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Dichlorodiphenyltrichloroethane (DDT) and its metabolites in house dust, soil and selected food crops from indoor residual sprayed areas of Apac and Oyam Districts, Uganda

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Dichlorodiphenyltrichloroethane (DDT) levels and its metabolites are reported in 75 soil and 75 vegetable samples from the vicinity of homesteads sprayed with DDT during indoor residual spraying (IRS) pilot exercise, carried out in 2008 in Apac and Oyam districts, Uganda as a measure to control malaria. The samples were randomly and conveniently collected in 2020 from selected villages of the districts, extracted using solid dispersion and multi-residue methods for soil and vegetable samples, respectively and analyzed using GC-ECD and GC-MS. DDT residues were detected in all samples collected. The Σ DDT ($\mu\text{g kg}^{-1}$) in all indoor house dust, outdoor soil samples from villages of Apac and Oyam districts and control areas were 69.9 ± 20.9 and 8.9 ± 4.7 , 113.4 ± 22.6 and 15.4 ± 4.3 , and 1.13 ± 0.27 and 1.8 ± 0.9 , respectively. Σ DDT were significantly higher ($p < 0.05$) in the study areas than the control areas. In vegetables, Σ DDT ($\mu\text{g kg}^{-1}$) ranged between 1.7 ± 0.3 and 8.3 ± 4.0 and 2.3 ± 0.4 and 11.4 ± 7.5 in the study and control areas, respectively. *Abelmoschus esculentus* and *Gynandropsis gynandra* had the highest levels. The results suggest that IRS had an effect on DDT levels in the environment. However, Σ DDT in vegetables were significantly below ($P < 0.05$) the EU EMRL, thus, the results raise no concern regarding the potential health effects of DDT to the residents. Nevertheless, due to bioaccumulation effects of DDT, to control malaria, measures like Ecohealth rather than IRS of DDT should be embraced.

Key words: Dichlorodiphenyltrichloroethane metabolites, indoor residual spraying.

INTRODUCTION

Dichlorodiphenyltrichloroethane (DDT) is the oldest known synthetic organochlorine insecticide which has been

intensively used in agriculture, either alone or as a mixture with other insecticides in public health arena to

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control malaria, typhus and other insect-borne diseases (Van den Berg et al., 2017). DDT like other organochlorine pesticides (OCPs) is persistent in the environment for years and maintains their toxic properties (Agbeve et al., 2014; Nyaundi et al., 2019). Due to being bio-accumulative, lipophilic with low biodegradation in nature, DDT can cause adverse effects on humans such as malfunctions in immune system, cancers, birth defects, reproductive system deformations, endocrine disruptions (Fosu-Mensah et al., 2016) among other effects (Muhugija et al., 2017). DDT is known to be persistent organic pollutant (POP), and its use is restricted to public health purposes (UNEP, 2020) with permission and strict control from the Stockholm convention secretariat for vector control. This means that although banned in many countries world-wide, DDT continues to be used especially in indoor residual spraying (IRS) in a number of countries including Uganda (Van den Berg et al., 2017).

The use of DDT in Uganda dates back to the 1950s when it was used as the main pesticide against pests and vectors up to mid-1980's (Nnamuyomba et al., 2014); although its use world-wide was stopped in early 1960s owing to international concern of its bioaccumulation effects and the subsequent resistance of vectors against the chemical (Wasswa et al., 2011). However, on behalf of the Ugandan government, the Ministry of Health by the approval of Uganda's National Environment Management Authority (NEMA) (USAID, 2007) through the National Malaria Control Program (NMCP) (Acquaye and Chandonait, 2014), supported by president's malaria initiative decided to re-introduce DDT use through IRS program in 2008 as a means to control malaria. In Uganda, malaria is highly endemic and it puts to approximately 90% of the population at risk and causes half of the inpatient pediatric deaths. To use DDT, the ministry argued that the insecticide is cheap, effective and its insecticidal properties are credited for saving over 100 million lives of people since 1944 to date globally mainly from malaria. Apac and Oyam districts which were among the most malaria-prone districts in the country were selected and sprayed by DDT in April 2008 to pilot the IRS programme (RTI, 2008).

The use of DDT in malaria control raises serious health and environmental concerns because the chemical and its metabolites can persist for years within the sprayed domestic environment (Van Dyk et al., 2010). Consequently, residents can be exposed to the residues through a number of pathways including inhaling indoor dust, outdoor soil and food intake (Tao et al., 2009) for instance, leafy vegetables around the sprayed homesteads might be contaminated with DDT residues by both root uptake from contaminated soils and spray-drift from application during IRS. Therefore, consumption of such contaminated vegetables makes the people susceptible to the numerous deleterious effects of the insecticide (Ssemugabo et al., 2022) because ingestion

of food has been recognized as the major pathway for human exposure to DDT (Tao et al., 2009).

After IRS programme in 2008 in Apac and Oyam districts, no comprehensive and justifiable study has been performed to determine the levels of DDT and its metabolites in the environment and agricultural produce in this area. Therefore, this study was aimed at assessing the levels of DDT residues in soils, leafy vegetables (food crops) around the sprayed homesteads and indoor soil dust from the sprayed houses of selected villages of Apac and Oyam districts of Northern Uganda.

MATERIALS AND METHODS

Study area

The areas of study were the two districts (Apac (01°59'N 32°32'E) and Oyam (02°14'N 32°23'E)) in the Northern region of Uganda. The two districts are bordering each other, with Oyam being located to the Northwest of Apac (Figure 1). Inset is the location of the areas selected in the study in Uganda.

