue monitoring not only for these pesticides but also for other commonly used pesticides in agriculture is recommended to undertake a more comprehensive risk assessment.

8.1.4. Importance of food processing for the reduction of pesticide residues in food

Does household processing such as washing, roasting, brewing, and doughing and baking have an effect on residues of pesticides in raw food items (RQ7)?

To avoid food safety issues related to pesticides, organic farming is being propagated in the world. However, organic farming is not well practiced in most developing countries for several reasons, such as the increasing population size, lower yields from agricultural production due to the effect of pests, low awareness of the pesticide users on organic practices and wrong perception of the farmers about pesticides. Most farmers in Ethiopia considering pesticides are the best option to increase agricultural production. In addition to this, there are critics that organic farming use more land compared to conventional agriculture (Seufert et al., 2012). Therefore, it is still important to look for other solutions to tackle food safety problems related to pesticide residues in agricultural crops and drinking water.

One advantage for consumers is that food processing minimizes pesticide residues in food which had been shown in this work. The food processing operations such as washing, peeling, cooking, blanching and baking play a great role in the pesticide residue reduction in food (Elkins, 1989). As a result, food processing at household and industrial level improve the current scenario of unsafe food (Kaushik et al., 2009).

Our study in **chapter five** indicates that household processing of coffee and teff reduces pesticides residues from the raw commodity. Even the high thermal resistant pesticide, DDT and its metabolites showed a significant decrement particularly during the processing of coffee. This was mainly due to the frequent exposure of the coffee to heat during the roasting and brewing processes. Baking is also the most effective household processing for the reduction of pesticides in teff. The PFs for both crops were found to be less than one, which is an indication of a reduction of pesticide residues compared to the raw commodity. The percent reduction varies from processing to processing. The process with the application of heat such as roasting, brewing and baking has the greatest impact on the pesticide residue reduction. The effects of food processing is influenced by the physicochemical properties of pesticides such as the solubility, thermal degradation, octanol-water partition coefficient and volatility (Holland et al., 1994; Kaushik et al., 2009).

In conclusion, stewardship to enforce the law and create awareness by the government towards the pesticide users, strict monitoring, processing of food before consumption and risk evaluation are important tools to sustain consumer safety of the Ethiopian population.

8.2. Conclusions

While pesticides are important for the control of pests, they may cause side effects on human health and the environment. Developing countries, in particular Ethiopia suffers from problems related to pesticides use. Despite this fact there are little attention on potential research areas, lack of law enforcement or poor policy directions and relevant actions to at least minimize the risks related to pesticides. The goal of this dissertation was to identify problems related to pesticide contamination in agricultural produce, water, khat, human exposure due to consumption and to assess the effect of food processing on level of pesticides in food. These makes a novel contribution to the literature by demonstrating that human exposure can be associated with the consumption of pesticide contaminated food, drinking water and other agricultural crops which pose a threat to health.

To sumerize the key issues, the following conclusions are drawn based on the findings of the PhD study:

- ✓ It can be concluded that food items, water and khat are often contaminated with pesticides that may arise from agricultural and public health use of pesticides and the presence of obsolete stocks in Ethiopia (answered RQ2, RQ6 and RQ7).
- ✓ Some pesticide residues in the foods were above the legal reference doses which reflect the maximum allowable concentration in food. This indicates that good agricultural practice (GAP) is perhaps not be well practiced in Ethiopia (answered RQ1 and RQ2).

- ✓ From the results of human exposure and consumer risk assessment, it is concluded that human beings, in particular infants are exposed to non-authorized pesticides such as DDT from consumption of their complementary diet (answered RQ3, RQ4 and RQ5).
- ✓ Consumers are daily exposed to pesticides from drinking water, in which the contamination may come from the surrounding environment (answered RQ4, RQ5 and RQ6).
- ✓ Khat, being a stimulant plant which has naturally an impact on consumer's health, is also contaminated with pesticides such as DDT. This could pose a double burden on human health (answered RQ4, RQ5 and RQ7).
- ✓ Mutual consumption of food items, which are contaminated with multiple pesticides belonging to a common mechanism of action group (CMGs), shows a cumulative health effect on the Ethiopian population (answered RQ 9 and RQ10).
- ✓ In general, developing a continuous pesticide monitoring program, law enforcement such as on time implementations of policies and regulations, networking within different stakeholders, stewardship and creating awareness to pesticide users, promoting the use of integrated pest management (IPM) in Ethiopia are important to minimize problems related to human exposure to pesticides and associated health risks.
- ✓ Strong regulation regarding pesticides import, use, storage and continued pesticide monitoring in food and other environmental samples seems vital to assure the safety of consumers in Ethiopia.

