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# Dichloro-Diphenyl-Trichloroethane and Benzene Hexachloride Metabolite Residues in Blood of Khat (Catha edulis) Chewers and Agricultural Workers in Wondogenet district, Sidama National Regional State, Ethiopia

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**ABSTRACT:** Ethiopia is the world's largest producer and exporter of khat. However, pesticides are used intensively to protect khat from destruction of pest and increase its product, which is leading exposure to agricultural workersandkhat chewers. The objective of this paper was to evaluate the levels of Dichloro-Diphenyl-Trichloroethane (DDT) and Benzene Hexachloride (BHC) Metabolite Residues in Blood samples of Khat (*Catha edulis*) Chewers and Agricultural Workers in Wondogenet district, Sidama National Regional State, Ethiopia using appropriate standard techniques. Data obtained show that in chewers blood samples the concentration was ranked as follows: *p,p'*-DDE (14.26µg$L^{-1}$) >*p,p'*-DDT(5.88µg$L^{-1}$) > Gamma BHC (3.378µg$L^{-1}$) >Beta BHC (2.336µg$L^{-1}$)>Delta BHC (0.506 µg$L^{-1}$).Whereas ,in sprayer workers the concentration was ranked as follow:*p,p'*-DDE (13.245µg$L^{-1}$) >*p,p'*-DDT(5.468 µg$L^{-1}$) > Gamma BHC (3.13µg$L^{-1}$) >Beta BHC (2.169 µg$L^{-1}$) >Delta BHC (0.469 µg$L^{-1}$) and in farmers blood sample the concentration of pesticides was as follow;*p,p'*-DDE (0.193µg$L^{-1}$) >*p,p'*-DDT(0.192µg$L^{-1}$) > Gamma BHC (0.11µg$L^{-1}$) >Beta BHC (0.07µg$L^{-1}$) >Delta BHC (0.02µg$L^{-1}$).There is significant difference in mean concentration of delta BHC,gammaBHC,betaBHC,,*pp*DDE and *pp*DDT in blood serum among spray workers and farmers as well as khat chewers and farmers.Furthermore, onepesticides:*pp*DDE had residues above ADI in blood serum of khat chewers and spray workers.Therefore, vigilant monitoring pesticide levels in khat is imperative for addressing environmental and public health concerns. It is essential for policymakers to take appropriate measures at this critical time to safeguard public health against the threats posed by pesticide pollution in Khat cultivation.

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Organochlorine pesticides are extensively used in agriculture and public health to protect plants against pests, weeds, and illnesses, as well as humans from diseases carried by vectors. (Damalas, 2009), Because most sprayed pesticides are harmful, they spread throughout the environment and have an

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adverse effect on agricultural workers' and customers' health when they consume treated crops (Afata*et al*., 2021). Pesticides are poisonous and detrimental to living things at certain concentrations. Enzymatic activity and metabolism are impacted by pesticides when they reach the human body (Yassi*et al*., 2001). Numerous studies have demonstrated that OCs interact with the endocrine system, producing a variety of physiological consequences that might have an impact on both human and animal health (Munhozde-Toro *et al*., 2006).Inhalation, skin contact, and ingestion are the main ways that pesticides are exposed to humans (Damalas and Koutroubas, 2016). Pesticide exposure is frequently exacerbated as a result of farmers' misunderstanding of possible health concerns, improper application techniques, and absence of protective gear during application (Khan *et al*., 2015). Numerous health issues might arise from the use of pesticides without knowledge of their toxicity and without taking any precautions (Yassi*et al*., 2001). The manufacturing and use of these substances were outlawed in industrialized nations in the 1970s due to the negative effects they were seen to have on both humans and animals (Jaraczewska*et al*., 2006), and their usage was restricted in many other countries. Nevertheless, due to their persistent and bio accumulative characteristics, they are still found in biological and environmental samples from all over the world.

Khat is a flowering plant and it is also known as Bushman's tea, native to eastern and southeastern Africa. Ethiopia's khat output has grown significantly in recent years due to export and domestic feeding needs. A variety of pesticides are routinely utilized nationwide to manage common pests in order to safeguard crop output. In addition, the abuse and misuse of pesticides is frequent in Ethiopia, with farmers in south regions of the nation sometimes employing outmoded pesticides like DDT and other organochlorine pesticides. Hence, the objective of this paper is to evaluate the levels of Dichloro-Diphenyl-Trichloroethane (DDT) and Benzene Hexachloride (BHC) Metabolite Residues in Blood samples of Khat (*Catha edulis*) Chewers and Agricultural Workers in Wondogenet district, Sidama National Regional State, Ethiopia.

## MATERIALS AND METHODS

*Study setting:* The study was conducted in the Wondogenet district of the Sidama region of Ethiopia. The distance from the capital city of Ethiopia, Addis Ababa, is 265 km. In accordance with the population projections for Ethiopia, the Wondogenet district had a total population of 240,182 in 2023 (CSA, 2023). The area is situated at 7°06′–7°07′N latitude and 38°37′–38°42′E longitude, with an altitudinal range between 1600 and 2580 meters above sea level (Tasew and Abayneh, 2017). The study area's climate is typified by the Woyna-Degaagroclimatic type. The mean annual precipitation and temperature of the study area are 1210 mm and 20 °C, respectively (Tasew and Abayneh, 2017). Wondogenet is one of Ethiopia's most promising khat-producing areas, particularly within the Sidama Region. All kebeles within the district engage in khat production, utilizing it as a primary source of income. To safeguard khat from pests and enhance its productivity, farmers in Wondogenet employ a range of pesticides, with the majority of agricultural workers and regular khat chewers exposed to these chemicals.

