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Exposure to DDT and its metabolites from khat (*Catha edulis*) chewing: Consumers risk assessment from southwestern Ethiopia

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ABSTRACT

Khat (*Catha edulis*) is one of the most consumed plant in the horn of African countries. However, it is a stimulant plant that has several side effects on the health of consumers. On top of that, the khat leaves used for human consumption are often contain contaminants such as pesticide residues. The present study aims to investigate the level of DDT residue and its metabolites (p'p-DDE, p'p-DDD, o'p-DDT and p'p-DDT) in khat samples and to undertake exposure assessment to consumers. The khat samples were collected from local markets in southwestern Ethiopia. Consumption survey was undertaken using 24 h recall method for both male and female khat consumers. The finding showed that 80% of the khat samples contained DDT and its metabolites. Some of the residues were above the maximum residue limit (MRL) set by Food and Agricultural Organization (FAO). The concentration of p'p-DDE and p'p-DDT in khat were in the range of 0.033–0.113 and 0.010–0.026 mg/kg, respectively. High concentration of the metabolite (p'p-DDE) compared to the parent compound (p'p-DDT) revealed the historical use of DDT in the study area. Probabilistic exposure analysis indicated that the mean and 97.5 percentile (P97.5), of the estimated daily intake of total DDT were 0.002 and 0.006 mg/kg bw/day, respectively. The study concluded that khat consumers are exposed to the stimulant effect of the plant as well as DDT and its metabolites in Jimma zone.

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 illness propagated by insects such as malaria and typhus (Beceiro-González et al., 2012). However, the residue of the applied pesticides remains in the environment and can contaminate water, soil, air and food. The contamination of food items by hazardous substances, in particular the presence of residues of persistent organochlorine pollutants are 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 certain diseases (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, particularly, in developing countries like Ethiopia 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 relative stability and bioaccumulation properties, DDT can be transferred and magnified to higher trophic levels through the food chain (Man et al., 2013).

Exposure to pesticide residues from food consumption is assumed to be five times 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 reduce the pesticide residues (Daba et al., 2011). Khat chewers consume the fresh leaf without any processing which could exposed the consumers to pesticides.

Khat (*Catha edulis*) is a flowering evergreen tree or large shrub of

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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 from time to time (Dessie, 2013a). 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; Dessie, 2013a; Douglas et al., 2011). This could be the 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 that are estimated to be 10 million people 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 fresh 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 used DDT and other pesticides as khat growing chemicals. Despite all these problems, only few studies are published and presenting very high concentrations of p,p'-DDT, ranging from 141.2 to 999.0 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 indicated that up to 44.8 μg/kg residue of p'p-DDT is detected in khat samples (Ligani and Hussen, 2014). However, the work of Ligani and Hussen estimated the daily intake of DDT metabolites without assessing the consumption rate and without the application of probabilistic risk assessment techniques. In addition, Jimma zone is one of the khat growing areas in the southwestern part of Ethiopia where khat chewing is a deep-rooted tradition for majority of the population (Damena et al., 2011). Moreover, khat growers in southwestern Ethiopia apply DDT on leaves so that it can stay shiny and attractive to the customers. Investigating DDT residue on khat and undertake exposure assessment to consumers in such areas could give a new insight about the level of environmental and human health risks. Therefore, the main aim of the present study is to determine the exposure of khat chewers to DDT and its metabolites in khat using khat consumption and residue data.

2. Materials and methods

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, 2013b). The study area includes six districts of Jimma zone (Sekoru, Kersa, Saka, Dedo, Mana, and Gomma) which are considered as a source of Khat for local markets (Fig. 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 1780 m 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) where the total number of household is 85,260.

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 Jimma town. A total sample size of 423 khat chewers were selected using a single population proportion formula. To maximize the sample size, prevalence of khat chewers was considered to be 50%. Confidence interval of 95% and 5% margin of error was taken. Ten percent non-response rate was also added to the sample size for 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,

The total sample size was then calculated to be 423 considering a 10% of non-response rate.