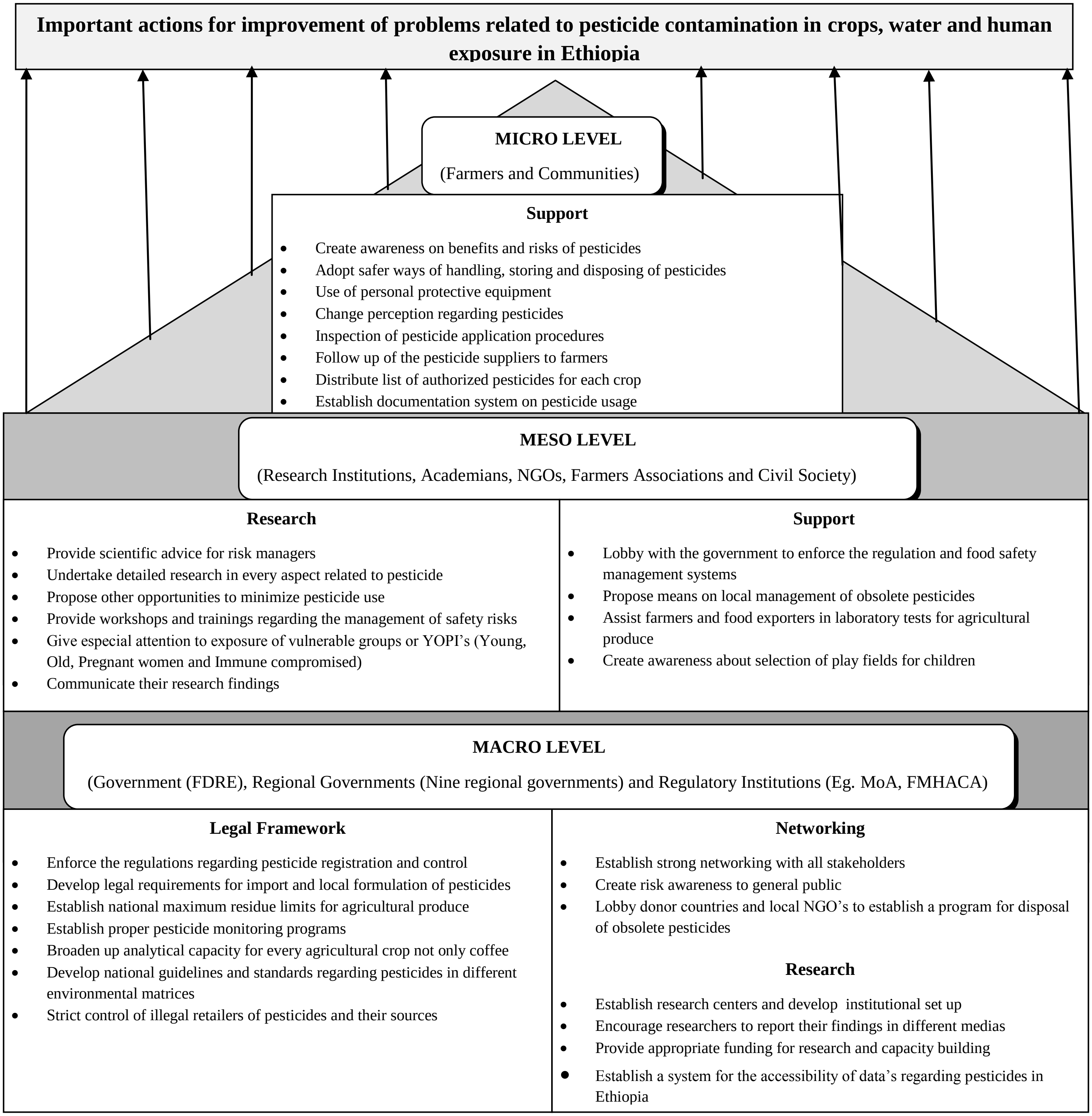
The following roadmap (**figure 8.2)** indicates proposed actions at different levels of the chain to minimize the problem related to human exposure to pesticides in Ethiopia.