*Ethical consideration:* The Department of Biology Ethical Review Board of Hawassa University College of Natural and Computational Science provided ethical clearance and permission for this project with a RERC reference number: CNCS-REC017/24. After that, acquired letter of consent was delivered to the district agricultural office in Wondogenet district. Prior to sample collection, consent was obtained from each individual. Subsequently, a letter of authorization was obtained from the university and delivered to the district agricultural office in Wondogenet. Prior to sample collection, informed consent was obtained from each individual.

*Study design:* The study was conducted among two distinct groups of individuals in the Wondogenet District of Ethiopia: agricultural workers and khat chewers. Furthermore, a laboratory-based study was conducted to assess the concentration of pesticides in the serum of the study participants.

*Blood sampling:* To determine the metabolites of DDT and BHC residue in serum, blood samples were procured from 90 healthy male subjects between the ages of 18 and 55 years. The cohort was divided into three groups: 30 farmers, 30 spray workers, and 30 regular khat chewers. The farmers are engaged in khat production and have no history of pesticide spraying or khat consumption. Spray workers and khat chewers were selected based on their experience of spraying pesticides and regularly chewing khat for > 5 years. The trained lab technician collected 10ml of blood using butterfly needles from each subject's veins of inner forearm. The samples were centrifuged, transferred to polyethylene tubes and placed in an ice-cold box. They were transported to JijeLaboglass Pvt. Ltd. and stored at -20°C until further analysis.

*Extraction and clean up:* DDT and BHC metabolites were extracted from blood samples using the method by Agarwal(1976). A 4-ml blood sample was diluted with 25 ml of distilled water and 2 ml of saturated brine and transferred to a 60-ml separator funnel. The extraction was carried out by adding 10 ml of acetone and 10 ml of n-hexane in a 1:1 ratio. The mixture was shaken three times for approximately three minutes, with pressure being released occasionally, and then allowed to settle until distinct layers formed. After that, the upper (non-polar) layers were obtained by repeatedly removing the lower (polar) layers three times. Finally, the three combined extracts were passed through anhydrous sodium sulfate in order to eliminate residual polar solvents.

The process of dispersive solid-phase extraction cleanup (dSPE) process was carried out as follows: A 15-milliliter Bond ElutQuEChERS AOAC tube was filled with 2 milliliters of the acetonitrile layer. The dSPE tube comprised 400 mg of PSA, 1,200 mg of anhydrous magnesium sulfate, 400 mg of C18, and 400 mg of GCB. Furthermore, each tube was provided with two milliliters of n-hexane. After securely capping the tubes, they were subjected to a one-minute vortexing process. Subsequently, the tubes were subjected to five-minute centrifugation at 4,500 rpm using a standard centrifuge. Finally, a 2-ml auto-sampler vial was filled with an aliquot of the top hexane layer in preparation for GC-MS injection.

*Pesticides Residues Analysis Method (GCMS/MS analysis):* Residues of metabolites of DDT and BHC in the blood serum of khat chewers and agricultural workers were targeted to determine their concentration.

To this end, a GC Agilent 7890B equipped with an auto-sampler G4513A was used, which was connected to an Agilent Technologies, Inc., CA, USA, Triple Quadrupole mass detection system operating at 7000C GC/MS. To facilitate analyte dissociation and a highly passive transport pathway into the sensor, a 30-meter-long capillary GC column with an internal diameter of 0.25 mm and a film thickness of 0.25 μm was employed. This column was manufactured by Agilent J and W DB-5ms Ultra Inert.

Helium (99.999%) was employed as the carrier gas, with a flow rate of 1.2 mL/min for the column. At 280°C, the injector and interface were configured for splitless injection of 1 μL. Subsequently, the GC column's eluent was supplied to a 70 eV source of ionization by electron impact, which had a 280°C source temperature. A selected ion monitoring mode was employed for the examination. Instrumental responses were analyzed and conducted using Agilent Technologies MassHunter software.

*Data quality assurance:* To ensure the quality of the data, a representative sample of blood was taken from regular khat chewers, spray workers, and farmers. The samples were analysed using certified analytical techniques and standard processes with graded products. At each stage of the process, samples were stored in a refrigerator until the subsequent step was initiated. Prior calibration of the instrument was conducted before the serum samples were analyzed by GC, and all glassware was thoroughly cleaned, rinsed, and handled correctly throughout the extraction and clean-up procedure.

*Statistical analysis:* The data were subjected to cleaning and entered into the SPSS software, version 24, for analysis. The concentrations of metabolites of DDT and BHC were expressed as arithmetic means with standard deviations. A one-way ANOVA test was employed to ascertain the existence of a statistically significant difference between the groups. Subsequently, post-hoc tests (Tukey's HSD) were conducted to determine which specific group pairs exhibited a significant discrepancy from one another.

## RESULTS AND DISCUSSIONS

*Method validation:* The organochlorine pesticide residues detected in the blood serum samples were quantified using a calibration curve established using a reference mixture of 7 OCPs at concentrations of 5, 20, 40, 60, and 200 $\mu gL^{-1}$ .For the method's validation, limit of quantifications (LOQs) and limit of detections (LODs) were established. According to GetachewDinede*et al*. (2023), the values of LOD and LOQ were calculated as 3.3 and 10 times the intercept's standard deviation over the regression line's slope, respectively.