In Apac district, two villages: (Aboko parish) and Olamia A (Ongoceng parish); and Arocha swamp were selected for sampling, while in Oyam district, two villages: Abang and Arukolong (Pukica parish); and Tochi swamp were selected for sampling. The villages were randomly selected on the basis of ease to locate the sprayed homesteads which could be a representation of the 92.4 and 93.1% sprayed houses in Apac and Oyam districts, respectively (RTI, 2008), while the swamps were the ones at the lower ends of the villages sampled where runoffs are deposited over time.

Karuma Wildlife Reserve (KWR), which is part of Murchison Falls protected area (2° 14' 3"N, 32° 14' 47"E) was used as the control site. KWR is located in Kiryandongo district in Western Uganda. The KWR was chosen as the control site for the study of soils because the area was anticipated to be free from direct exposure of insecticides such as DDT since it was gazetted in 1964 (Behangana et al., 1997). Gwara village in Kiryandongo district was another control area for the study of indoor house dust, outdoor soils and leafy vegetables because no IRS in 2008 took place in that area. The homesteads were randomly selected for house indoor dust, outdoor soils and leafy vegetables sampling.

Sample collection

Soils (50 samples), indoor dust (25 samples) and the selected leafy vegetables (75 samples) around the selected sprayed homesteads were randomly and conveniently collected from selected villages in Apac and Oyam districts and from the control areas in June, 2020. Indoor dust samples were obtained by scratching the indoor walls using a chisel. The dust obtained was swept using a broom from the very house, collected and transferred into an aluminium foil then into well labelled sampling bags, and then placed into a clean dry bucket. The outdoor soil samples around the sprayed homesteads were obtained by judgemental sampling technique from the sampling points located in three different directions around the sprayed house at 2 to 5 m and/or 10 m away from the sprayed and control house at a depth of up to 10 cm using Auger handle, mixed thoroughly and a representative amount transferred into aluminium foil then into a well labelled sampling bag, and then put into a clean dry bucket. Soil samples from Arocha and Tochi swamps and KWR were obtained at approximately over 500 m away from the main roads in these areas, 20 m apart in different directions at a depth of up to 10cm

Table 1. Mean percentage recoveries for DDT pesticide residue isomers from soil and vegetables.

Pesticide residue	Soil samples (n=8)		Vegetable samples (n=5)	
	2.0 µg kg ⁻¹	4.0 µg kg ⁻¹	2.0 µg kg ⁻¹	4.0 µg kg ⁻¹
<i>p,p'</i> -DDT	79.33±6.31	82.94±5.05	77.61±6.75	76.86±5.99
<i>o,p'</i> -DDT	80.60±6.90	78.19±5.86	85.57±2.14	79.86±4.08
<i>p,p'</i> -DDE	79.13±5.78	81.27±5.40	76.83±4.10	79.49±2.08
<i>o,p'</i> -DDE	81.37±5.35	77.75±5.75	80.86±6.07	78.45±4.89
<i>p,p'</i> -DDD	78.75±6.41	80.08±3.89	76.77±5.04	80.02±6.46
<i>o,p'</i> -DDD	81.56±5.48	79.25±4.51	76.83±7.30	79.42±4.05

The mean values are presented with ± standard deviation.

Source: Primary data

through a plug of glass wool into an evaporation flask. The extract was then subjected to a clean-up process using the florisil technique (Kegley and Laura, 1998). Briefly, a glass column (15 cm × 4 mm i.d) was plugged with glass wool rinsed with cyclohexane, packed with 10 g florisil followed by 4 g anhydrous sodium sulphate, then conditioned with 2 ml acetone/hexane (1:9 v/v), followed by hexane (5 ml). The soil extract was then added to the column, eluted with 10 ml acetone/hexane (1:9 v/v), followed by 10 ml hexane and the eluate was collected in a 100 ml evaporation flask. The eluate was then concentrated on a rotary evaporator at 40°C followed by a gentle stream of nitrogen gas to dryness and then reconstituted in cyclohexane (2 ml) for gas chromatograph (GC) analysis.

Extraction and cleanup of vegetable samples

A multi-residue method DFG S19 (Specht et al., 1995) for the determination of pesticides residues in vegetables and fruits was followed during the extraction of vegetables for DDT residues. 25 g of the chopped homogenized vegetable was mixed with acetone (50 ml), 50 ml ethyl acetate/cyclohexane (1:1 v/v) followed by 50 g of sodium sulphate and then blended for 5 min. The extract obtained filtered over sodium sulphate (10 g), 20 ml of saturated aqueous sodium chloride solution was added, mixed vigorously, hand shaken for 1 min, and allowed to settle. The upper organic layer was Pasteur pipetted into a flask containing sodium sulphate (15 g), the mixture was vigorously hand shaken for 30 s and then filtered over sodium sulphate through Whatman GF/C Glass Micro-fibre filters and rinsed with cyclohexane/ethyl acetate (1:1 v/v) solvent mixture. All the filtrates (supernatant) were transferred into a 100 ml evaporation flask, concentrated to about 4 ml on a rotary evaporator at 40°C and then divided into two portions (each of 2 ml), one for florisil clean up and another for acid treatment.