2.3. Consumption survey

Prior to the consumption survey, IRB clearance was obtained from the College of Health Sciences at Jimma University. After determination of the sample size, a consumption survey was undertaken to assess the daily intake of khat by 423 consumers. The consumers were identified from the 423 households selected by random number generator from the list of households having at least one khat chewer. Then survey was conducted on the khat chewers from 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 (return back after two weeks to same household) 24 h recalls method. Face-to-face interviews were done by trained interviewers using structured and pretested questionnaire for two non-consecutive days separated by fifteen days. The amount of chewable khat leaves 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) with his/her body weight (kg). The body weight of the khat chewers was obtained by interviewing the study participants.

2.4. Data handling and analysis

The data entry was done using EpiData version 3.1. 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 the probabilistic exposure analysis method, in the @risk statistical software Microsoft Excel 2010.

2.5. Khat sampling

A total of 60 khat leaves (with tender stem) samples were collected from local markets in Jimma zone southwestern Ethiopia where the sellers were interviewed regarding the source of the khat samples. Chewable parts of khat samples (50–100 g each)

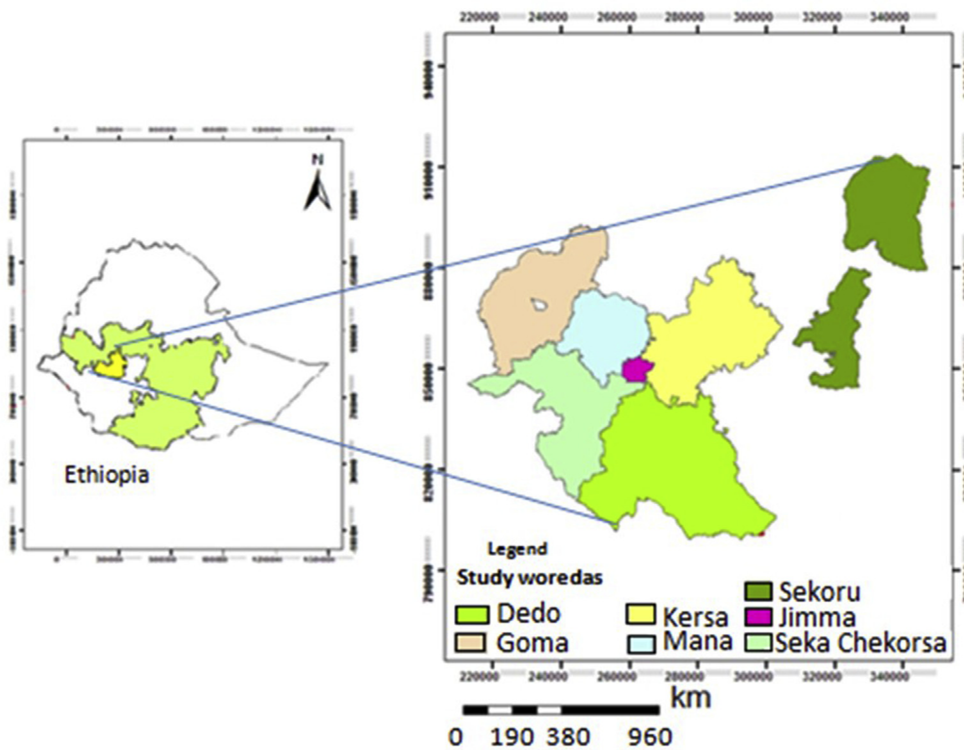

Fig. 1. Study area descriptions of Jimma zone, Southwestern Ethiopia.

were packed in polyethylene plastic bags and labeled 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.

2.6. Reagents and materials

Analytical grade acetone (99.5%), ethyl acetate (99.5%) and n-hexane (95%) obtained from BDH limited Poole (BDH AnalaR®), were used as solvent for extraction of the khat samples. Sodium chloride (NaCl) and anhydrous sodium sulfate (Na_2SO_4) was used for removal of water from the sample. Solid Phase Extraction (SPE) with column chromatography cleanup, 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.

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). 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.

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 Fig. 2.