The concentration achieved with the substance's starting concentration was used to determine the recoveries of each analyte in each sample (Gure*et al*., 2014). Method validation showed that the calibration curves for each analyte exhibited a linear distribution of concentration between 5 and 200 $\mu gL^{-1}$. Regression coefficient determinations for each pesticide ($R^2$) values ranged from 0.9912 to 0.9977. All OCPs that were found had LODs and LOQs ranged between 0.018 to 0.038 and 0.055to 0.116, respectively.With the exception of alpha BHC, all OCP percentage recoveries within the permissible range (98.98% and 115.9%), and all pesticide residues show reasonable accuracy with a relative standard deviation (RSD) of less than 20% (Table 1).

**Table 1:** Method validation parameter results in blood serum residue analysis.

| Organochlorine Pesticides | $R^2$ | Recovery (%) | LOD | LOQ | %RSD |
|---|---|---|---|---|---|
| Delta BHC | 0.9977 | 98.98 | 0.019 | 0.059 | 7.70 |
| Gamma BHC | 0.9947 | 104.85 | 0.029 | 0.089 | 3.52 |
| Beta BHC | 0.9967 | 100.78 | 0.023 | 0.069 | 8.25 |
| Alpha BHC | 0.9965 | 156.38 | 0.029 | 0.089 | 8.26 |
| DDE_pp | 0.9949 | 100.93 | 0.029 | 0.088 | 5.35 |
| DDT_pp | 0.9980 | 99.78 | 0.018 | 0.055 | 10.60 |
| DDD_pp | 0.9912 | 115.9 | 0.038 | 0.116 | 0.50 |

*Note: LOD = limit of detection in µgL-1LOQ = limit of quantification in µgL-1; RSD= residual standard deviation , Recovery%= mean recovery and $R^2$ = regression coefficient*

This study included 90 human subjects of which 30 participants from each regular khat chewers, spray workers and farmers producing khat. This study targeted 7 metabolites and analyzed for blood samples from collected from regular khat chewers, spray workers and farmers producing khat in Wondogenet district. Out of the metabolites of DDT and BHC in blood samples of khat chewers and spray workers,5 of them were detected and the two undetected were Alpha BHC, *pp*'DDD. In chewers blood samples the concentration was ranked as follows: *p,p'*-DDE (14.26µgL$^{-1}$) >*p,p'*-DDT(5.88 µgL$^{-1}$) > Gamma BHC (3.378µgL$^{-1}$) >Beta BHC (2.336 µgL$^{-1}$) >Delta BHC (0.506 µgL$^{-1}$).Whereas ,in sprayer workers the concentration was ranked as follow:*p,p'*-DDE (13.245µgL$^{-1}$) >*p,p'*-DDT(5.468 µgL$^{-1}$) > Gamma BHC (3.13µgL$^{-1}$) >Beta BHC (2.169 µgL$^{-1}$) >Delta BHC (0.469 µgL$^{-1}$) and in farmers blood sample the concentration of pesticides was as follow;*p,p'*-DDE (0.193µgL$^{-1}$) >*p,p'*-DDT(0.192 µgL$^{-1}$) > Gamma BHC (0.11µgL$^{-1}$) >Beta BHC (0.07 µgL$^{-1}$) >Delta BHC (0.02µgL$^{-1}$)(**Table 2**).

**Table 2:** The percent of farm workers, regular khat chewers and pesticide-spray workers having different pesticide residues and mean concentration of residues (µgL$^{-1}$) in their blood serum

| DDT and BHC Metabotes | ADI [µgL-1] | **Khat chewers** | | **Spray workers** | | **Farmers** | |
|---|---|---|---|---|---|---|---|
| | | Range | Mean | Range | Mean | Range | Mean |
| *pp'* DDE | 10 | 9.7356-38.7644 | 14.2644$^a$ | 10.7544-37.7456 | 13.2456$^a$ | 6.407 -6.7927 | 0.1927$^b$ |
| *pp'* DDT | 10 | 0.7107-12.4893 | 5.8893$^a$ | 1.1313-12.0687 | 5.4687$^a$ | 6.4076-6.7924 | 0.1924$^b$ |
| *pp'* DDD | 10 | ND | ND | ND | ND | ND | ND |
| Delta BHC | - | 1.474 - 2.4856 | 0.506$^a$ | 1.510 - 2.449 | 0.469$^a$ | 1.9561- 2.0039 | 0.0239$^b$ |
| Gamma BHC | - | 0.4413 - 7.1987 | 3.3787$^a$ | 0.6827- 6.9573 | 3.1373$^a$ | 3.7108 -3.9292 | 0.1092$^b$ |
| Beta BHC | - | 0.2336 - 4.9064 | 2.3364$^a$ | 0.4004 - 4.7396 | 2.1696$^a$ | 2.426 - 2.574 | 0.0740$^b$ |
| Alpha BHC | - | ND | ND | ND | ND | ND | ND |

*Values with different letters with in a row are significantly different at p < 0.005 level,ND means Not detected*