The clean-up of extracts from vegetable samples was by florisil (Åkerblom, 1995). Briefly, 10 g florisil packed in the column was conditioned using 5 ml acetone/hexane (1:9 v/v) followed by 5 ml hexane. The first 2 ml portion of the vegetable extract (obtained as described earlier) was dissolved in hexane (2 ml), eluted with 10 ml acetone/hexane (1:9 v/v). The eluate was then concentrated on a rotary evaporator at 40°C followed by a gentle stream of nitrogen gas to dryness and then reconstituted in cyclohexane (2 ml) for gas chromatograph (GC) analysis.

For acid treatment, five soil and five vegetable extracts were selected and treated with sulphuric acid as described by Åkerblom (1995) to access the stability of DDT and its metabolites in the samples analysed. DDT residues were detected in the acid treated portions.

GC chemical analysis of sample extracts

The GC (Varian 3800-CP) equipped with ECD and SPB-1 0.53 mm i.d. × 60 m non-polar capillary column was used for analysis of sample extracts and later interchanged with Wcot fused silica (30 m × 0.25 mm i.d) coated CP-Sil 19 CB semi-polar capillary column, for confirmation. The GC temperature program was as follows: initial temperature 90°C (held for 1 min), raised to 180°C at a rate of 30°C min⁻¹, and then raised to 260°C at a rate of 4°C min⁻¹ (held constant for 10 min), for both non-polar and semi-polar columns. The injection port and ECD detector temperatures were 230 and 300°C, respectively. 1 µl of the sample was injected, hydrogen gas and nitrogen gas at 1.2 and 30 ml min⁻¹ flow rates was used as the carrier and makeup gases, respectively. A Turbochrom (Perkin-Elmer Corporation, 1989-1995, USA) 4.0 chromatography work station was used for chromatographic data processing. The GC-ECD calibration linear plots were of peak area against the concentration of the standards. Identification of DDT residues was based on retention time as well as external standard method. Selected samples were analyzed by GC-MS for structural confirmation of the DDT residues.

Quality assurance and quality control

Recoveries, precision and accuracy were determined by addition of standard method at two spiking levels of 2 and 4 µg/ml of a mixture of standards (*p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE, *o,p'*-DDE, *p,p'*-DDD and *o,p'*-DDD) into the soil (from KWR) and vegetable samples (*S. melongena*) obtained from control site, kept overnight, extracted as of the study samples and analysed in triplets (ICH, 1995; EPA, Method 8000C, 2003). The mean percentage recoveries for soil and vegetable samples with standard deviations are shown in Table 1.

From the percentage recoveries, the data was not corrected for use because the average recoveries obtained were within the acceptable limits of 70 to 120% (Åkerblom, 1995) or 70 to 130% (USEPA, 2003). The close values of mean percent recoveries were also indices of good accuracy (IUPAC, 1994) while the low standard deviation values signified good precision (Ripp, 1996) of the analytical process used in the study.

To determine the lower limit of detection, LLD (sensitivity of GC-ECD), three soil samples from the control site were fortified at a lower concentration of 0.02 µg/ml of the mixture of standard DDT insecticide, and analysed in triplicate (ICH, 1995). LLD was estimated as 3.29 times standard deviation (Eaton et al., 1995) because for chromatographic techniques, the response of an analyte is known to gradually change over time (Table 2). The DDT

Table 2. Computation of lower levels of detection (LLD = 3.29SD), n = 3.

Component DDT residue	Mean±SD ($\mu\text{g kg}^{-1}$)	LLD ($\mu\text{g kg}^{-1}$)
<i>p,p'</i> -DDT	0.0053±0.0017	0.0056
<i>o,p'</i> -DDT	0.0011±0.0009	0.0029
<i>p,p'</i> -DDE	0.0037±0.0022	0.0073
<i>o,p'</i> -DDE	0.0025±0.0016	0.0053
<i>p,p'</i> -DDD	0.0021±0.0008	0.0028
<i>o,p'</i> -DDD	0.0028±0.0026	0.0085

SD = Standard deviation

Source: Primary data

residues below the LLD were reported as not detected (nd).

Data analysis

The data obtained for the levels of all the six isomers of DDT, the mean total DDT (Σ DDT) as well as the significant values between the DDT levels from the control and the exposed areas were calculated using Excel for mean and standard deviation or standard error values and for a one-tailed *t*-test. Standard deviation was used in the quality control data analysis while standard error was used for sample extracts data analysis. The level of significance was set at (5%). The DDT/DDE ratio was calculated to inspect the presence of DDT parent molecule as compared to its metabolites (Gonzalez et al., 2003).

RESULTS AND DISCUSSION

Levels of DDT residues in indoor soil/dust samples

The levels of DDT and its metabolites detected in the indoor dust samples from the sprayed houses selected from villages of Apac and Oyam districts and the control Gwara village, Kiryandongo district are summarized in Table 3.

