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Organochlorine Levels in Human Milk and Risks to Exposed Breastfed Infants in Nandi County, Kenya

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ABSTRACT

Lipophilic persistent organochlorines (OCs) accumulate in the human adipose tissues and exposed lactating mothers can put breastfeeding infants at health risk. The study aimed to estimate the excretion levels of OCs in human milk and potential health risks to exposed breastfed infants. Between February and July 2019, 74 lactating mothers, across Nandi County, volunteered sociodemographic information and human milk samples. Milk lipids and OCs residues were extracted into an n-Hexane: Acetone (3:1) mixture using the Soxhlet technique. Twenty percent of the extract was gravimetrically analyzed to estimate milk lipid content and 80% with not more than 240 mg estimated lipids, was chromatographically purified by eluting through neutral Aluminium Oxide using 170 mL n-Hexane for GC-EI-MS/MS analysis. DDTs and PCBs residues occurred in 70.3% and 68.9% samples respectively while chlordane, endosulfan, and HCHs were each observed in less than 50% samples. The chemicals' average occurrence levels ranged from 0.04 to 7.30 ng/g lw. Statistically, there were no significant, $p>0.05$, differences in the average levels of OCs across the county, and between maternal parities and age groups. However, the average levels of OCs demonstrated significant, $P<0.05$, decreases over time in Kenya. The daily intake of chemicals by breastfed infants was computed for each of the sum OC groups. Three (4.05%) infants were exposed to residues of sum indicator PCBs above 20 ng/kg/day, the risk level given by the Agency for Toxic Substances and Disease Registry. The findings demonstrated that humans are moderately exposed to banned OCs in Nandi County and therefore underscore the need for effective control and monitoring of the safety of human food from environmental contaminants.

Key Words: Human Milk, Infants, Organochlorine Pesticides, Polychlorinated Biphenyls, Risk Assessment

INTRODUCTION

Abiotic and biotic ecological units are exposed to chemicals which include anthropogenic organochlorines (OCs) that have been intentionally or unintentionally introduced and widely distributed (Ritter et al., 2013; Thompson et al., 2017; Zeilmaker et al., 2020). Owing to their stable and lipophilic nature the chemicals have since been classified as persistent organic pollutants (POPs) (Manzetti et al., 2014). The majority of these chemicals are semi-volatile organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) (Zeilmaker et al., 2020). Organochlorines can; migrate and be deposited at remote places from their point of production or applications, persist in soil, water, sediments, and rocks, and accumulate in biological mediums in the environment and human adipose tissues (Alharbi et al., 2018).

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Organochlorines biomagnify up the food chain once they are ingested by invertebrates, consequently, humans at the top of the trophic accumulate much higher levels compared with those in the environment (Loganathan & Kannan, 2018).

Global studies traced back to the 1960s deduced that under appropriate conditions, definite exposure to OCs cause various human health hazards and environmental effects ranging from subtle biological changes: birth defects; dysfunctional immune, respiratory, nervous, hormone, and endocrine systems, increased cancer risk, reproductive disorders, genotoxicity; to death (Alharbi et al., 2018; Mostafalou & Abdollahi, 2013). The human and environmental health effects of the chemicals let to establishment of global treaties and particularly the Stockholm Convention which came up with strategies to eliminate or strictly control selected chemicals that includes a number of anthropogenic OCs (KNIP, 2014). Kenya has, since 1986, banned or restricted the release of a number of Stockholm Convention listed chemicals (PCPB, 2018).

The banned or control OCPs and PCBs were widely applied in huge amounts in agriculture and industry between 1945 and 1986 across Kenya (Saoke, 2005). Good agricultural opportunity in Nandi County led to large-scale farming of tea, coffee, sugarcane and cereals which involved widespread use of OCPs (Mungai & Wang, 2019). The widespread use of these slow degrading products for several years resulted in their ubiquity in the environment (Abong'o et al., 2015; Kaigwara et al., 2002; Lalah et al., 2003; Madadi et al., 2021; Makokha et al., 2018; Mungai & Wang, 2019; Ndunda et al., 2018; Olisah et al., 2020; Oluoch-Otiego et al., 2016; Omwenga et al., 2016; Saoke, 2005). However, there is no data on the occurrence and levels of OCs in humans in Nandi County.

