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Risk of DDT residue in maize consumed by infants as complementary diet in southwest Ethiopia

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HIGHLIGHTS

- Total DDT was detected in all maize samples
- More than three fourth of maize samples have total DDT concentration above MRL
- The mean and P 97.5 estimated daily intake of total DDT were above PTDI
- Deterministic and probabilistic assessment results were not significantly different

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ABSTRACT

Infants in Ethiopia are consuming food items such as maize as a complementary diet. However, this may expose infants to toxic contaminants like DDT. Maize samples were collected from the households visited during a consumption survey and from markets in Jimma zone, southwestern Ethiopia. The residues of total DDT and its metabolites were analyzed using the Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method combined with dispersive solid phase extraction cleanup (d-SPE). 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 guidance value in this case the provisional tolerable daily intake (PTDI). All maize samples ($n = 127$) were contaminated by DDT, with a mean concentration of 1.770 mg/kg, which was far above the maximum residue limit (MRL). The mean and 97.5 percentile (P 97.5) estimated daily intake of total DDT for consumers were respectively 0.011 and 0.309 mg/kg bw/day for deterministic and 0.011 and 0.083 mg/kg bw/day for probabilistic exposure assessment. For total infant population (consumers and non-consumers), the 97.5 percentile estimated daily intake were 0.265 and 0.032 mg/kg bw/day from the deterministic and probabilistic exposure assessments, respectively. Health risk estimation revealed that, the mean and 97.5 percentile for consumers, and 97.5 percentile estimated daily intake of total DDT for total population were above the PTDI. Therefore, in Ethiopia, the use of maize as complementary food for infants may pose a health risk due to DDT residue.

1. Introduction

Dichlorodiphenyltrichloroethane (DDT) was an effective insecticide until it was banned in most industrialized countries since the late 1970s. 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 human health risks of DDT (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, the dietary intake of DDT is the main route of human exposure because, 90% of the residue stored in a human body is due to

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consumption of different food items (Center for food safety, 2006). Studies in different countries indicated 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 found residues of total DDT and its metabolites in 59% of wheat grains and in 78% of wheat flour in different geographic regions of India. A recent study in Ethiopia, also indicated that many staple food items have been contaminated with DDT (Mekonen et al., 2014). In addition to the dietary exposure indoor residual spraying (IRS) of pesticides for vector control may be another source of human exposure (Yewhalaw et al., 2011). 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).

Infants are very susceptible when exposed to toxic pesticides like DDT compared to adults. 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 a potential risk on the infants' neurodevelopmental growth at the age of 6–12 months (Eskenazi et al., 2006). Infants can also be exposed to DDT during breast milk feeding. A study done in India indicated that concentration of total DDT in breast milk for some infants were above the tolerable daily intake which will have a health risk (Bedi et al., 2013). Additionally, infants are also 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 months (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, like DDT which is actually banned for agricultural use. A recent study done in southwest Ethiopia indicated that, DDT was found above the maximum residue limit (MRL) in maize samples collected from Jimma zone (Mekonen et al., 2014). If the maize is contaminated with DDT and its metabolites, infants consuming it may be exposed and face health risks. Pesticide application often results in residues in food which may cause a health risk for human (Akoto et al., 2013). So consumer risk assessment is an