Micro-level: at this level farmers and communities who are working to secure food should act. These group of the production chain (chain function stage) need support to improve their production systems and update their knowledge on pesticide handling to minimize pesticide related risk.

Meso-level: scientific people, NGO's and different associations who support both micro and macro levels (chain supporters). These groups can support financially or giving scientific and policy advice.

Macro-level: decision makers or enablers/regulation of the processes in the chain, such as policy makers, implementers of the regulations, giving certifications and others.

If the use of pesticides causes human exposure and health risks, what will be the next step (RQ10)?



*FDRE = Federal Democratic Republic of Ethiopia, MoA = Ministry of Agriculture, FMHACA = Food, Medicine and Health Care Administration, and Control Authority, NGOs = Non-governmental Organizations

Figure 8.2. Specific recommendation for intervention at three levels of chain to minimize pesticide related problems in Ethiopia

8.2. Future research prospects

The study deals about human exposure and risks to pesticides from consumption of food, drinking water and other consumer products. The contamination of the pesticides may not be limited to food and drinking water. Further research in this area should focus on the other environmental compartments such as soil, air and biota living in the aquatic and soil environment. Detailed analyses of pesticide residues in these environmental compartments followed by environmental risk assessment (ERA) is recommended, particularly for those pesticides which are able to persist in the environment and have the ability to biomagnify through the food chain.

This study focuses on the estimation of human exposure based on residues and consumption data; this does not indicate the real amount of uptake of these chemical pesticides in the general blood circulation and/or target organs or tissues. Therefore, further study is needed using biomarkers to identify the biological and toxicological available fractions of pesticides in the human body.

In Ethiopia, there is no national consumption data base, for this study we used a consumption survey on the 24-hr recall method for the assessment of the daily consumption by infants and khat consumers. However, this might not give all the details of the usual dietary intake, so further research and new food surveys about the Ethiopian diet over more days of consumption representing the general population is of interest.

This study considers that the feeding status of most Ethiopian people is quite similar. However, there are some cultural differences, so undertaking a national consumption survey based on the different seasons and assessing human exposure to pesticides in all regions of the country is needed to evaluate the pesticide exposure of the general population in the country.

Food processing has an effect on the residues of pesticides detected in the study for two commonly consumed food items (teff and coffee). Further study is required to include also the effect of other household and industrial food processing steps among different food items and for other pesticides present.

Pesticide residue analyses is important to crop protection, research, environmental monitoring, public health protection and legislative enforcement, to be conversant with the legislations of importing countries, to be a competitive exporter of agricultural products, and to participate effectively in international and regional trade. Therefore, future work should also be focused on the establishment of a certified analytical laboratory for pesticide residue analysis in food and other environmental matrices in the region according to internationally accepted quality assurance (QA) and quality control (QC) procedures.

This study identifies the commonly applied pesticides regardless of how they are applied for different purposes; further research is needed to monitor the spray habits of Ethiopian farmers and pesticide applicators in general. This has to create of awareness in the community by giving information on the good agricultural practices and the application of the right dose and right pesticide for a specific crop pest of disease.

Finally future work has to be done, to create awareness to the regulatory bodies and decision makers, about the problems related to pesticide residues in food and other environmental samples, means of transport of these pesticides in the environment (run-off, drift and volatilisation), problems related to the accumulation of obsolete stocks (control of the local formulation and importation) and the importance of continuous pesticide monitoring to enforce the application of integrated pest management (IPM) and pesticide regulations in Ethiopia.