In our study, among the detected 5 metabolites of DDT and BHC, One had residues above ADI in blood serum of khat chewers and spray workers, these was*pp'*DDE, which is injurious to health.Blood serum samples from khat chewers, spray workers and farmers showed the mean concentration sequence of DDT metabolites: *pp'*DDE>*pp'*DDT. But *pp'* DDD was not detected in all groups. To distinguish between recent and historical exposure, Ruiz-Suarez *et al*.(2014) used the ratio of *pp'* DDT/ *pp'* DDE, if the ratio is less than one, it indicates high persistence in the environment and ongoing bio-magnification whereas it is greater than one, in shows both continuous and continued exposure to DDT (Antonio *et al*.,2013). Accordingly, the mean ratio of *pp'* DDT/ *pp'*DDE across all study groups was less than one; this indicates high persistence in the environment and ongoing bio-magnifications. In this particular study, the mean levels of *pp'*DDE in blood from khat chewers, spray workers and farmers were 14.2644 µgL$^{-1}$, 13.2456 µgL$^{-1}$and 0.1927 µgL$^{-1}$, respectively. The mean concentration of *pp'*DDE in the blood serum of farmers in the present study(0.1927 µgL$^{-1}$) was lower than previous studies conducted in flower farm workers in Ethiopia (Mekonen*et al* 2023), in malaria epidemic community in Mexico(Ruiz-Suarez *et al*.,2014), in Tunisia (Ben-Hassine*et al*.,2014) , in Ghana( Ntow*et al*.,2008) and in Pakistan(Khwaja*et al*.,2013) which reported that *pp'* DDE in blood serum was found to be 38 µgL$_{-1}$, 8 µgL$^{-1}$, , 169 µgL$^{-1}$, 7.1 µgL$^{-1}$ and 5 µgL$^{-1}$, respectively. Similarly, the result of the present study was lower than the previous study done on serum of farmers in Ethiopia, Sudan, India and Mexico, which reported that *pp'* DDE was found to be 20 µgL$^{-1}$(Afata*et al*.,2021), 618 µgL$^{-1}$(Elbashir*et al*.,2015), 15.6 µgL$^{-1}$(Bedi*et al*.,2015), and 8 µgL$^{-1}$(Ruiz-Suarez *et al*.,2014), respectively.

The mean concentration of *pp'* DDE in blood serum of spray workers in the present study(13.2456 µgL$^{-1}$) was comparable with a study of sprayers in Bethanda district of Punjab, India, where the mean concentration of *pp'* DDE was found to be 13.22

$\mu gL^{-1}$(Bedi*et al*.,2015). However, the result of the present study was higher than the previous study in Pakistan, where the mean concentration of *pp'* DDE was found to be below detection limit. The mean residue level of *pp'* DDE in the blood serum of khat chewers in the present study was 14.2644 $\mu gL^{-1}$. The mean level of *pp'* DDE in blood serum of khat chewers was higher than the ADI(10 $\mu gL^{-1}$) set by which is risky for the human health.

The detected residual concentrations of *pp'* DDE was significantly different among khatchewers and farmers as well as among spray workers and farmers. But there was no significant difference in mean residual concentration of *pp'* DDE among khat chewers and spray workers, this may be due to high exposure to *pp'* DDE for khat chewers through regular chewing until they find the stimulating effect from khat and high exposure for spray workers was during spray on crops are directly exposed to pesticides while mixing, handling and spraying as well as through contaminated soil, air, drinking water, eating food and smoking at work places.
In this study, the mean concentration of *pp'* DDT in blood from khat chewers, spray workers and farmers were 5.8893 $\mu gL^{-1}$,5.4687 $\mu gL^{-1}$, and 0.1924 $\mu gL^{-1}$,respectively. The mean concentration of *pp'* DDT in the blood serum of farmers in the present study(0.1924 $\mu gL^{-1}$)was lower than in a study of flower farm workers in Ethiopia, where mean concentrations of *pp'* DDT in blood serum were found to be 81.5 ± 83.5 $\mu gL^{-1}$(Mekonen*et al*., 2023); 31.585 $\mu gL^{-1}$ (Chang, *et al*.,2017) in China, and, 3 $\mu gL^{-1}$ in Pakistan (Khwaja*et al*.,2013), these all study highlighting occupational exposure risks. Similarly, the result of the present study was lower than the previous study done in Ethiopia, and in Mexico which reported that *pp'* DDT was found to be 270$\mu gL^{-1}$ (Afata*et al*.,2021), and 200 $\mu gL^{-1}$ (Ruiz-Suarez*et al*.,2014) respectively. However, the finding of the present study was comparable with a study of African-American farmers in North Carolina reported a median plasma *pp'* DDT concentration of 7.7 µg/L, with a range from 0.2 to 77.4 $\mu gL^{-1}$, indicating significant exposure to *pp'* DDT and its metabolites .The cause for this difference might be due to individual exposure histories and environmental factors. The mean level of *pp'* DDT in the blood serum of farmers is influenced by several interrelated factors, including agricultural practices, dietary exposure, and socioeconomic conditions. Studies indicate that previous pesticide application history significantly affects current serum concentrations of organochlorinepesticides (Brock *et al*.,1998) and high levels of DDT were detected in dietary samples from farms with a history of pesticide use (Brock *et al*., 1998). The uptake of DDT from contaminated soils into crops can also contribute to dietary exposure, further elevating serum levels in farmers (Gaw*etal* .,2008).
Unlike the finding of the present study, which reported mean concentration of *pp'* DDT in the blood serum of spray workers (5.4687 $\mu gL^{-1}$), other studies on vegetable farming community in Ghana, on cotton growing workers in Bathinda district of Punjab, India and cotton producers in Sudan, reported mean concentration of *pp'* DDT in blood serum undetected (BDL) (Ntow,2001) , (Bedi*et al*.,2015) and (Elbashir*et al*.,2015) respectively. However, the finding of the present study was higher than investigations in Rio de Janeiro state Brazil, which revealed that *pp'* DDT in blood samples from agricultural workers ranged 1.8 $\mu gL^{-1}$-4.4 $\mu gL^{-1}$, indicating the presence of this pesticide in human body (Paumgartten*et al*.,1998). Similarly, the result of the present study was lower than the previous study done in Ghana, and on sprayers on malaria control operation in Brazilian Amazon region, which reported that *pp'* DDT was found to be 9.1 $\mu gL^{-1}$(Ntow*et al*.,2008),and 231 $\mu gL^{-1}$(Ferreira *et al*.,2011), respectively. The mean level of *pp'* DDT in the blood serum of sprayers can be influenced by several factors: including duration and frequency of exposure, use of personal protective equipments, environmental conditions and personal hygiene(Rudzi*et al*.,2022). The mean concentration of *pp'*DDT in blood samples of spray workers and farmers was significantly different, which indicates that there may be due to the exposure difference between the groups since khat chewers and spray workers were more exposed than farmers. There is no significant difference in mean concentration of *pp'* DDT in blood samples among sprayers and khat chewers. But there is significant difference in mean concentration of *pp'* DDT in blood samples among sprayers and farmers. Although DDT is banned worldwide, the presence of residues in the blood samples indicates that widespread pollution with this pesticide could be due to extensive use in this khat fields illegally. The mean concentrations of *pp'* DDT, was significantly different among spray workers (5.4687$\mu gL^{-1}$) and farmers (0.1924 $\mu gL^{-1}$).