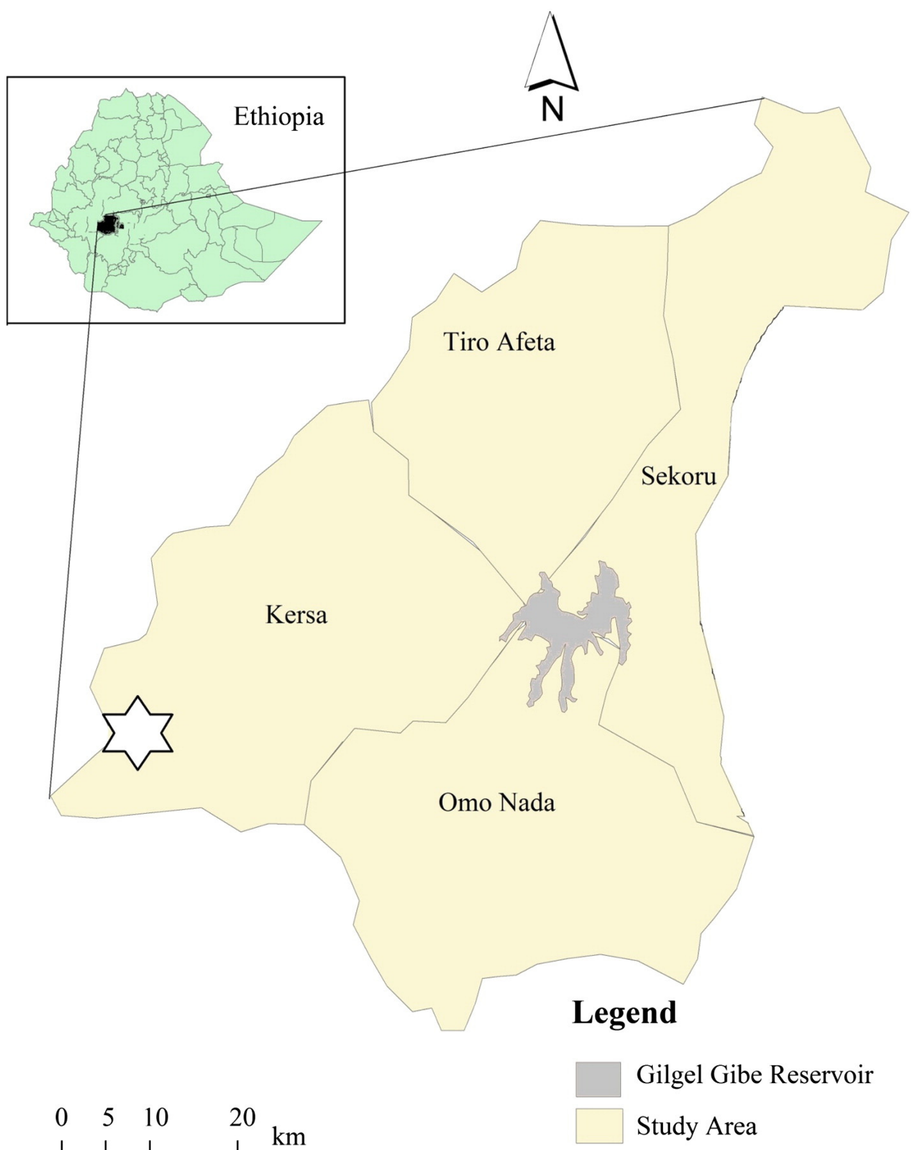

Fig. 1. Map of the study area in southwest Ethiopia.

important step for regulation of pesticide use on food crops (Hamilton et al., 2004). Despite all these problems, there is no study that has estimated the exposure and risks of infants to pesticides, 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.

2. Materials and methods

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 the country (Fig. 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, sorghum and teff are the commonly grown food crops in the area. Among those cereals, maize production ranks first in Jimma zone (Gebremedhin et al., 2007). In addition, maize is the commonly used staple food item in Ethiopia particularly in Jimma zone (Sori and Ayana, 2012).

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 preharvest season (June to September), 2011. 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 the 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). The weight of the infants was measured when infants are naked or with light clothes using mother–child digital scales and recorded to the nearest 0.1 kg (SECA Uniscale, Hamburg, Germany).

2.3. Maize sampling

A total of 127 maize samples were collected during the preharvest season. A first batch of 59 samples came from households interviewed during the dietary assessment. In these households, the 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 buy it at the local markets, a second batch of 68 maize samples were collected from four different markets (Sekoru, Omo-Nada, Kersa and Tiro Afeta). The maize sampling from the market was done according to (Mekonen et al., 2014). Maize samples (250 g each) were packed in black polyethylene plastic bags, labeled accordingly and transported to the laboratory. Then the samples were stored at −20 °C until analysis.