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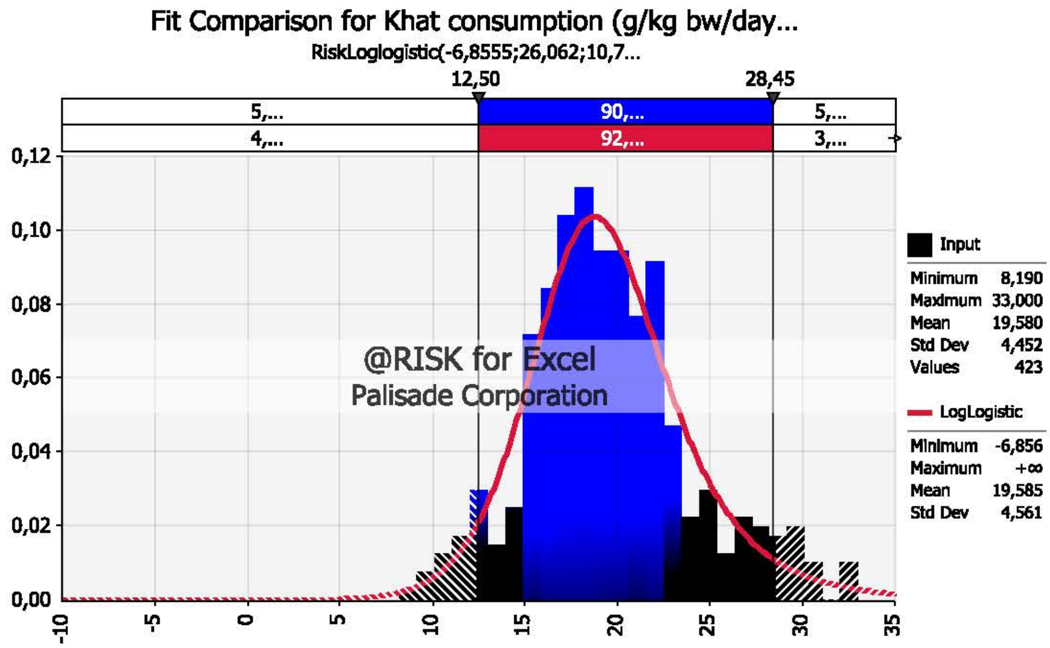