This study, therefore, analysed for the occurrence and levels of thirty-eight OCs in human milk conveniently obtained from lactating mothers at maternal and child health clinics across Nandi County. In Kenya, OCs have been analysed in human milk in the 1980s and '90s through academic research (Kanja et al., 1986; Kinyamu et al., 1998) and between 2008 and 2019 through global monitoring programmes (Madadi et al., 2021). All these investigations analysed selected OCs and found on average 87.8% occurrence prevalence. OCs in human milk reflects the donors body burden and fundamentally estimates the levels ingested by breastfed infant (WHO, 2015).

The OCs analysed for were; aldrin, chlordane, l, l, l-Trichloro-2,2-bis(4-chlorophenyl) ethane (DDT), dieldrin, endosulfan, endrin, heptachlor, *cis/trans*-heptachlor epoxide, hexachlorobenzene (HCB), hexachlorocyclohexane (*δ, β, δ* & *γ* HCH also referred to as lindane) earlier named as benzene hexachloride (BHC; a misnomer), methoxychlor, mirex and indicator polychlorinated biphenyls (iPCBs) indicator congers: 28, 52, 101, 138, 152 and 180.

MATERIALS AND METHODS

Study Location

This cross-sectional study was conducted in Nandi County which is a rural area situated in the Western lower region of Rift Valley and on the Eastern side of Lake Victoria Basin where agricultural practices are undertaken with significant use of pesticides. The county consists of six sub counties which were treated as distinct sampling locations. The county comprises of rolling hills to the West, the Kapsabet Plateau, the Tindiret Volcanic Mass, King'wal Swamp, and the Nyando Escarpment on the Southern border. The county covers an area of 2,856 sq. Km and lies between latitudes 0° 4' and 0° 38' S, and longitudes 36° 38' and 37° 20' E with an altitude of between 1,777 and 5,199 meters above the sea level (Fig. 1) (NCIDP, 2018a). The county's population was estimated as 845,863 persons sparsely distributed into 342 persons per square kilometre with an annual growth rate of 2.2%. An estimated 3.9% of the total population live in urban centres of Kapsabet and Nandi Hills (ASDSP, 2016; NCIDP, 2018b).

Demographic Data and Sample Collection

Target sample size (≈96) was estimated using Eq. 1 previously used by Jaykaran Charan and Tamoghna Biswas (Charan & Biswas, 2013). An accuracy error of 20% was assumed (Kothari, 2004).

The participants in this study were healthy mothers who were breastfeeding single infants between 2.1 and 10.4 weeks old. The mothers were all born in Kenya and had resided in the study sub-counties for not less than five years. Each participant demonstrated acceptance by signing a consent form and proper representation was arranged for mothers who were unable to give consent, after proper explanation about the study was provided.

$$n = \frac{(Z(1-\alpha)/2)^2 NP(1-P)}{\Delta\, 2(N-1) + (Z(1-\alpha)/2)^2 P(1-P)} \quad (1)$$

Where: N = Population size, n = Sample size, P = Population proportion, Z = Level of confidence, Δ = Degree of accuracy expressed as proportion, α = Significance level.