The mean residue level of *pp'* DDT in the blood serum of khat chewers in the present study was 5.8893 $\mu gL^{-1}$.Pesticide residue can be found in human blood as a result of direct and indirect exposure (Subramaniam and Solomon, 2006). Our previous study have also detected levels of a variety of pesticide residues including *pp'* DDT, which reported the mean concentration ranged 31.021 $\mu gKg^{-1}$ to 36.21 $\mu gKg^{-1}$ in khat leaves from selected

Khat-growing farmlands (Roriso*et al*. 2024). This means that excessive levels of intake of cultivated khat can potentially lead to accumulation of the pesticide residues in the human blood and perhaps disrupt the normal biochemistry and metabolic functions of man. The level of *pp'* DDT residue in the blood serum of khat chewers can be influenced by several factors including frequency and quantity of Khat chewing, pesticide use on Khat plants, Source of Khat, metabolism and health of the chewer, environmental factors.(Roriso*et al*.,2024).

In this particular study, the mean residual level of delta BHC residues in the blood serum of khat chewers, spray workers and farmers, were reported 0.5056 $\mu gL^{-1}$, 0.4694 $\mu gL^{-1}$ and 0.0239 $\mu gL^{-1}$, respectively. The mean concentration of delta BHC in the blood serum of farmers in the present study (0.0239 $\mu gL^{-1}$) was higher than in a study from agricultural farmers spraying complex mixtures of pesticides in Guntur district of south india, where the mean concentration of delta BHC in serum samples was found to be ND (Jonnalagadda*et al*.,2012). However, our findings were much lower than the finding of the study in blood samples from Agriculturists who spray pesticide around Madurai, India by (Subramaniam and Solomon,2006), which reported BHC levels from 0.006 $\mu gL^{-1}$ to 130 $\mu gL^{-1}$. These studies highlight the presence of BHC in serum at different levels, indicating its persistence and potential health hazard. The detectable concentrations underline the need for continued monitoring and efforts to reduce the impact of this persistent organic pollutant on human health.

In this particular study detectable mean concentration of delta BHC in blood serum of sprayers in was reported (0.4694 $\mu gL^{-1}$), But in other studies on sprayers in Albeheira Governorate, Egypt, in blood of villagers involved in pesticide application at district vehari (Punjab), Pakistan, and in Bathinda district of Punjab,India, the mean levels of deltaBHC in serum samples were reported undetectable(BDL),(Nassar*et al*.,2016, Hayat *et al*.,2010 and Bedi*et al*.,2015), respectively

In our study, the mean level of deltaBHC in blood serum of khat chewers was reported (0.5056 $\mu gL^{-1}$). This finding of the present study was supported with our previous study on analysis of OCPs in chewable parts of khat on similar study area, which reported deltaBHC to be 0.021 µg/kg(Roriso*et al*., 2024). This is due to, the level of delta BHC in serum samples of khat chewers depends on several factors particularly the source of khat for chewers. The mean concentrations of delta BHC, was significantly different. According to the results of Post hoc Tukey HD test, the mean concentration of delta BHC was significantly different among khat chewers and farmers as well as among spray workers and farmers.

In present study, the mean residual level of gamma BHC levels in the blood of khat chewers, spray workers and farmers were 3.3787 $\mu gL^{-1}$,3.1373 $\mu gL^{-1}$, and 0.1092 $\mu gL^{-1}$, respecively. The mean levels of gamma BHC in blood serum of farmers in the present study ( 0.1092 $\mu gL^{-1}$) was lower than studies on farmers and non-farmers in Suru local government area, Kebbi state ,Nigeria(Osesua*et al*., 2018) which reported the mean level of gamma BHC to be 0.026 $\mu gL^{-1}$.Similarly another study conducted from agricultural farmers in Guntur district of south India by (Jonnalagadda*et al*.,2012) which reported the mean level of gamma BHC to be 18 $\mu gL^{-1}$.