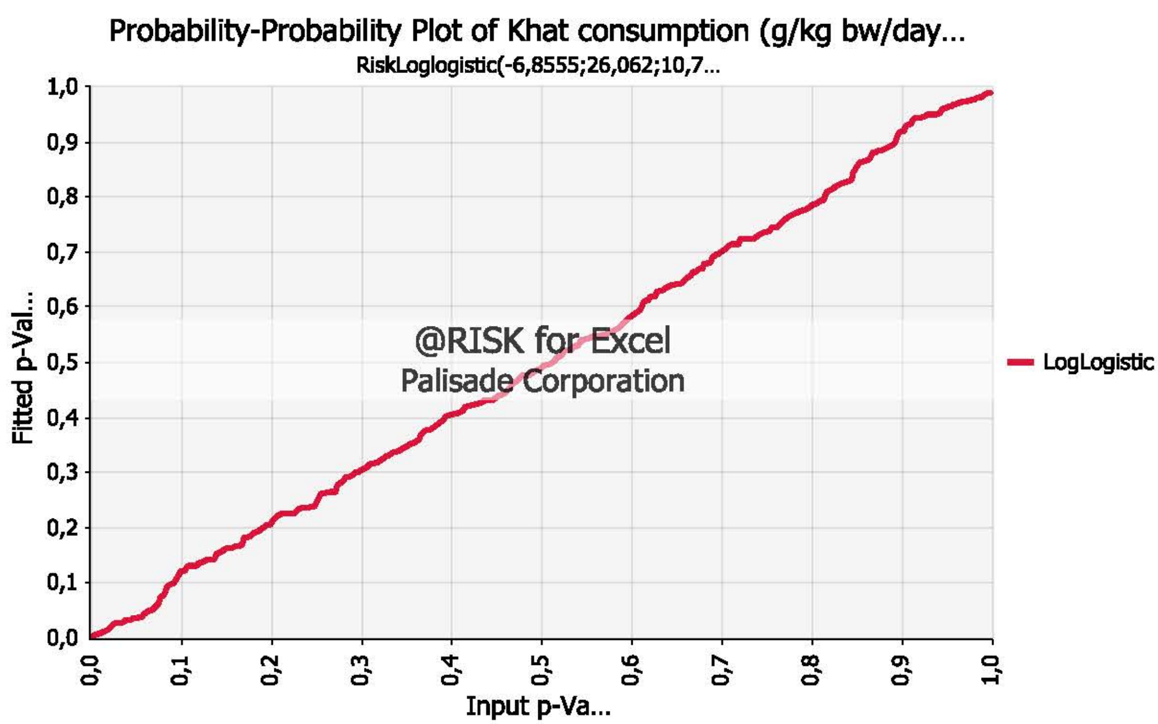
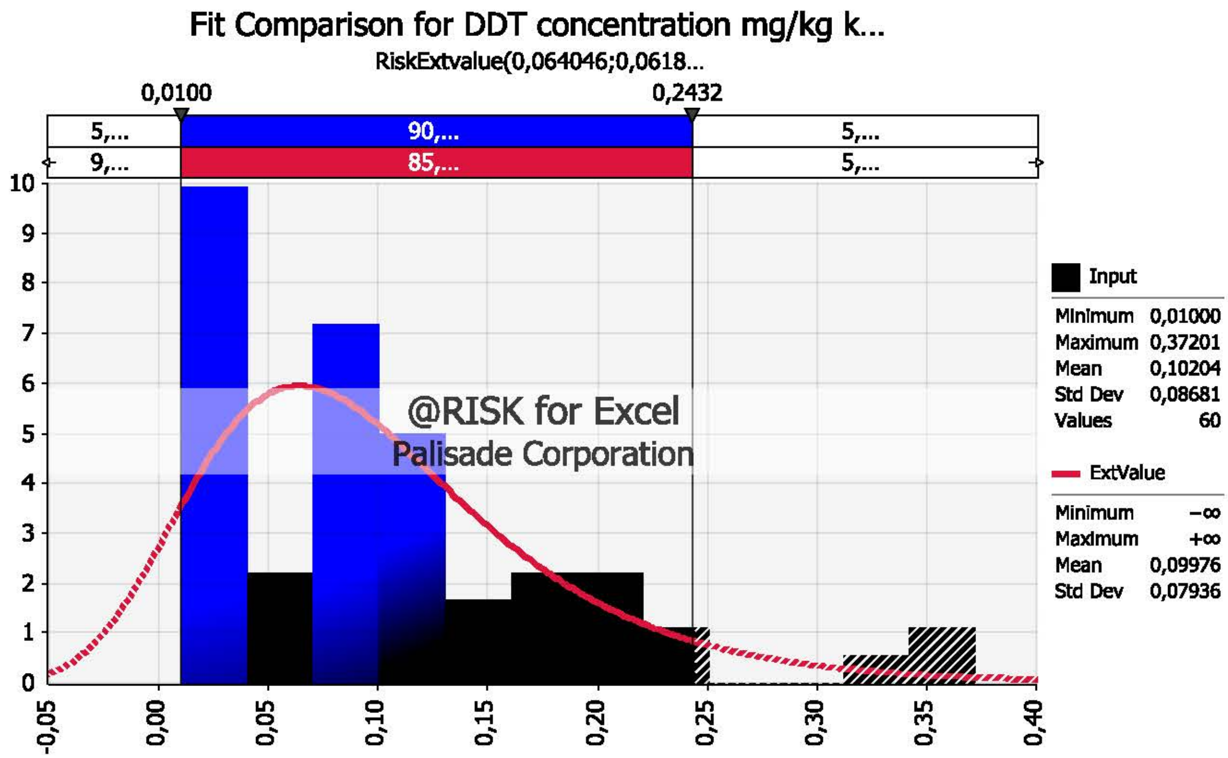
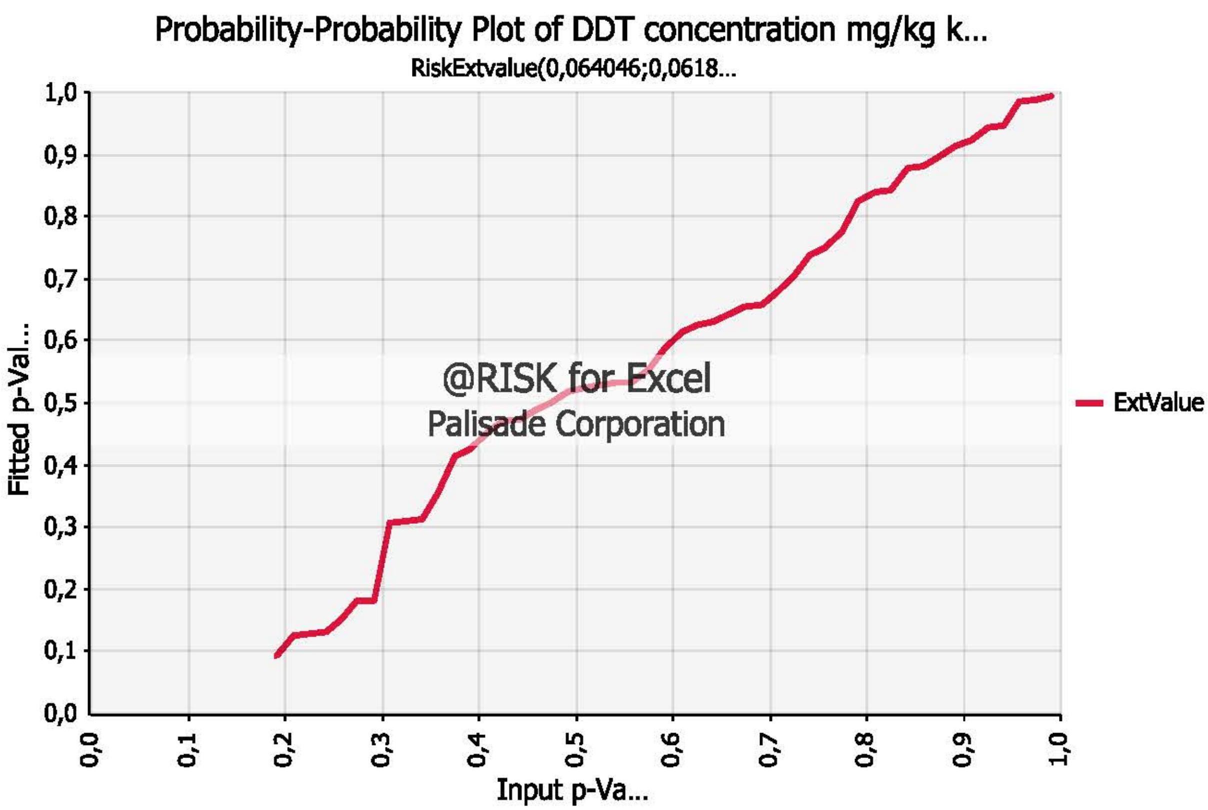
Annexes