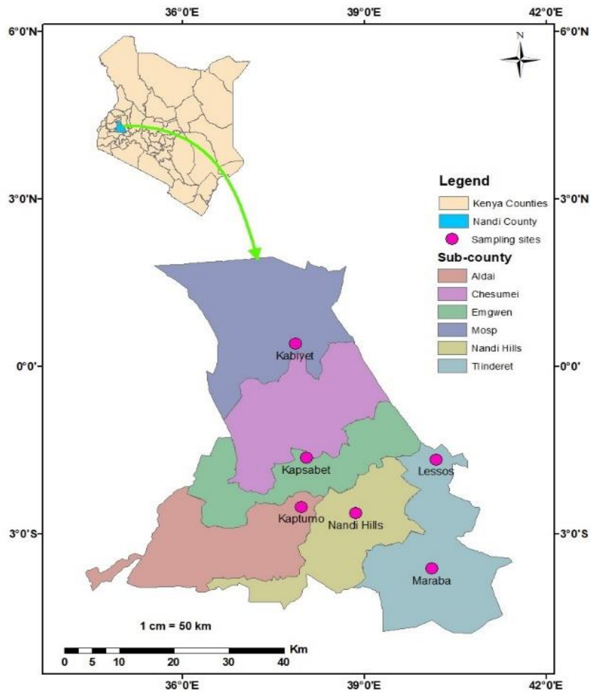


Figure 1: Map of Nandi County

Under the guidance of a trained medical staff, at the Maternal and Child Health Clinics (MCHCs) across Nandi County, 74 breastfeeding mothers provided sociodemographic data and manually expressed between 15- and 50-mL milk samples. The participating mothers provided information guided by pre-prepared structured questionnaire, on mothers' age, occupation, smoking and dietary habits, parity, weight, height, and infants' weight, age and breastfeeding frequency (WHO/UNEP, 2007). Mothers' milks were expressed into 50 mL glass vials with a Polybutylene Terephthalate (PBT) cap and Polytetrafluoroethylene (PTFE) seal all pre-cleaned scrupulously using soap and hot water followed by thorough rinse with deionized water and overnight oven drying. Each glass vial and cap were uniquely labelled prior milk expression, which were handle as bio-hazardous material and appropriately stored in the freezer compartment of the MCHCs refrigerator awaiting shipment to the laboratory.

Without allowing to thaw the milk samples were shipped at night packaged on dry ice, in a clean cool box to the laboratory where they were kept frozen at -20 ℃ awaiting analysis.

Chemicals and Standards

Custom mixed 40 OCPs and six iPCBs standards each at 100 µg/mL in Toluene, from Restek International, were used to prepare 200 ng/mL stock standard solution using n-hexane. Glass wool, HPLC grade solvents n-Hexane and Isooctane, analytical grade reagents Acetone, Anhydrous Sodium Sulphate, Silica gel, Alumina Oxide, and ultra-pure (MS grade) water from the water purification system (ELGA, UK) were purchased from Scielabs Chemical Suppliers Kenya.

Pre-preparation of chemicals

Anhydrous sodium sulphate, neutral aluminum oxide, and silica gel were baked at 200 ℃ in a Memmert hot air oven for twelve hours and cooled in a desiccator before use (Kanangire et al., 2015).

Preparation of standards solutions

200 ng/mL stock standard solution was used to prepare calibration standards of 0.8, 2.5, 5, 10, 25, 50, 75 and 100 ng/mL. Calibration standards in 20-mL amber-coloured glass vials were labelled to indicate preparation date, concentration, and net weight and stored at -20 ℃ for frequent use in GC-EI-MS/MS analysis. Owing to solvent loss, the standards were often checked against the chromatogram which were obtained from the original standard solutions. A deviation of more than 5% was considered inappropriate and new calibration solutions were prepared.

Preparation of recovery test samples

Bovine milk samples were spiked with appropriate volume of stock standard solution to achieve concentrations of 4, 5, 10, 25, 50 and 100 ng/g lw per chemical. Spiked Bovine milk samples were mixed thoroughly, stored at -20 ℃ for not less than 12 hours before analysis.

Chemical Analysis

The Institute of Environmental Studies, 2014 protocol 2 for the analysis of PCBs and OCPs (Institute of Environmental Studies, 2014) was used with slight modification in order to extract and clean OCs from human and spiked bovine milk samples. In summary, duplicate weights of five grams of thawed and homogenized milk samples were each mixed accurately with 25 g of previously activated Na_2SO_4, on an n-Hexane-washed aluminium foil and then pulverized to powder. The resultant powders were covered with aluminium foil and allowed to dry further overnight then grounded in a mortar to free-flowing powders. Each of the free-flowing powder was quantitatively refluxed in the Soxhlet apparatus for 16 hours by using 200 mL n-Hexane: Acetone (3:1, v/v) extraction mixture. The Soxhlet extracts were then concentrated into about 5 mL by using BÜchi Rotavapor set at 40 ℃ after adding 2 mL of Isooctane. 20% aliquot of each Soxhlet extract was subjected to gravimetric analysis as described by Linderholm et al. (2010) to determine the lipid content of each milk sample and the remaining 80% was clean-up using aluminum oxide chromatography for GC-EI-MS/MS Analysis.