All the indoor dust samples collected from selected villages of Apac and Oyam districts contained detectable residues of DDT and its metabolites, while DDE and DDD residue isomers were above detection limits in 50% of the total indoor dust samples from the control village of Gwara. The levels of Σ DDT detected in indoor soil dust samples collected from Apac and Oyam district were 69.9 and 113.4 $\mu\text{g kg}^{-1}$ dry weight (dw), respectively, while 1.13 $\mu\text{g kg}^{-1}$ dw from Gwara village. The levels of Σ DDT detected in indoor dust samples from selected villages of Apac and Oyam districts were significantly higher ($P < 0.05$) than those from Gwara village. The significant difference in the Σ DDT levels confirms that high DDT residues levels such as *p,p'*-DDT are as a result of the recent application of DDT insecticide in the houses during IRS in the villages of Apac and Oyam districts in 2008. The ratios of the mean levels of *p,p'*-DDT to *p,p'*-DDE (4.0) for villages of Apac, and that of *p,p'*-DDT to *p,p'*-DDD (1.8) for villages of Oyam districts also justifies the

recent spraying of DDT, since such ratios greater than one (1) suggest recent application of DDT in the environment (Gonzalez et al., 2003). The results therefore, raise concerns regarding human exposure to the contaminated indoor dust, particularly the exposure to children during play activities and other behavioral characteristics such as ingestion of non-food items and regular hand-to-mouth contact (Roberts and Ott, 2006; Hwang et al., 2008; Van Dyk et al., 2010). The results also raise concern regarding the possible presence of the insecticide residues in indoor air (Manaca et al., 2012) which could lead to DDT exposure through inhalation by the occupants.

Levels of DDT residues in outdoor soil samples

Levels of DDT residues and its metabolites detected in soil samples collected around the sprayed homesteads from selected villages of Apac, Oyam districts and from the control areas are summarized in Table 4.

The results show that soils around the sprayed homesteads are contaminated with DDT residues. The Σ DDT residue levels detected in soil samples collected around the sprayed houses from villages of Apac and Oyam districts were 8.9 and 15.4 $\mu\text{g kg}^{-1}$ fresh weight (fw), respectively. In the control sites, the Σ DDT levels detected in soil samples from Arocha swamp, Tochi swamp, KWR and around houses in Gwara village were 4.9, 1.14, 1.5 and 1.8 $\mu\text{g kg}^{-1}$ fw, respectively. All DDT residue isomers were detected in soil samples collected around the sprayed homesteads in villages of Apac and Oyam districts, respectively; while only DDE and DDD residue isomers were detected in soil samples obtained from the control areas. High levels of DDT and its metabolites were detected in the soil samples from selected villages of Apac and Oyam districts around the sprayed homesteads ($P < 0.05$) as compared to soil samples from the control areas (Gwara village and KWR). The higher residue levels of *p,p'*-DDT in soil samples from villages of both Apac and Oyam districts, confirms a recent application or an occasional exposure of DDT insecticide around the homesteads. Similarly, the

Table 3. Summary of levels of DDT residues and DDT metabolites ($\mu\text{g kg}^{-1}$) in house indoor dust from sprayed houses of villages of Apac and Oyam districts and Gwara village.

Variable	Σ DDT	<i>p,p'</i> -DDT	<i>o,p'</i> -DDT	<i>p,p'</i> -DDE	<i>o,p'</i> -DDE	<i>p,p'</i> -DDD	<i>o,p'</i> -DDD	$\frac{p,p'-DDT}{p,p'-DDE}$	$\frac{p,p'-DDT}{p,p'-DDD}$
House indoor dust: Apac (n = 10)									
Mean \pm S.E	69.9 2 \pm 20.91	43.03 \pm 14.65	30.15 \pm 9.89	10.75*	99.87 \pm 45.75	121.29 \pm 75.18	4.25 \pm 3.26		
Min-Max	0.91 - 429.16	4.99 - 112.50	8.09 - 55.27	10.75 - 10.75	0.91 - 325.35	1.08 - 429.16	0.98 - 7.51	4.00	0.35
F (%)	100	70	50	10	90	60	20		
House indoor dust: Oyam (n = 10)									
Mean \pm S.E	113.45 \pm 22.67	29.2 2 \pm 9.24	nd	107.46 \pm 70.95	139.15 \pm 44.52	15.97 \pm 10.48	180.7 4 \pm 37.67		
Min-Max	0.76 - 599.80	1.11 - 75.90	nd	1.78 - 599.80	2.66 - 419.32	4.97 - 36.92	0.76 - 337.48	0.27	1.83
DF (%)	100	70	0	80	100	30	100		
Gwara: House indoor dust (n = 5)									
Mean \pm S.E	1.13 \pm 0.27	nd	nd	0.87 \pm 0.08	0.34 \pm 0.12	1.80 \pm 0.40	2.16 \pm 0.51		
Min-Max	0.08 - 3.17	nd	nd	0.80 - 0.94	0.08 - 0.69	1.39 - 2.20	1.57 - 3.17	0.00	0.00
DF (%)	100	0	0	40	100	40	60		

Σ DDT = Mean of (*p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE, *o,p'*-DDE, *p,p'*-DDD and *o,p'*-DDD) in all house indoor dust samples; S.E = standard error; Min-Max = minimum to maximum residue value detected (range); DF (%) = percentage detection frequency; nd = not detected, * = level from one sample.

Source: Primary data

difference in the levels of DDT and its metabolites is attributed to the application of DDT in 2008 during IRS processes in the two districts because the DDT residues levels detected in this study were generally higher than those detected in soil around homesteads during the baseline study ($14 \mu\text{g kg}^{-1}$) (RTI, 2008). The results therefore show an increase in the concentration of DDT and its metabolites in the soils as a result of IRS. The Σ DDT mean levels detected in the soil samples collected around the sprayed homesteads in this study, were however lower than those reported by Van Dyk et al. (2010) [$25 \mu\text{g kg}^{-1}$ outdoor soil] and Manaca (2012) [up to 130 ng g^{-1} wall dwellings] conducted after IRS in Vhembe district, South Africa and Machica district, Mozambique, respectively. Van Dyk et al. (2010) study was

carried out after two months of IRS while Manaca (2012) study was conducted after one year when DDT was applied through IRS. The differences in the Van Dyk's and Manaca's findings from those of the current study could be attributed to the twelve years interval taken after the 2008 IRS in Apac and Oyam districts.