The mean levels of gamma BHC in blood serum of sprayers in this study (3.1373 $\mu gL^{-1}$) was higher than the findings of studies on sprayers in Albeheira Governorate, Egypt (Nassar*et al*.,2016), in Bathindadistrict of Punjab, India (Bedi*et al*.,2015) and in blood samples of villagers involved in pesticide application at district Vehari (Punjab) Pakistan (Hayat *et al*.,2010), where mean levels of gamma BHC in blood serum were found to be below detection limit. On the contrary, the result of the present study was lower than the previous study done in farmers in Guntur district of south India,which reported that gamma BHC was found to be 18 $\mu gL^{-1}$. The mean concentrations of gamma BHC, was significantly different. According to the results of Post hoc Tukey HD test, the mean concentration of gamma BHC was significantly different among khat chewers and farmers as well as among spray workers and farmers. The cause for this difference might be due to individual exposure histories, and environmental factors.

The mean residue level of gammaBHC in the blood serum of khat chewers in the present study was 3.3787 $\mu gL^{-1}$.In this study, the mean residual level of beta BHC residue in the blood from chewers, spray workers and farmers was 2.3364 $\mu gL^{-1}$,2.1696 $\mu gL^{-1}$, and 0.0740 $\mu gL^{-1}$, respectively. Alpha BHC was not detected in all blood samples of khat chewers, spray workers and farmers.

The mean levels of beta BHC in blood serum of farmers in this study (0.0740 $\mu gL^{-1}$) was lower than the previous study in Guntur district on agricultural farmers, which reported mean concentration of beta BHC in the blood serum to be 34 $\mu gL^{-1}$. In contrast, the result of this studies was higher than the earlier studies done in Bathanda district of punjab,India(Bedi*et al*.,2015), in farmers in AlbehieraGovernorate,Egypt(Nassar*et al*.,2016) and on villagers involved in pesticide application at district vehari(punjab) Pakistan, which reportedbelow detection limit(BDL).

In this particular study, the mean residual level of beta BHC residue in the blood serum of sprayers (2.1696 $\mu gL^{-1}$) was higher than the previous study on villagers involved in pesticide application at district Vehari, Pakistan(Hayat *et al*.,2010) and on sprayers in Albeheira Governorate Egypt, which reported the mean levels of beta BHC in blood serum samples undetected. However, the finding of this study was lower than the previous studies in agricultural farmers spraying complex sample mixture of pesticides in Guntur district of south India (Jonnalagadda*et al*., 2012), andin agriculturists around Madurai, India (Subramaniam and Solomon,2006), which reported mean levels of beta BHC in the blood serum to be 34 $\mu gL^{-1}$ and 6 $\mu gL^{-1}$-13 $\mu gL^{-1}$, respectively. The mean concentrations of beta BHC, was significantly different among khat chewers, spray workers and farmers. According to the results of Post hoc Tukey HD test, the mean concentration of delta BHC was significantly different among khat chewers and farmers as well as among spray workers and farmers. These studies highlight the presence of BHC in serum at different levels, indicating its persistence and potential health hazard. The significance difference in mean concentration of delta BHC, gamma BHC, and beta BHC in blood samples of khat chewers and spray workers with farmers indicates the exposure difference between the groups since khat chewers and spray workers were more exposed than farmers. The detectable concentrations underline the need for continued monitoring and efforts to reduce the impact of this persistent organic pollutant on human health.

The mean residue level of beta BHC in the blood serum of khat chewers in the present study was 2.3364$\mu gL^{-1}$. In our study Alpha BHC residue concentration was not detected for serum samples of khat chewers, sprayers and farmers. In contrast other study on agricultural farmers spraying complex sample mixture of pesticides in Guntur district of south India, reported high mean concentration of alpha BHC in serum samples 52 $\mu gL^{-1}$(Subramaniam and Solomon, 2006). However, in other study on serum samples of agricultural farmers spraying complex mixtures of pesticides in Guntur district of south India (Jonnalagadda*etal*.,2012) reported much higher mean concentration of alpha BHC levels of 52 $\mu gL^{-1}$.

The mean concentrations of delta BHC, alpha BHC, and beta BHC, were significantly different among khat chewers and farmers as well as among spray workers and farmers. The difference in mean concentration of delta BHC,gamma BHC, and beta BHC in blood samples of khat chewers and spray workers with farmers indicates the exposure difference between the groups since khat chewers and spray workers were more exposed than farmers.

*Conclusion:* One metabolite, *pp'*DDE, showed residue levels above the ADI in the blood serum of khat chewers and spray workers out of the five DDT and BHC metabolites that were found in our investigation. The highest concentration of *pp'*DDE was found among the identified metabolites. In chewers blood samples, the concentration was ranked as follows: *p,p'*-DDE (14.26$\mu gL^{-1}$) >*p,p'*-DDT(5.88 $\mu gL^{-1}$) > Gamma BHC (3.378$\mu gL^{-1}$) >Beta BHC (2.336 $\mu gL^{-1}$) >Delta BHC (0.506 $\mu gL^{-1}$).Whereas ,in sprayer workers the concentration was ranked as follow:*p,p'*-DDE (13.245$\mu gL^{-1}$) >*p,p'*-DDT(5.468 $\mu gL^{-1}$) > Gamma BHC (3.13$\mu gL^{-1}$) >Beta BHC (2.169 $\mu gL^{-1}$) >Delta BHC (0.469 $\mu gL^{-1}$) and in farmers blood sample the concentration of pesticides was as follow;*p,p'*-DDE (0.193$\mu gL^{-1}$) >*p,p'*-DDT(0.192 $\mu gL^{-1}$) > Gamma BHC (0.11$\mu gL^{-1}$) >Beta BHC (0.07 $\mu gL^{-1}$) >Delta BHC (0.02$\mu gL^{-1}$).Therefore, vigilant monitoring pesticide levels in khat is imperative for addressing environmental and public health concerns. It is essential for policymakers to take appropriate measures at this critical time to safeguard public health against the threats posed by pesticide pollution in Khat cultivation.