Annex 1: Model out puts from probabilistic exposure analysis of DDT in khat






Fit Comparison for DDT concentration mg/kg k...
RiskExtvalue(0,064046;0,0618...
0,0100
0,2432
5,...
90,...
5,...
9,...
85,...
5,...
Input
Minimum 0,01000
Maximum 0,37201
Mean 0,10204
Std Dev 0,08681
Values 60
ExtValue
Minimum −∞
Maximum +∞
Mean 0,09976
Std Dev 0,07936
@RISK for Excel
Palisade Corporation


Probability-Probability Plot of DDT concentration mg/kg k...
RiskExtvalue(0,064046;0,0618...
Fitted p-Val...
Input p-Va...
ExtValue
@RISK for Excel
Palisade Corporation

Annex 2: @risk detailed statistics for the intake of DDT due to consumption of khat from Monte-Carlo simulation model

Summary statistics	Intake of DDT in khat (mg/kg bw/day) Output (Sim#1) Sheet1!G8	Intake of DDT in khat (mg/kg bw/day) Output (Sim#2) Sheet1!G8	Intake of DDT in khat (mg/kg bw/day) Output (Sim#3) Sheet1!G8	Intake of DDT in khat (mg/kg bw/day) Output (Sim#4) Sheet1!G8	Intake of DDT in khat (mg/kg bw/day) Output (Sim#5) Sheet1!G8
Minimum	-0.0023	-0.0023	-0.0023	-0.0023	-0.0023
Maximum	0.0211	0.0211	0.0211	0.0211	0.0211
Mean	0.0019	0.0019	0.0019	0.0019	0.0019
Std Deviation	0.0017	0.0017	0.0017	0.0017	0.0017
Variance	0. 0001	0. 0001	0. 0001	0. 0001	0. 0001
Skewness	1.4735	1.4735	1.4735	1.4735	1.4735
Kurtosis	7.1615	7.1615	7.1615	7.1615	7.1615
Errors	0	0	0	0	0
Mode	0.0010	0.0010	0.0010	0.0010	0.0010
5% Perc	-0.0007	-0.0007	-0.0007	-0.0007	-0.0007
10% Perc	0.0002	0.0002	0.0002	0.0002	0.0002
15% Perc	0.0004	0.0004	0.0004	0.0004	0.0004
20% Perc	0.0006	0.0006	0.0006	0.0006	0.0006
25% Perc	0.0008	0.0008	0.0008	0.0008	0.0008
30% Perc	0.0009	0.0009	0.0009	0.0009	0.0009
35% Perc	0.0011	0.0011	0.0011	0.0011	0.0011
40% Perc	0.0013	0.0013	0.0013	0.0013	0.0013
45% Perc	0.0015	0.0015	0.0015	0.0015	0.0015
50% Perc	0.0016	0.0016	0.0016	0.0016	0.0016
55% Perc	0.0018	0.0018	0.0018	0.0018	0.0018
60% Perc	0.0020	0.0020	0.0020	0.0020	0.0020
65% Perc	0.0022	0.0022	0.0022	0.0022	0.0022
70% Perc	0.0025	0.0025	0.0025	0.0025	0.0025
75% Perc	0.0027	0.0027	0.0027	0.0027	0.0027