2.4. Reagents and materials

Analytical grade acetonitrile (99.9%) was supplied by VWRPROLABO, and high-performance liquid chromatography grade n-hexane (95%) and acetone (98.5%) were obtained from ALLtech. Thermo Fisher Scientific supplied magnesium sulfate ($MgSO_4$) to remove water from organic solvent, sodium acetate (NaAc) to absorb remaining water content in samples, 50-mL polypropylene centrifuge tubes, and 15-mL d-SPE tubes packed with primary and secondary amines (PSAs) for the removal of organic acids and polar pigments among other compounds, and octadecyl (C18) to remove lipids and sterols from the matrix. Standards of DDT metabolites (p,p′-DDE (99.9%), p,p′-DDD (99.3%), o,p′-DDT (100%), and p,p′-DDT (99%)) of highest analytical purity were obtained from Supelco and delivered by Sigma-Aldrich Logistics.

2.5. Extraction and clean up of maize samples

The maize samples were analyzed for the presence of total DDT and its metabolites (p,p′-DDE, p,p′-DDD, o,p′-DDT and p,p′-DDT). The extraction and cleanup of the maize samples were done using modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method with dispersive solid phase extraction cleanup (d-SPE). The analytical procedures were as follows: 1) 10 g of comminuted and homogenized sample of maize, was weighed in 50-mL centrifuge tubes on an analytical balance (Sartorius); 2) 10 mL of deionized water was added; 3) 15 mL of acetonitrile containing 1% glacial acetic acid (v/v) was added in each sample using a solvent dispenser; 5) the tube was tightly capped and shaken gently for 1 min to facilitate contact between the solvent and the sample; 6) 6 g of anhydrous $MgSO_4$ and 1.5 g NaAc were added, and the sample was shaken vigorously by hand for 5 min; 7) the sample was centrifuged at 3000 rpm for 5 min; 8) for clean-up, the upper 10 mL was put into a d-SPE tube containing 300 mg PSA, 900 mg $MgSO_4$, and 150 mg C18 and shaken by hand for 30 s, and then step 7 was repeated; 9) a 5-mL aliquot of cleaned extract was taken and evaporated to dryness using a rotary evaporator (N18673 Rotavapor; Buchi) at a temperature of 40 °C; 10) the cleaned extract was reconstituted with 2 mL n-hexane:acetone (9:1, v/v) for solvent exchange; and 11) the extract was then put into an auto sampler vial for GC analysis.

2.6. Analytical equipment

Total DDT and its metabolites were determined by capillary gas–liquid chromatography with electron capture detector (GC–ECD; Agilent Technologies 6890N) and an auto sampler. An HP-5 capillary column (30 m; 0.25 mm inner diameter; 0.25 mm film thickness) coated with 5% phenyl methyl siloxane (model 19091J-433; Agilent) was used in combination with the following oven temperature program: initial temperature of 80 °C, ramped at 30 °C min^{-1} to 180 °C, ramped at 3 °C min^{-1} to 205 °C, held for 4 min, ramped at 20 °C min^{-1} to 290 °C, held for 8 min, ramped at 50 °C min^{-1} to 325 °C. 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) and the results were compared with the MRL (0.1 mg/kg) which is set by the Codex Alimentarius (FAO/WHO, 2013).