2.9. Analytical equipment

Total DDT and its metabolites were determined by capillary gas–liquid chromatography with electron capture detector

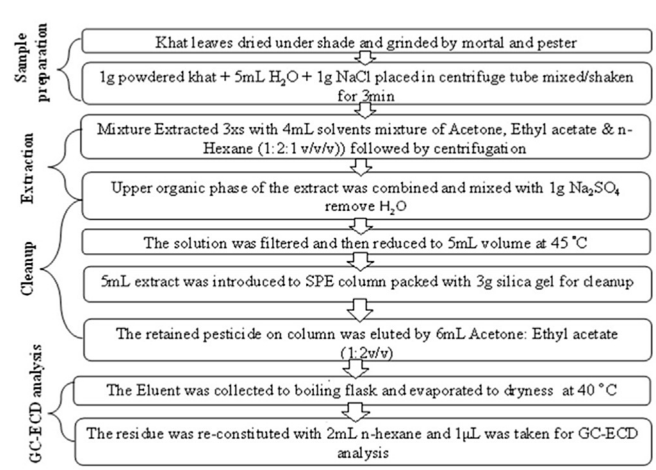

Fig. 2. Flow-diagram for the sample preparation, extraction, cleanup and analysis.

(GC–ECD; Agilent Technologies 6890N) in similar chromatographic conditions with the previous study worked out by (Mekonen et al., 2015) as explained in chapter four. After the analysis, the concentration of total DDT was determined by summing up its metabolites (p,p'-DDT, p,p'-DDE, p,p'-DDD and o,p'-DDT). The results were compared with the maximum residue limit (MRL).

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 and total DDT was not detected in some of the khat samples. According to Medeiros Vinci and colleagues (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, 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). The estimated daily intake of total DDT from consumption of khat was assessed at upper, medium and lower bound scenarios using the probabilistic exposure analysis. As there was no significant difference in the lower, medium and upper bound scenarios in the exposure analysis ($P\text{-value}<0.05$) the results of the probabilistic exposure analysis were only done for the upper bound (worst case) scenarios.

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, in which the consumption and residue distributions were combined to give an exposure distribution. Monte-Carlo simulation ran with 100,000 iterations.

The results from the probabilistic exposure analysis were compared with the provisional tolerable daily intake (PTDI) of 0.01 mg/kg bw/day of DDT for every agricultural crop. The mean and the P 97.5 values were considered as the average and high consumer exposure. The results above the health based reference value were taken as an indication for a human health risk.

3. Results

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 ($P = 0.141$). According to this study, 64.4% of the chewers were in the age group between 20 and 39 years. Regarding the marital status of the participants, 53.0%, 33.3%, 9.5% and 4.2% were married, single, widowed or 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. Regarding the occupational status of the participants, 40.2%, 22.9%, 9.7%, 9.5%, 7.8% were government employed merchants, daily labors, house wife's and students, respectively. 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 (2.4 USD) per day to buy khat. Among the consumers, 26.2% and 24.5% of the respondents spent respectively three up to seven days per week on chewing khat and about 33.8% of the participants spent four hours per day 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.

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 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 and isomers 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.

The concentration of the total DDT in an increasing order of khat samples as indicated in Fig. 3. As it is noticed from the figure, some of the samples contained total DDT residue above the MRL set by FAO/WHO, while more than three fourth of the khat samples contained a total DDT above the European Commission (EC) MRL.

The mean concentration of total DDT and its metabolites for six different sample sources in southwestern Ethiopia are presented in Table 2. From the results, the mean total DDT residue in khat was generally higher in samples from Sokoru (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.

From the Post-hoc tests presented below in the Box-Whisker plot (Fig. 4), there is a significant difference in the concentration of DDT metabolites in the khat samples ($P\text{-value} < 0.0001$). The metabolite p,p'-DDE is distributed more in khat samples and is contributing more in the sum of the total DDT.

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 may be considered as a health risk related to DDT.

3.3.1. Probabilistic exposure analysis

Table 3 presents the probabilistic estimates of total DDT intake from consumption of khat (mg/kg bw/day). 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 of total DDT from consumption of khat is 0.002 ± 0.003 mg/kg bw/day,

Table 1
Occurrence of DDT and its metabolites in khat samples. ND= None Detected, LOD = Limit of Detection.