Summary statistics	Intake of DDT in khat (mg/kg bw/day) Output (Sim#1) Sheet1!G8	Intake of DDT in khat (mg/kg bw/day) Output (Sim#2) Sheet1!G8	Intake of DDT in khat (mg/kg bw/day) Output (Sim#3) Sheet1!G8	Intake of DDT in khat (mg/kg bw/day) Output (Sim#4) Sheet1!G8	Intake of DDT in khat (mg/kg bw/day) Output (Sim#5) Sheet1!G8
80% Perc	0.0031	0.0031	0.0031	0.0031	0.0031
85% Perc	0.0035	0.0035	0.0035	0.0035	0.0035
90% Perc	0.0041	0.0041	0.0041	0.0041	0.0041
95% Perc	0.0051	0.0051	0.0051	0.0051	0.0051
Filter Minimum					
Filter Maximum					
Filter Type					
# Values Filtered	0	0	0	0	0
Target #1 (Value)	1				
Target #1 (Perc%)	1				
Target #2 (Value)	0.0061				
Target #2 (Perc%)	0.975				
Target #3 (Value)	0.0086				
Target #3 (Perc%)	0.995				
Target #4 (Value)					
Target #4 (Perc%)					

Annex 3: Curriculum Vitae

1. Personal detail

Name: Seblework Mekonen Shegen
Birth date: 11 Sept.1979
Sex: Female
Marital status/number of children: Married/two children
Address: P.O. Box 807, Jimma, Ethiopia.
Email: seblework2001@yahoo.com, Seblework.mekonen@ju.edu.et

2. Qualification and current position

Assistant professor since June 2014, at Jimma University, Ethiopia

3. Education

From Sept. 2007 – Sept. 2009
Qualification: Master of Science in Environmental Sanitation
Institution: Ghent University, J. Plateaustrat 22, 9000 Ghent, Belgium

From Sept. 2002 - July 2006
Qualification: Bachelor degree in Pharmacy
Institution: Jimma University, P.O. Box 378, Jimma Ethiopia

From Oct. 1997 - Oct. 1999
Qualification: Diploma in Pharmacy
Institution: Jimma Institute of Health Sciences, P.O. Box 378, Jimma Ethiopia

4. Professional carrier

From Sept. 2009 - May 2014
Position: Lecturer, Teaching and research

Major responsibilities: Teaching Bachelor and Master Program students. Main subjects I am involved are Human Toxicology, Environmental Toxicology, and Environmental Impact of Pesticides, promoter for under graduate and masters students when doing their thesis.

Name of Employer: Jimma University, P.O. Box 378, Jimma Ethiopia

From Sept. 2007 – Aug. 2007

Position: Senior Pharmacist, teaching and supervision of students

Major responsibilities: working as senior pharmacist supervising all pharmaceutical cares and medications in Jimma University Specialized Hospital. I also have been supervising intern pharmacy students during their practical training in the Hospital.

Name of Employer: Jimma University Specialized Hospital, P.O. Box 378, Jimma, Ethiopia

From Sept. 2001 - Sept. 2002

Position: Druggist, dispensing drugs

Major responsibilities: supervising all pharmaceutical cares and medications in Jimma University model pharmacy. I also have been supervising intern pharmacy students during their practical training

Name of Employer: Jimma University Model Pharmacy, P.O. Box 378, Jimma, Ethiopia

From Dec. 1999 - May 2001

Position: Pharmacy Technician

Major responsibilities: Dispensing drugs, giving health information for communities, supervising private drug stores, venders

Name of Employer: Sagure Health Centre, Arsi Zone Health Bureau, Sagure, Ethiopia

5. Conference presentation: Oral and poster

Oral presentation on the **7th International symposium of Ghent-African Platform-GAPSYM7**, with the theme ‘Africa and Food, Challenges, Risks and Opportunities’. Organized by Ghent University, December 6.