*Declaration of Conflict of Interest:*The authors declare no conflict of interest.

*Data Availability Statement:*Data are available upon request from the first author or corresponding author or any of the other authors.

## REFERENCES

Afata, TN; Mekonen, S; Tucho, GT(2021).Evaluating the level of pesticides in the blood of small-scale Farmers and its associated risk factors in western Ethiopia. *Environ.Health. Insights*.*15*: 1-13. DOI:10.1177/11786302211043660

Agarwal, HC; Pillai, MKK;Yadav, DV; Menon, KB; Gupta, RK(1976). Residues of DDT and its metabolites in human blood samples in Delhi, India.*Bull.World.Health. Organ*. 54(3):349-351.

Antonio, TA; Edith, RPN; Rogelio, FR; Fernando, DB; Catalina, OA; Nelinho, PMI(2013). Assessment of persistent organic pollutants levels in blood samples from Quintana Roo, Mexico. *Int.J.Hyg. Environ Health*. *216*(3):284-289.

Bedi, JS; Gill, JPS;Kaur, P; Sharma, A;Aulakh, RS(2015). Evaluation of pesticide residues in human blood samples from Punjab (India). *Vet.World*. *8*(1):66.doi: 10.14202/vetworld.2015.66-71

Ben Hassine, S;Hammami, B;BenAmeur, W;ElMegdiche, Y;Barhoumi, B; El Abidi, R; Driss, MR (2014). Concentrations of organochlorine pesticides and polychlorinated biphenyls in human serum and their relation with age, gender, and BMI for the general population of Bizerte, Tunisia. *Environ.Sci.Pollut.Res*.21(10):6303-6313.DOI: 10.1007/s11356-013-1480-9

Brock, JW;Melnyk, LJ; Caudill, SP; Needham, LL; Bond, AE(1998). Serum levels of several organochlorine pesticides in farmers correspond with dietary exposure and local use history. *Toxicol.Ind.Health*. *14*(1-2):275-289.DOI: 10.1177/074823379801400117

Chang, C; Chen, M;Gao, J;Luo, J; Wu, K; Dong, T; Zhou, K; He, X; Hu, W;Wu, W; Lu, C(2017). Current pesticide profiles in blood serum of adults in Jiangsu Province of China and a comparison with other countries. *Environ. int*. 102:213-222.DOI: 10.1016/j.envint.2017.03.004

CSA (2023).Central statistical agency of Ethiopia population projection.

Damalas, CA; Koutroubas, SD(2016). Farmers' exposure to pesticides: toxicity types and ways of prevention. *Toxics*. 4(1): 1.doi: 10.3390/toxics4010001

Damalas, CA(2009). Understanding benefits and risks of pesticide use. *Sci. Res. Essays*. 4(10):945-949.http://www.academicjournals.org/sre

Dinede, G; Bihon, W; Gazu, L;FoukmeniokMbokou, S; Girma, S; Srinivasan, R;Roothaert, R; Grace, D;Gashaw, H. ; Knight-Jones, TJ(2023). Assessment of pesticide residues in vegetables produced in central and eastern Ethiopia. *Front. Sustain.Food. Syst*. *7*:1143753.https://doi.org/10.3389/fsufs.2023.1143753

Elbashir, AB;Abdelbagi, AO;Hammad, AM;Elzorgani, GA; Laing, MD(2015).Levels of organochlorine pesticides in the blood of people living in areas of intensive pesticide use in Sudan. *Environ.Monit. Assess.* 187 (68 ): 1-10.https://doi.org/10.1007/s10661-015-4269-0

Ferreira, CP; De-Oliveira, ACA; Paumgartten, FJ(2011). Serum concentrations of DDT and DDE among malaria control workers in the Amazon region. *J. Occup.Health*. *53*(2):.115-122.DOI: 10.1539/joh.o10026

Gaw, SK; Kim, ND;Northcott, GL; Wilkins, AL; Robinson, G (2008). Uptake of ΣDDT, arsenic, cadmium, copper, and lead by lettuce and radish grown in contaminated horticultural soils. *J .Agric.Food.Chem*.56(15):6584-6593.DOI: 10.1021/jf073327t

Gure, A; Megersa, N; Retta, N(2014). Ion-pair assisted liquid–liquid extraction for selective separation and analysis of multiclass pesticide residues in environmental waters. *Anal.Methods*. *6*(13): 4633-4642. DOI: 10.1039/c4ay00285g

Hayat, K;Ashfaq, M; Ashfaq, U; Saleem, MA(2010). Determination of pesticide residues in blood samples of villagers involved in pesticide application at District Vehari (Punjab), Pakistan. *Afr. J. Environ. Sci .Tech*. 4(10):666-684.