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

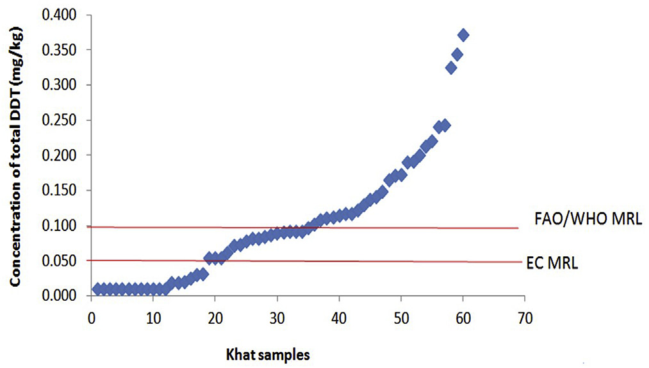

Fig. 3. Concentration of total DDT in an increasing order of the khat samples. Horizontal lines indicate maximum residue limit (MRL).

while the P 97.5 is 0.006 mg/kg bw/day.

4. Discussion

The Mann Whitney *U* test indicated that the distribution of average khat consumption (g/kg bw/day) is the same across the sex categories (p-value > 0.05). This is an indication of similar exposure of DDT for both female and male khat chewers. 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 problems and influence of peers, which may motivate them to chew khat. Additionally, this indicates that 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.

In the present monitoring study, results showed that a high residue of DDT in the chewable parts of khat from the investigated areas were investigated. In all the khat samples from the six agricultural areas contained residues high above the EC maximum residue limit (MRL) for total DDT. As reported in figure three, more than three fourth of the khat samples contain total DDT concentrations above the EC MRL. MRLs are not safety levels but indicates legal issues in relation to pesticide use such as illegal use of obsolete or banned pesticides; the use of sub-standard formulations; or contamination from various sources including uses to protect public health, etc (Fussell, 2016; PRC Secretariat, 2009). The residue value above MRL is an indication of illegal use of DDT in the study area for different purposes such as indoor residual spraying (IRS) in the framework of malaria control or any other control (Székács et al., 2015). Jimma zone is known to be one of a malaria endemic areas in Ethiopia (Karunamoorthi and Hailu, 2014). Therefore, the occurrence of DDT in khat might be due to malaria control activities which has been resulted from its persistent nature as a pollutant in the environment (Bempah et al., 2012).

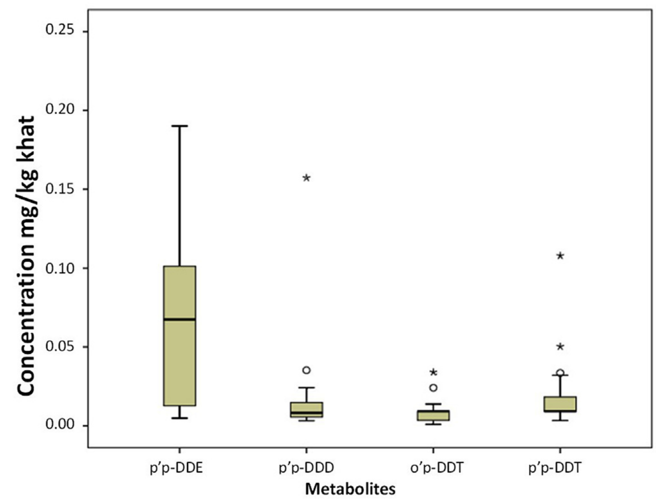

Fig. 4. Distribution of the concentration of DDT metabolites in khat samples.