Further still, the high ratios of the mean levels of *p,p'*-DDT to *p,p'*-DDE and that of *p,p'*-DDT to *p,p'*-DDD of above 1.0 (Table 4) also confirm a recent application of DDT around homesteads in these districts. Normally, a ratio of DDT/DDE or DDT/DDD greater than 1 reflects a recent application of DDT in the environment (Gonzalez et al., 2003), which could be probably due to the spray-drift from DDT application (Van Dyk et al., 2010) during the IRS in 2008.

The DDT metabolites (*p,p'*-DDE, *o,p'*-DDE, *p,p'*-DDD and *o,p'*-DDD) detected in the soil samples collected around the sprayed houses could be due to the on-going transformation of DDT applied during IRS in 2008 to these stable metabolites. The conversion of DDT to its metabolites could be due to aerobic or anaerobic degradation (Aislabie et al., 1997; Muhugija et al., 2017), dehydrochlorination to DDE and reductive dechlorination to DDD by micro-organisms in the environment (Fox et al., 1998; Mwangi et al., 2010). Levels of DDE and DDD isomers ($23.4 \mu\text{g kg}^{-1}$ for *o,p'*-DDE from villages in Oyam and $66.2 \mu\text{g kg}^{-1}$ for *o,p'*-DDD from villages in Apac) were detected because they are chemically stable metabolites of the insecticide (Manaca et al., 2012) and thus are persistent in the environment.

Table 4. Levels of DDT Residues and DDT metabolites ($\mu\text{g kg}^{-1}$) in soils around sprayed homesteads from selected villages of Apac and Oyam districts and Gwara village.

Variable	Σ DDT	<i>p,p'</i> -DDT	<i>o,p'</i> -DDT	<i>p,p'</i> -DDE	<i>o,p'</i> -DDE	<i>p,p'</i> -DDD	<i>o,p'</i> -DDD	$\frac{p,p' - \text{DDT}}{p,p' - \text{DDE}}$	$\frac{p,p' - \text{DDT}}{p,p' - \text{DDD}}$
Apac-Outdoor soils (n=10)									
Mean \pm S.E	8.97 \pm 4.46	8.68*	5.84 \pm 4.75	0.20*	4.18 \pm 2.72	5.37 \pm 4.57	66.26*		
Min-Max	0.20 - 66.26	8.68-8.68	1.09 - 10.59	0.20 - 0.20	1.24 - 15.03	0.31 - 23.61	66.26 - 66.26	43.40	1.62
DF (%)	60	10	20	10	50	50	10		
Apac-Arocha swamp soils (n=5)									
Mean \pm S.E	4.98 \pm 1.98	nd	nd	5.49 \pm 2.47	2.97*	nd	nd		
Min-Max	1.26 - 11.82	nd	nd	1.26 - 11.82	2.97 - 2.97	nd	nd	0.00	0.00
DF (%)	80	0	0	80	20	0	0		
Oyam-Outdoor soils (n=10)									
Mean \pm S.E	15.49 \pm 4.37	17.51 \pm 13.25	4.02 \pm 0.86	1.54*	23.45 \pm 9.61	12.49 \pm 5.31	nd		
Min-Max	0.17 - 52.88	4.26 - 30.76	3.16 - 4.89	1.54 - 1.54	0.17 - 52.88	0.54 - 30.85	nd	11.37	1.40
DF (%)	70	20	20	10	60	50	0		
Oyam-Tochi swamp soils (n=5)									
Mean \pm S.E	1.14 \pm 0.94	nd	nd	1.63 \pm 1.38	0.15*	nd	nd		
Min-Max	0.15 - 3.01	nd	nd	0.25 - 3.01	0.15 - 0.15	nd	nd	0.00	0.00
DF (%)	40	0	0	40	20	0	0		
Control homesteads-Gwara village-Outdoor soils (n=10)									
Mean \pm S.E	1.81 \pm 0.92	nd	nd	3.88 \pm 3.25	0.93 \pm 0.22	nd	1.08 \pm 0.30		
Min-Max	0.09 - 13.58	nd	nd	0.09 - 13.58	0.36 - 1.86	nd	0.64 - 1.65	0.00	0.00
DF (%)	70	0	0	40	70	0	30		
Control site-KWR soils (n=10)									
Mean \pm S.E	1.59 \pm 0.39	nd	nd	0.16*	1.33 \pm 0.13	1.06 \pm 0.25	3.21 \pm 0.04		
Min-Max	0.16 - 3.25	nd	nd	0.16 - 0.16	1.16 - 1.59	0.81 - 1.30	3.17 - 3.25	0.00	0.00
DF (%)	70	0	0	10	30	20	20		

Σ DDT = Mean of (*p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE, *o,p'*-DDE, *p,p'*-DDD and *o,p'*-DDD) in soil samples; S.E = standard error; Min-Max = minimum to maximum residue value detected (range); DF (%) = percentage detection frequency; nd = not detected, * = level from one field sample.