Jaraczewska, K; Lulek, J; Covaci, A; Voorspoels, S; Kaluba-Skotarczak, A; Drews, K; Schepens, P(2006).Distribution of polychlorinated biphenyls, organochlorine pesticides and polybrominateddiphenyl ethers in human umbilical cord serum, maternal serum and milk from Wielkopolska region, Poland. *Sci.Total. Environ*. 372(1):20-31.DOI: 10.1016/j.scitotenv.2006.03.030

Jonnalagadda, PR; Jahan, P;Venkatasubramanian, S; Khan, IA; Prasad, AYE; Reddy, KA; Rao, MV; Venkaiah, K; Hasan, Q(2012).Genotoxicity in agricultural farmers from Guntur district of South India—a case study. *Hum.Exp.Toxicol*. 31(7):741-747.DOI: 10.1177/0960327111408151

Khan, M; Damalas, CA( 2015).Occupational exposure to pesticides and resultant health problems among cotton farmers of Punjab, Pakistan. *Int. J. Environ.Health. Res*. 25(5):508-521.DOI: 10.1080/09603123.2014.980781

Khan, M; Mahmood, HZ; Damalas, CA (2015). Pesticide use and risk perceptions among farmers in the cotton belt of Punjab, Pakistan. *Crop.Protection*. 67:184-190.https://doi.org/10.1016/j.cropro.2014.10.013

Khwaja, S; Mushtaq, R; Mushtaq, R; Yousuf, M;Attaullah, M; Tabbassum, F; Faiz, R(2013).Monitoring of biochemical effects of organochlorine pesticides on human health. *Health*.2013. **DOI:** 10.4236/health.2013.58182

Mekonen, S; Belete, B; Melak, F; Ambelu, A(2023). Determination of pesticide residues in the serum of flower farm workers: A growing occupational hazards in low income countries. *Toxicology.Reports*. *10*: 293-300.https://doi.org/10.1016/j.toxrep.2023.02.012

Muñoz-de-Toro, M;Beldoménico, HR;García, SR; Stoker, C; De Jesús, JJ;Beldoménico, PM; Ramos, JG; Luque, EH(2006).Organochlorine levels in adipose tissue of women from a littoral region of Argentina. *Environ. Res*. 102(1):107-112.https://doi.org/10.1016/j.envres.2005.12.017

Nassar, AM;Salim, YM; Malhat, FM (2016).Assessment of pesticide residues in human blood and effects of occupational exposure on hematological and hormonal qualities.*Pak. J .Biol. Sci*.19(3):95-105.DOI: 10.3923/pjbs.2016.95.105

Ntow, WJ( 2001). Organochlorine pesticides in water, sediment, crops, and human fluids in a farming community in Ghana. *Arch. Environ.Contam.Toxicol*.40:557–563(2001). https://doi.org/10.1007/s002440010210

Ntow, WJ; Tagoe, LM; Drechsel, P; Kelderman, P; Gijzen, HJ; Nyarko, E(2008). Accumulation of persistent organochlorine contaminants in milk and serum of farmers from Ghana. *Environ. Res*. 106(1):17-26.DOI: 10.1016/j.envres.2007.05.020

Paumgartten, FJR; Delgado, IF; Oliveira, ES; Alleluia, IB;Barretto, HH;Kussumi, TA (1998). Levels of organochlorine pesticides in the blood serum of agricultural workers from Rio de Janeiro State, Brazil. *Cad.Saude.Publica*. 14(suppl3):S33-S39.DOI: 10.1590/s0102-311x1998000700005

Roriso, SS;Sota, SS;Yimer, G.T; Berego, YS.(2024).Organochlorine pesticide residues level in khat and possible human health risks to chewers in Wondogenet district, Sidama regional state, Ethiopia. *AGBIR*.2024;40(3):1078-1085.DOI:10.35248/0970-1907.24.40.1078-1085

udzi, SK; Ho, YB;Tan, ESS;Jalaludin, J; Ismail, P(2022). Exposure to airborne pesticides and its residue in blood serum of paddy farmers in Malaysia.*Int. J. Environ. Res. Public.Health*. 19(11):6806.DOI: 10.3390/ijerph19116806

Ruiz-Suárez, LE; Castro-Chan, RA;Rivero-Pérez, NE; Trejo-Acevedo, A; Guillén-Navarro, GK;Geissen, V; Bello-Mendoza, R(2014).Levels of organochlorine pesticides in blood plasma from residents of malaria-endemic communities in Chiapas, Mexico. *Int. J .Environ. Res. Public.Health*. 11(10):10444-10460.doi: 10.3390/ijerph111010444

Soomro, AM;Seehar, GM;Bhanger, MI; Channa, NA( 2008). Pesticides in the blood samples of spray-workers at agriculture environment: The toxicological evaluation. *Pakistan.J.Analyt.Envirol.Chemi*. 9(1) :6.

Subramaniam, K; Solomon, J(2006). Organochlorine pesticides BHC and DDE in human blood in and around Madurai, India. *Indian.J.Clin.Bio.chem*. 21:169-172.doi: 10.1007/BF02912936

Suru, I(2018). Evaluation of pesticide residues in blood samples of farmers and non-farmers in selected agricultural communities in suru local government area, kebbi state, nigeria. *Afri.J. Environ.Nat.Science. Res*. 1(2):20-30.

Tasew, W; Abayneh, H(2017). Practices and challeges of domestic biogas technology use: The Case of, Wondogenet District and Hawassa City Administration, Sidama Zone, Southern Ethiopia, Ethiopia. *J.Resourc.Dev.Manag*.*37*:7-17.

Yassi A; Kjellstrom T; Kok TK; Gudotli TL.(2001).Basic Environmental Health, World Health Organization. London, U.K.: Oxford University Press.