2.7. Analytical method validations

The limit of detection (LOD) and the limit of quantification (LOQ) were determined by preparing matrix spikes at a low level near the expected detection limit. The LOD and LOQ, the precision of the method (repeatability), were determined by spiking DDT and its metabolites with a concentration of 0.1 mg/L in 6 replicates in blank maize samples. The LOD and LOQ were calculated by multiplying the standard deviation from the replicates by 3 and 10, respectively (Butler et al., 2008). The relative standard deviation was calculated by dividing the standard deviation with the average concentration. The linearity was determined by preparing a stock solution of pure standards of the pesticides studied and diluting them to produce different concentrations. The standard solutions of the pesticide were run on GC–ECD under the set chromatographic conditions to produce 5-point calibrations ranking from 0.01 mg/L to 1 mg/L. The recovery tests were done by spiking a mixture of DDT metabolic products (p,p′-DDE, p,p′-DDD, o,p′-DDT, and p,p′-DDT). A pesticide standard was spiked into laboratory blank samples of maize to give 0.25 mg/g, and recovery was done based on

4 replicates. The spiked samples were left for 1 h before extraction to allow the pesticides to partition into the matrix (Bempah et al., 2012).

2.8. 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 ($P > 0.05$) 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 a box and a whisker plot.

To evaluate the safety of consumers regarding pesticide residues, exposure was assessed and compared to toxicological limits (Claeys et al., 2011). For the present study, the exposure assessment was done by assuming that the food processing (backing 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 DDT concentration in maize (worst case scenario). As recommended by the European Food Safety Authority (2011), 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 infant population (consumers and non-consumers). The inclusion of non-consumers was used to assess the chronic consumer exposure. The Mann–Whitney U test was applied to compare the estimated daily intake of total DDT by the infants from deterministic and probabilistic exposure analyses.

2.9. Deterministic exposure assessment

The dietary exposure of infants living in Jimma zone, Ethiopia to total DDT (mg/kg bw/day) was calculated deterministically based on the maize consumption data (g/kg bw/day) and DDT residue data (mg/kg maize). In this approach, the dietary exposure was computed by multiplying a single value of consumption and DDT concentration. The mean, P50, P75, P 90, and P 97.5 consumption was combined with the mean, P 50, P 75, P 90 and P 97.5 total DDT concentration as explained by McKinlay et al. (2008).

2.10. 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 and residue distributions were combined into an exposure distribution (mg/kg bw). 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 assessments were compared with the corresponding PTDI of 0.01 mg/kg bw/day of DDT set by the joint meeting of Food and Agricultural Organization and World Health Organization (FAO and WHO, 2007).

Table 1
Method validation results from spiked blank maize samples ($n = 6$).

Pesticides	Spiked conc. (μg/g)	Recovery (%)	RSD (%)	LOD (μg/g)	LOQ (μg/g)
p,p′-DDE	0.5	77.2	1	0.011	0.040
p,p′DDD	0.5	102	2	0.030	0.091
o,p′-DDT	0.5	86	1.4	0.020	0.052
p,p′-DDT	0.5	94	1.6	0.019	0.061

3. Results and discussion

3.1. Socio demographic results

The percentage of male and female infants (6–12 months of age) were 61% and 39%, respectively. 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 ($P = 0.354$).

3.2. Method validation results

The method validation results are indicated in Table 1. From the results the average recovery of DDT metabolites were in the accepted analytical range (70–120%) with reliable precision (%RSD < 20) (Document N° SANCO/12495/2011, 2011). The LOD and LOQ were in the range of 0.01–0.03 and 0.04–0.09 μg/g, respectively. This may indicate to us that the QuEChERS method is accurate and precise to detect DDT residue in maize.

3.3. Concentration of total DDT and its metabolites

The distribution of total DDT from the two sample sources (households and markets) was not significantly different ($P = 0.321$) as indicated in Fig. 2. This result revealed that the contamination of maize samples with DDT was similar whether the infants consumed either from household or market sources.

As indicated in Fig. 3 below, total DDT was detected (LOD = 0.019 mg/kg) in all maize samples. More than three fourth of the maize samples had a residue of total DDT above the corresponding MRL (0.1 mg/kg) (FAO/WHO, 2013). The residue load above MRL indicated that there may be illegal 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 historical use for indoor residual spraying (Yewhalaw et al., 2011). This may be also explained by the persistent nature of DDT as a contaminant in the environment (Bempah et al., 2012). Additionally, the detection of DDT may come from environmental contamination with dumped obsolete pesticides in different sites of Ethiopia (Amera and Abate, 2008).