Source: Primary data

The chemical stability of the DDT metabolites partially explains why only residue isomers of DDE and or DDD were detected in soil samples

collected from Arocha and Tochi swamps in Apac and Oyam districts respectively, KWR and around houses in Gwara village where IRS of DDT was not applied in 2008.

The presence of the DDE and DDD residue isomers in the control sites could have been due to the continuous degradation of the parent DDT isomers to their metabolites following the past spraying of DDT in Uganda to control agricultural pests in the 1960s (Wasswa et al., 2011). The past spraying of DDT in the control sites is supported by the ratios of *p,p'*-DDT to *p,p'*-DDE and *p,p'*-DDD (Table 4) which are all less than one and such ratios suggest a previous application of DDT in the environment (Gonzalez et al., 2003). The presence of the residue isomers of DDE and DDD in soil samples collected from KWR may also be attributed to atmospheric transport of the residues from other places where DDT had been used (Ssebugere et al., 2010) since the wildlife reserve was gazetted in 1964 (Bahengana et al., 1997) and no practices such as agriculture, settlement, that could have been the source of DDT is allowed in the area. From other studies, Ssebugere et al. (2010), Hellar-Kihampa (2011) and Muhugija et al. (2017), reported total DDT up to 50 µg/kg dw, ranging from 22.6 ± 2.1 to 1452 ± 1.1 µg/kg dw and 7.5 to 564.2 µg/kg dw in soils from South-Western Uganda, sugarcane plantation in Tanzania, and Eastern Lake Tanganyika basin in Tanzania, respectively which supports the fact that DDT accumulate in soils.

Levels of DDT residues in leafy vegetables

The levels of DDT residues and its metabolites are summarized in Table 5. The results show that leafy vegetables are contaminated with DDT residues. In villages from Apac district, the ΣDDT detected in *S. lycopersicum*, *S. melongena*, *H. sabdariffa*, *A. esculentus*, and *G. gynandra* samples were 1.7, 2.9, 2.1, 8.3 and 4.4 µg kg⁻¹ fresh weight (fw), respectively. All residues of DDT isomers were detected in *A. esculentus* and *G. gynandra*, residues of DDE isomers were detected only in *S. lycopersicum* and *H. sabdariffa* while those of *o,p'*-DDT and *p,p'*-DDE isomers were below detection limits in *S. melongena* samples. In leafy vegetables from selected villages of Oyam district, the respective levels of ΣDDT detected in *S. lycopersicum*, *S. melongena*, *H. sabdariffa*, *A. esculentus*, and *G. gynandra* samples were 10.3, 2.3, 3.4, 4.1 and 11.4 µg kg⁻¹ fw. All DDT isomers were detected in *G. gynandra* while only residues of DDE and DDD isomers were detected in *S. lycopersicum* and *H. sabdariffa* vegetable samples. In Gwara village (control site), only DDE and DDD isomers were above detection limits with the highest mean level in µgkg⁻¹ fw of 12.2 for *o,p'*-DDD in *S. lycopersicum* samples and the lowest level being 0.4 for *o,p'*-DDD in *H. sabdariffa* samples. The highest and lowest mean ΣDDT levels in vegetable samples from

Gwara village were 5.8 µg kg⁻¹ in *S. lycopersicum* and 0.5 µg kg⁻¹ in *A. esculentus*.

The vegetable samples from the villages in the study districts were mainly contaminated with the insecticide residues in the order DDE > DDT > DDD. *G. gynandra*, *A. esculentus*, and *S. melongena* were the most contaminated vegetables in both study areas and the control site, probably because the plants have abilities to absorb the residues into their tissues in a short time (Gitari et al., 2018). The contamination of these plants is likely to be as a result of root uptake of DDT residues and its metabolites present in the soil, as opposed to aerial precipitation of DDT during IRS (Van Dyk et al., 2010). The high mean levels of DDE and DDD detected in vegetable samples from the study areas were not significantly different ($p > 0.05$) from the mean levels of DDE and DDD in vegetable samples from the control site, probably because similar plant tissues are capable of absorbing insecticides (Gitari et al., 2018; Ngabirano and Birungi, 2021) at the same rate. ΣDDT levels from the current study were however lower than those reported by Van Dyk et al. (2010) of 43 µgkg⁻¹ in vegetables from Vembe district S. Africa, two months after IRS of DDT.

Ingestion is the most possible route for human exposure to DDT in non-malaria areas (Tao et al., 2009). Studies have shown that ingestion through diet is responsible for 94.9% of the total uptake of DDT while 5.1% is attributed to inhalation and dermal contact (Guo et al., 2005). This means that since the vegetables are contaminated with DDT residues and its metabolites probably due to the IRS process, the people of villages of Apac and Oyam districts may be exposed to DDT residues and its metabolites via ingestion, dermal and inhalation pathways. Vegetables such as *S. lycopersicum*, *S. melongena*, *H. sabdariffa*, *A. esculentus*, and *G. gynandra* are among the main diet of the people of villages in Apac and Oyam districts. Results of analysis of the vegetable samples from the selected villages of the two districts indicated that these plants contain levels of DDT and its metabolites especially *p,p'*-DDE and *o,p'*-DDE. High levels of *p,p'*-DDT as compared to *p,p'*-DDE and *p,p'*-DDD, was detected in *S. melongena*, *A. esculentus*, and *G. gynandra* samples and the ratios of *p,p'*-DDT to *p,p'*-DDE were up to 144.1 and 1.8 for villages from Apac and Oyam districts, respectively. On the other hand, the ratios of *p,p'*-DDT to *p,p'*-DDD for *S. melongena*, *A. esculentus*, and *G. gynandra* samples vegetable samples were recorded to be up to 38.4 from villages of Apac district and 1.8 in Oyam district. According to Gonzalez et al. (2003), such high ratios confirm accumulation of the pesticide residues in these vegetables as a result of the recent spraying of DDT in the two districts.