Oral presentation on the **4th annual conference of Jimma University** with the theme of meeting national development challenges through Science, Technology and Innovations, February 7-8 and certificate of participation obtained.

Poster presentation on the **65th International Symposium on Crop Protection**, organized by Ghent University, The submitted abstract was published in the proceedings of the Communication and Agricultural biological Sciences, Vol.78(2), 1-386 (2013). Website: http://WWW.fbw.ugent.be

Oral presentation on a conference **Food and Power, the hunger games in sub-Saharan African Politics**, The commemoration of The World Food Day.Oct.16, 2014.

Oral presentation on the **26th annual conference of Ethiopian Public Health Association (EPHA**) and certificate of participation obtained

Poster presentation on the **68th international symposium on crop protection**, organized by Ghent University, May 17, 2016

6. Professional affiliation

Member of Ethiopian Pharmaceutical Association (EPA)

Member of Ethiopian Public Health Association (EPHA)

Member of Ethiopian Environmental Health Association (EHA)

7. Short term Training

Basic training on infection prevention and patient safety for higher education teaching staff organized by USAID, December, 2011.

Training on "Malaria control and Artemisinin based combination Therapy (ACT) Pharmacovigilance "organized by Drug Administration and Control Authority of Ethiopia (DACA) in collaboration with Federal Ministry of Health (FMOH) and Ethiopian Pharmaceutical association (EPA), August, 2007

Pre-service Comprehensive ART training, organized by school of pharmacy Jimma University in collaboration with Drug Administration and Control Authority of Ethiopia (DACA) and Management Science for Health (MSH)/RPM Plus-Ethiopia, July, 2006

Training on "Module Writing in Higher Education" organized by Jimma University Academic Development and Resource Center (ADRC), November, 2009

Training on Business process Re-engineering (BPR), organized by Jimma University, May, 2007

8. List of Publications

Mekonen S, Ambelu A and Spanoghe P, 2014. Pesticide residue evaluation in major staple food items of Ethiopia using the QuEChERS method: A case study from the Jimma zone. Environmental Toxicology and Chemistry, Vol. 33, No. 6, pp. 1294–1302.

Mekonen S. Lachat C., Ambelu A., Steurbaut W., Kolsteren P., Jacxsens L., Wondafrash M., Houbraken M. and Spanoghe P.(2015), Risk of DDT residue in maize consumed by infants as complementary diet in southwest Ethiopia. Science of the Total Environment; Vol.511: 454–460.

Mekonen S, Ambelu A and Spanoghe P, (2015). Effect of Household Coffee Processing on Pesticide Residues as a Means of Ensuring Consumers' Safety. Journal of Agricultural and Food chemistry, DOI: 10.1021/acs.jafc.5b03327

Mekonen S, Argaw R., Simanesew A., Houbraken M., Senaeve D., Ambelu A. and Spanoghe P (2016). Pesticide residues in drinking water and associated risks to consumers in Ethiopia. Chemosphere 162 (2016) 252e260, doi.org/10.1016/j.chemosphere.2016.07.096

Mekonen S, Maniales.W.S and Ambelu, 2014. Importance of labeling and patient knowledge to ensure proper care during drug dispensing: A case study from a tertiary hospital in Ethiopia

Ambelu A, **Mekonen S**, Koch M, Addis T, Boets P et al, 2014. The application of predictive modeling for determining Bio-Environmental factors affecting the distribution of Black flies (Diptera: Simuliidea) in Gilgel Gibe watershed Southwest, Ethiopia. PLos One 9(11), e112221.doi 10.1371/Journal.Pone.0112221

Getaneh Z, **Mekonen S** and Ambelu A, 2014. Exposure and health risk assessment of lead in the communities of Jimma town, Southwest Ethiopia. Bulletin of Environmental contamination and Toxicology, doi 10.1007/s0028-014-1293-7

Ambelu A, **Mekonen S** & Gebresilassie A, Malu and Karunamoorthi K.A, 2013. Physicochemical and Biological characteristics of two Ethiopian wetlands. Wetlands 33:691–698

Amasa W, Santiago D, **Mekonen S** and Ambelu A, 2012. Are cosmetics used in developing countries safe? Use and dermal irritation of body care products in Jimma twon, Southwest, Ethiopia. Journal of Toxicology Volume 2012, Article ID 204830, 8 pages, doi:10.1155/2012/204830