Distribution of DDT metabolites in the maize samples is indicated in Fig. 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. Among the metabolites of total DDT, the concentrations of o′p-DDT and p′p-DDT were significantly higher ($P < 0.0001$) than the rest of the metabolites. However, no significant difference was observed in concentrations between o′p-DDT and

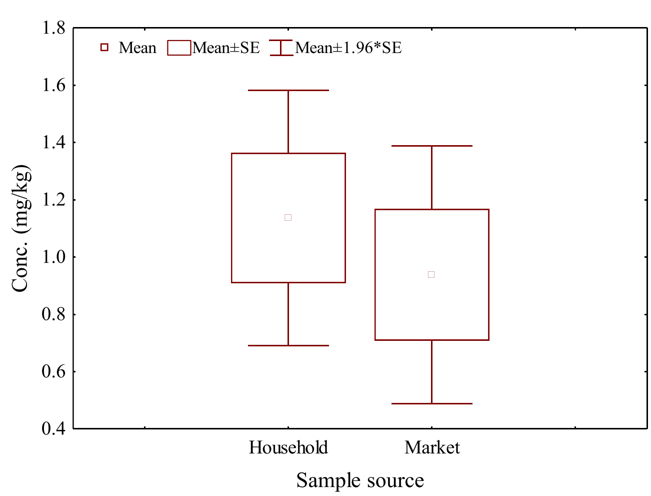

Fig. 2. The concentration distribution of total DDT between household ($n = 59$) and market maize samples ($n = 68$), SE = standard error.

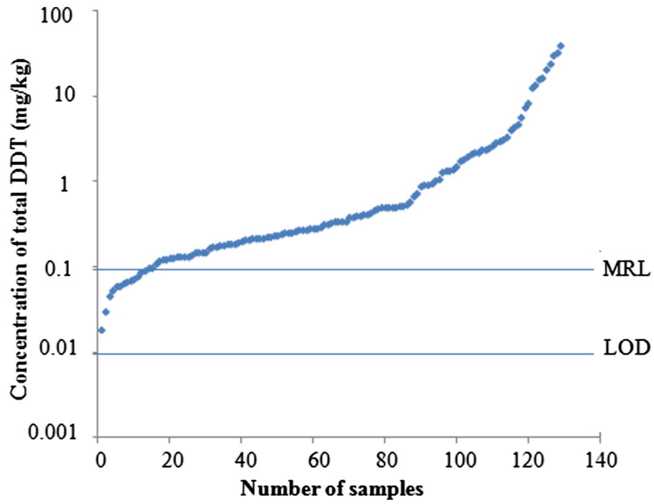

Fig. 3. Concentration of DDT in an increasing order in 127 maize samples (LOD = limit of detection, MRL = maximum residue limit).

p′p-DDT, and p′p-DDD and p′p-DDE (P = 0.125). Similar findings were observed with the study done in southwest Ethiopia (Mekonen et al., 2014). The high concentration of p′p-DDT indicated that there may be a recent use of DDT in the study area as has been explained by the European Food Safety Authority (Alexander et al., 2012).

3.4. Exposure assessment

Deterministic and probabilistic analyses were worked out to evaluate whether the level of exposure of infants to total DDT from maize consumption exceeds the PTDI. The mean and the P 97.5 of the estimated daily intake were used to represent the average and high consumer exposure scenario, respectively. A DDT intake above the PTDI may be considered as there will be a chronic health risk for the exposed infants.

3.4.1. 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 2 presented the calculation of the DDT concentration in maize, maize consumption, and estimated daily intake of DDT by Ethiopian infants using the deterministic exposure analysis.