The baseline study conducted by RTI (2008), in which crops including vegetable samples from Apac and Oyam districts were analysed, revealed that DDT residues were below detection limits. The current study has, however,

Table 5. Levels of DDT residues and its metabolites ($\mu\text{g kg}^{-1}$) in selected leafy vegetables around selected homesteads from selected villages of Apac and Oyam districts and Gwara village.

Parameter	Σ DDT	<i>p,p'</i> -DDT	<i>o,p'</i> -DDT	<i>p,p'</i> -DDE	<i>o,p'</i> -DDE	<i>p,p'</i> -DDD	<i>o,p'</i> -DDD	$\frac{p,p'-DDT}{p,p'-DDE}$	$\frac{p,p'-DDT}{p,p'-DDD}$
Leafy vegetables from villages of Apac districts (n=10)									
<i>S. lycopersicum</i>									
Mean \pm S.E	1.72 \pm 0.39	nd	nd	1.10 \pm 0.20	2.33 \pm 0.60	nd	nd		
Min-Max	0.82 - 3.38	nd	nd	0.82 - 1.49	1.31 - 3.38	nd	nd	0.00	0.00
DF (%)	50	0	0	30	30	0	0		
<i>S. melongena</i>									
Mean \pm S.E	2.92 \pm 0.96	7.75*	nd	nd	2.37 \pm 1.28	2.34*	1.61 \pm 0.67	--	3.31
Min-Max	0.01 - 7.75	7.75-7.75	nd	nd	0.01 - 4.39	2.34 - 2.34	0.93 - 2.28		
DF (%)	40	10	0	0	30	10	20		
<i>H. sabdariffa</i>									
Mean \pm S.E	2.19 \pm 0.51	nd	nd	2.93*	1.95 \pm 0.63	nd	nd		
Min-Max	1.06 - 3.17	nd	nd	2.93 - 2.93	1.06 - 3.17	nd	nd	0.00	0.00
DF (%)	30	0	0	10	30	0	0		
<i>A. esculentus</i>									
Mean \pm S.E	8.39 \pm 4.04	23.06 \pm 8.46	nd	0.16 \pm 0.05	1.43 \pm 0.22	0.60 \pm 0.33	3.42*		
Min-Max	0.02 - 39.81	0.29 - 39.81	nd	0.12 - 0.21	1.21 - 1.65	0.02 - 1.51	3.42 - 3.42	144.13	38.43
DF (%)	60	40	0	20	20	30	10		
<i>G. gynandra</i>									
Mean \pm S.E	4.47 \pm 1.51	4.97 \pm 2.25	4.68*	1.62 \pm 0.54	6.97 \pm 4.73	1.53 \pm 0.77	3.39 \pm 1.34		
Min-Max	0.28 - 30.42	0.28 - 11.04	4.68-4.68	1.03 - 2.70	0.88 - 30.15	0.75 - 2.30	1.51 - 5.98	3.07	3.25
DF (%)	60	50	10	30	60	20	30		
Leafy vegetables from villages of Oyam districts (n=10)									
<i>S. lycopersicum</i>									
Mean \pm S.E	10.33 \pm 5.17	nd	nd	30.60*	2.40 \pm 0.69	nd	21.82*		
Min-Max	0.94 - 30.60	nd	nd	30.60 - 30.60	0.94 - 4.22	nd	21.82 - 21.82	0.00	0.00
DF (%)	40	0	0	10	40	0	10		
<i>S. melongena</i> L.									
Mean \pm S.E	2.30 \pm 0.43	1.65 \pm 1.39	nd	0.91 \pm 0.30	2.99 \pm 0.78	3.52 \pm 1.52	2.08 \pm 0.25	1.81	0.47
Min-Max	0.25 - 5.25	0.25 - 3.04	nd	0.49 - 1.49	1.14 - 5.25	2.00 - 5.04	1.83 - 2.33		

Table 5. Contd.