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 all khat samples in the present study is 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 analyzed. This revealed that, there was intensive application of DDT in our study area compared to Sidama zone. After application of DDT it can be the persistent nature of DDT and its metabolites in the environment with a half-life of 2–15 years (Mahdavian and Somashekar, 2013). As indicated in the box and whisker plot (Fig. 4), the metabolite p,p'- DDE is highly distributed in the khat samples. Several literature (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 could be a historical use of DDT. The parent DDT gets metabolized over time to p,p'-DDE and p,p'-DDD, while recent application of DDT in that environment did not really happened. Specifically, p,p'-DDE is only found in the environment as a result of contamination or breakdown of DDT which may leave soil and other earth materials contaminated with this metabolite (Agency for Toxic Substances and Disease Registry, 2002). Consequently, the khat leaves could be contaminated by dust or soil material. Hence, the contamination of khat in Jimma zone may most probably be due to historical use of DDT that were used for public health purposes as a control of disease vectors. In addition, the contamination may also be originated from the dumped obsolete pesticides containing organochlorine pesticides like DDT in large amount as explained by Daba et al. (2011). Due to this dumping, pesticides

Table 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
Deddo	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

Table 3
Probabilistic analysis, DDT concentration (mg/kg), khat consumption (g/kg bw/day) and estimated daily intake of total DDT (mg/kg bw/day). EDI = estimated daily intake.

Distributions	Mean	StDv	P 50	P 75	P 90	P 95	P 97.5	P 99.5
khat consumption	19.585	4.561	19.206	22.024	25.147	27.461	29.846	35.881
DDT concentration	0.100	0.080	0.085	0.142	0.208	0.255	0.301	0.407
EDI	0.002	0.002	0.002	0.003	0.004	0.005	0.006	0.009

may leak from the storage area to the different environmental compartments and contaminate agricultural products such as khat.

From the probabilistic exposure analysis, the mean concentration of total DDT exceeds the maximum residue limit (MRL) recently set by FAO/WHO for different agricultural food items which is 0.1 mg/kg (FAO/WHO, 2013), and the European commission (EC) MRL of 0.05 mg/kg (EFSA, 2012). 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 P 97.5 estimated daily intake was below the PTDI. 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. These consumers could be severely exposed to this toxic compound as the khat leaves often contain DDT. Such exposure to DDT may result bioaccumulation in the body followed by chronic health problems. On top of that, the khat consumers may have commutative exposure to DDT through consumption of other commonly consumed agricultural crops in Ethiopia like teff, maize, and red pepper which also contain DDT and its metabolites as reported by Mekonen et al. (2014). In that study, all the crops which are major staple food items in Ethiopia showed more than 0.1 mg of DDT/kg of the food sample. This implies that the khat consumers might also be exposed to DDT from various sources, in addition from khat, where the effect of bioaccumulation could severely affect the health of consumers in the long run, if not acute. As khat chewing is becoming a common practice by the majority of the population of Ethiopia, Somalia, Kenya, Yemen, and Djibouti, and Uganda, application of quality control procedures on the level of pesticide residue could be an important public health activity to safeguard consumers' health.

5. Conclusion

The present study investigated consumer exposure to DDT in khat in southwestern Ethiopia. From the results of the study, it was observed that 80 percent of the khat samples contained total DDT residues and some of the residues were above the MRL set by FAO/WHO where most are above the EC MRL. From the metabolites, p'p-DDE was detected in large numbers of the khat samples compared to the parent compound p'p-DDT. This may be due to the contamination of the khat samples from historical use of DDT in the study area. The mean and 97.5 percentile estimated daily intake (EDI) of DDT from probabilistic analysis was below the Acceptable Daily Intake (ADI). The risk index for khat consumers were below one (RI < 1) for the mean and for each of the percentiles values and characterize as no risk of total DDT from consumption of khat. But this does not guarantee for the safety of the khat consumers, because they may be exposed to DDT from other food sources. Continuous monitoring of pesticides including DDT in khat and other commonly used agricultural crops is important to assure the safety of consumers.

Author's contribution

SM design the study, carried out the risk assessment, drafted the manuscript. BN carried out khat consumption survey, prepare the khat samples for laboratory analyses and participated in drafting the manuscript. AA participated in the study design and reviewing of the manuscript. PS participated in the design of the study, reveiw of the manuscript and overall supervision of the laboratory results. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

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Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.yrtph.2017.05.008.

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