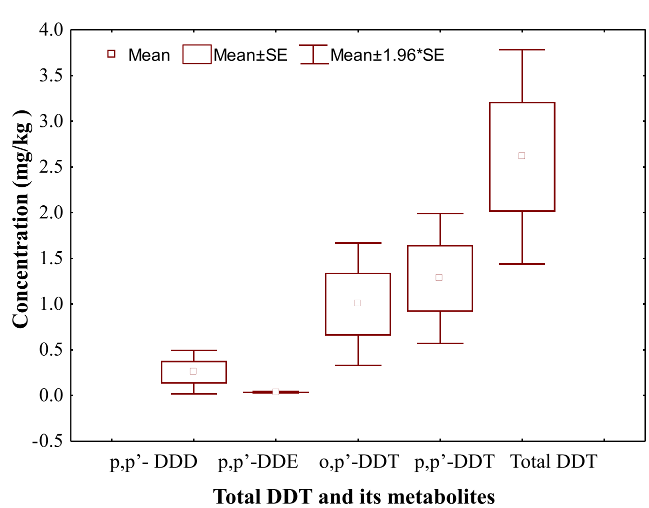

Fig. 4. Concentration distribution of total DDT and its metabolites in maize samples, SE = standard error.

Table 2
DDT concentration (mg/kg), maize consumption (g/kg bw/day) and estimated daily intake of total DDT (mg/kg bw/day) from the deterministic exposure assessment.

Deterministic analysis	Mean	P 50	P 75	P 90	P 95	P 97.5
DDT concentration	1.770	0.350	1.370	4.560	10.280	14.700
Consumption in consumers	5.985	4.160	7.500	13.930	19.770	21.050
Consumption in total population[a]	2.390	0.000	2.700	7.500	11.390	18.050
Estimated daily intake of DDT						
Consumers only	**0.011**	0.001	**0.01**	**0.064**	**0.203**	**0.309**
Total population[a]	0.004	0.0001	0.004	**0.034**	**0.117**	**0.265**

[a] Including non-consumer infants; bold indicated results above PTDI.

The mean and P 97.5 consumption were 5.980 and 21.050 g/kg bw/day and the mean and P 97.5 total DDT residue were 1.770 and 14.700 mg/kg maize, respectively. From the deterministic exposure assessment, the mean and P 97.5 estimated daily intake of total DDT were 0.011 and 0.309 mg/kg bw/day for infants consuming maize in which both values were above the PTDI, while 0.004 and 0.265 mg/kg bw/day were for both maize consumer and non-consumer infants. The P 97.5 estimated daily intake of total DDT for total population were also above the PTDI, while the mean estimated daily intake of total DDT for total population was less than PTDI. The median estimated daily intakes were 0.001 and 0.0001 mg/kg bw/day for maize consumers and total population, respectively.

3.4.2. Probabilistic exposure assessment

In the probabilistic exposure analysis, both maize consumption and DDT residue data were fitted in the @Risk software to identify the best fit distribution using Monte-Carlo simulation model (MC). From the result the P–P plot provided roughly a straight line joining the diagonals for both consumption (consumers only) and DDT concentration. During fitting of the distribution consumption of the total infant population (consumers and non-consumers), data deviations from the normal line were observed. This deviation may be due to the presence of zero consumption patterns. Table 3 presented the distribution consumption, distribution DDT concentration in maize and the estimated daily intake of total DDT by Ethiopian infants from probabilistic exposure analysis. The results revealed that the mean and P 97.5 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 P 97.5 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 from the probabilistic exposure analysis. The median (P 50) estimated daily intakes were 0.002 and 0.001 mg/kg bw/day for consumers only and total population from the probabilistic exposure assessment, respectively.

The median estimated daily intakes (P 50) of total DDT from both the deterministic and probabilistic exposure analyses were below the PTDI. The upper percentile (p 97.5) indicated that, the infants were exposed to high DDT concentrations for both consumers and total populations. This result is inconsistent with the work of Wason et al. (2013). Values above the health based guidance (>PTDI) indicated that there may be a health risk for the infants consuming maize as a

Table 3
DDT concentration (mg/kg), maize consumption (g/kg bw/day), and estimated daily intake of total DDT (mg/kg bw/day) from the probabilistic exposure assessment.