DF (%)	50	20	0	30	50	20	20		
<i>H. sabdariffa</i>									
Mean±S.E	3.49 ± 0.92	nd	nd	3.78 ± 0.91	2.83 ± 1.31	0.10*	6.40 ± 3.42		
Min-Max	0.10 - 9.82	nd	nd	2.27 - 5.42	0.89 - 7.97	0.10 - 0.10	2.99 - 9.82	0.00	0.00
DF (%)	50	0	0	30	50	10	20		
<i>A. esculentus</i>									
Mean±S.E	4.17 ± 2.06	1.47*	nd	nd	6.07 ± 4.18	nd	3.06*		
Min-Max	1.47 - 10.25	1.47 - 1.47	nd	nd	1.89 - 10.25	nd	3.06 - 3.06	--	--
DF (%)	20	10	0	0	20	0	10		
<i>G. gynandra</i>									
Mean±S.E	11.47 ± 7.58	4.44 ± 1.42	5.18 ± 2.97	14.03*	39.04 ± 36.05	2.63 ± 1.31	1.57 ± 0.14		
Min-Max	0.47 - 147.18	1.05 - 8.35	2.21 - 8.15	14.03 - 14.03	1.68 - 147.18	0.47 - 5.89	1.34 - 1.82	0.32	1.69
DF (%)	60	50	20	10	40	40	30		
Leafy vegetables from Gwara village (control site) (n=5)									
<i>S. lycopersicum</i>									
Mean±S.E	5.81 ± 2.78	nd	nd	nd	1.52 ± 0.77	1.44*	12.28 ± 2.62		
Min-Max	0.75 - 14.91	nd	nd	nd	0.75 - 2.28	1.44 - 1.44	9.66 - 14.91	0.00	0.00
DF (%)	60	0	0	0	40	20	40		
<i>S. melongena</i>									
Mean±S.E	0.89 ± 0.16	nd	nd	1.03*	0.95 ± 0.22	nd	0.78 ± 0.33		
Min-Max	0.22 - 1.37	nd	nd	1.03 - 1.03	0.56 - 1.32	nd	0.22 - 1.37	0.00	0.00
DF (%)	60	0	0	20	60	0	60		
<i>H. sabdariffa</i>									
Mean±S.E	0.75 ± 0.20	nd	nd	nd	0.71 ± 0.17	0.95 ± 0.67	0.48*		
Min-Max	0.28 - 1.61	nd	nd	nd	0.40 - 1.01	0.28 - 1.61	0.48 - 0.48	0.00	0.00
DF (%)	60	0	0	0	60	40	20		
<i>A. esculentus</i>									
Mean±S.E	0.53 ± 0.07	nd	nd	nd	0.48 ± 0.08	nd	0.59 ± 0.14		
Min-Max	0.38 - 0.73	nd	nd	nd	0.39 - 0.65	nd	0.46 - 0.77	0.00	0.00
DF (%)	80	0	0	0	60	0	40		

Table 5. Contd.

G. gynandra									
Mean±S.E	1.05 ± 0.22	nd	nd	1.22*	0.75 ± 0.22	1.21 ± 1.01	1.24 ± 0.15		
Min-Max	0.21 - 2.22	nd	nd	1.22 - 1.22	0.32 - 1.01	0.21 - 2.22	1.09 - 1.38	0.00	0.00
DF (%)	100	0	0	20	60	40	40		

Σ DDT = Mean of (p,p'-DDT, o,p'-DDT, p,p'-DDE, o,p'-DDE, p,p'-DDD and o,p'-DDD) in vegetable samples; S.E = Standard error; Min-Max = minimum to maximum residue value detected; DF (%) = percentage detection frequency; nd = Not detected; * = Level from one field sample.

Source: Primary data

revealed that twelve years after IRS, the mean Σ DDT levels in the selected food crops were high (ranged between 1.72 and 11.4 $\mu\text{g kg}^{-1}$). Nevertheless, the mean level of Σ DDT in the vegetables were significantly lower ($P < 0.05$) than the Maximum Residue Limit (MRL) set by European Union (EU) for DDT and its metabolites in vegetables like *S. lycopersicum*, *S. melongena*, *H. sabdariffa*, and *A. esculentus* (0.05 to 0.5 mg kg^{-1}) (EU Pesticide Database, 2020). The levels therefore do not pose a health threat to the consumers of the vegetables. However, since DDT and its metabolites are bio-accumulative (Agustina et al., 2008), long term health effects may arise due to DDT bio-accumulation, and over consumption or long-term exposure may lead to health effects especially in pregnant women and children (Nnamuyomba et al., 2014; Ssemugabo et al., 2022). Studies have reported that even in small doses, DDT has numerous impacts on the environment and human life (Birnbbaum, 2002) since man is at high trophic level in the food chain. Therefore, the people in the study villages of Apac and Oyam districts may be at risk with time due to the presence of DDT and its metabolites in the vegetables that they feed on, though cooking foods could reduce the amount ingested (Van Dyk et al., 2010) which in turn reduces its risks to humans (Muhugija et al., 2017).

Conclusion

In this study to assess the levels of DDT and its metabolites in indoor and outdoor environmental samples after the 2008 use of DDT in Malaria Control Program in Northern Uganda, all DDT isomers and the metabolites were above detection limits in indoor dust and outdoor soil samples collected from villages of Apac and Oyam districts except o,p'-DDT and o,p'-DDD which was below detection limits in some indoor dust and outdoor soils, respectively from villages of Oyam district. The Σ DDT residue levels detected in the indoor dust samples from Apac and Oyam districts were significantly higher than those from the unsprayed areas. Most of the leafy vegetables were also contaminated by DDT residues. The findings suggest that IRS of DDT conducted in 2008 led to an increase in the Σ DDT levels in the house indoor dust/wall, outdoor soils and leafy vegetables. The results however do not raise concern regarding the potential health effects of DDT to the consumers of the vegetables since the levels were significantly lower than the EU EMRL. However, the shift of the Σ DDT from below detection limits of the residues in the baseline study to detectable levels reported in this study raise health concerns due to potential bio-accumulation of the insecticide. The findings in this study therefore, calls for the re-evaluation of

the necessary safety regulations that forms part of the IRS protocol especially at national level, to prevent unnecessary contamination of the immediate domestic environment. It is also important to note that countries still facing the problem of malaria should seek for other measures such as Ecohealth to deal with malaria vectors rather than IRS using DDT because it does not render the environment free from being contaminated by the insecticide and its residues.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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