Probabilistic analysis	Mean	P 50	P 75	P 90	P 95	P 97.5
DDT concentration	1.77	0.415	1.29	4.015	7.771	13.155
Consumption among consumers	6.484	3.591	7.405	14.337	21.339	30.153
Consumption among total population[a]	2.273	1.639	3.296	5.487	7.144	8.802
Estimated daily intake of DDT						
Consumers only	**0.011**	0.002	0.006	**0.021**	**0.044**	**0.083**
Total population[a]	0.004	0.001	0.002	0.008	**0.017**	**0.032**

[a] Including non-consumer infants; bold indicated results above PTDI.

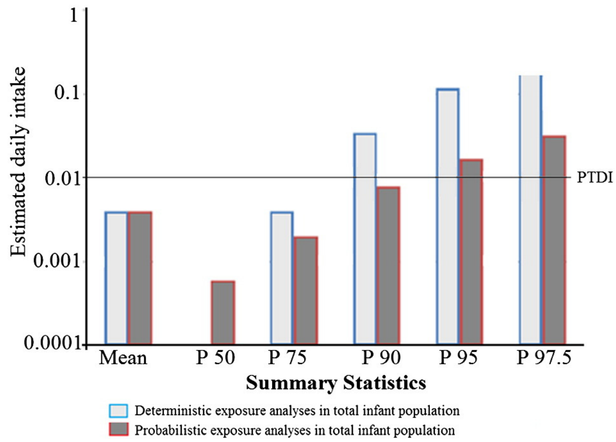

Fig. 5. Comparison of estimated daily intake of DDT (mg/kg bw/day) from deterministic and probabilistic exposure assessments. Y-axis in logarithmic scale.

complementary diet. Different studies explained that, infants' chronic exposure to organochlorine pesticides such as DDT, may develop health problems like attention deficient disorder, obesity and type 2 diabetics (Corin and Weaver, 2005; Polańska et al., 2013).

3.4.3. Comparison of estimated daily intake of total DDT for the total infant population from the deterministic and probabilistic exposure assessments

The estimated daily intake of total DDT by the Ethiopian infants from the deterministic and probabilistic exposure analyses was compared to see which method is appropriate to show the exposure scenario. The result of the comparison is presented in Fig. 5. The results revealed that, the mean estimated daily intake of total DDT for total infants from both deterministic and probabilistic exposure assessments were the same and below the corresponding PTDI. For the deterministic exposure assessment the P 90, P 95 and P 97.5, while for the probabilistic exposure assessment only the P 95 and P 97.5 estimated daily intakes of total DDT were above the PTDI. According to the European Food Safety Authority (EFSA, 2012), the deterministic exposure assessment showed a wide range of exposure values as it uses a point estimate for input and generate a point estimate for exposure, while the probabilistic exposure assessment uses distributions which are taking into account variability and uncertainties in exposure assessment. But to see whether there is a significant difference in estimating the daily exposure for the total infant population between the deterministic and probabilistic exposure assessments the Mann–Whitney U test was applied. The results revealed that there was 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 in their complementary diet even though the deterministic analysis showed a wide range in exposure values. So both exposure assessment methods can be appropriate for the determination of estimated daily exposure for the infants.

4. Conclusion

This study estimated the exposure and risk of infants to total DDT from maize consumption in their complementary diets. All the maize samples were contaminated with DDT, without significant difference between market and household sample sources. Both deterministic and probabilistic exposure analyses were appropriate to determine the estimated daily intake of total DDT by the infants. The deterministic and probabilistic exposure analyses indicated that, the average and high consumer estimated daily intakes of total DDT for consumers and high consumers for total population were above the health based guidance value. This could result in chronic health related 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 assure food safety.

Conflict of interest

The authors declare that there is no conflict of interest.

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