GC-EI-MS/MS Analysis

Triple quadruple GCMS-TQ8040 (Model GC-2010/TQ8040NCI Shimadzu, Japan) equipment, operated in Multiple Reaction Monitoring (MRM) mode was used. Chromatographic separation was achieved by using Phase ZB-5ms (5% phenyl and 95% Dimethylpolysiloxane), 30 m × 0.25 mm (i.d.), and 0.25 µm film thickness Capillary column from Restek, USA. High purity Helium gas (99.999%) at a constant flow of 1.69 mL min-1 was used as Carrier gas and Argon at a pressure range of 7.31-7.35 mTorr was used as collision gas for equipment operation conditions.

Analytical Quality Assurance and Quality Control

The accuracy of the analytical method was verified by using bovine milk samples spiked with OCs standards. The recovery of spiked OCs ranged from 71.44% for δ-HCH to 117.66% for β-endosulfan. The recovery results satisfied the limits of 60% and 130% given by residues quantitative methods document SANTE/12682/2019, 2019 (Pihlström et al., 2019). The precisions associated with OCs recoveries were good with RSDs ≤20%. The instrumental regression coefficients (r^2) for all OCs Chemicals were >0.990 showing a good fit to linearity within the calibration range.

The Method Detection Limit (LOD) defined as three times the noise response level at each OCs chemical elution time in a blank chromatogram were calculated following Eq. 2.

$$LOD = 3.3 \times \frac{\text{Response Standard Deviation}}{\text{Slope of calibration curve}} \quad (2)$$

The Method Quantification Limits (LOQs) used as the lowest level for reporting the OCs occurrence levels were obtained by multiplying noise levels at each chemical retention window by ten and were used as the lowest level for reporting the OCs occurrence levels.

Estimation of OCs Daily Intake by Infants

The estimated daily intake (EDI) of OCs residues in human milk by breastfed infants was calculated using Eq. 3. Infants' parameters used where: 700 g for milk consumed per day and 5 kg for an average six weeks old infant weight (Asamoah et al., 2018; Tsygankov et al., 2019).

$$EDI = \frac{C\text{ milk} \times 700\text{ g milk/day} \times C_{lipid}/100}{5\text{ kg body weight}} \quad (3)$$

Where: C_{milk} is OCs {i.e., ∑chlordane, ∑DDT, ∑endrin, ∑endosulfan, ∑HCH, ∑heptachlor, ∑methoxychlor, and ∑iPCBs} levels in ng/g milk lipid weight, and C_{lipid} is milk lipid content (% w/w).

Statistical Analysis

Mean, standard deviations, range and median were computed for sociodemographic and analysis data by using Microsoft EXCEL 2016. Statistically significant difference of OCs occurrence levels based on p-value of ≤0.05 was evaluated using ANOVA and Wilcoxon-Mann-Whitney test.

RESULTS

Study Participants' Demographic Information

Table 1 presents sociodemographic information of participants (mothers and infants). The mean lactation period of the mothers was 6.1 weeks and ranged from 2.1 to 10.4 weeks. The mothers were: 82.4% primiparous at mean age of 23.5 (range 15 to 35) years, average weight was 59.80 kg (range, 48 to 80 kg) with a mean height of 160 cm (range 147 to 175 cm), while the infants were on average 4.31 kg (range 2.60 to 5.80 kg). The multiparous mothers were breastfeeding either second, third or fourth child. The mothers' mean body mass index (BMI) was 23.28 (range 18.67 to 29.52). All mothers consumed a mixed diet consisting of cereals (100%), beans (100%), meat (97.3%), dairy products (97.3%), poultry products (94.0%), and fish (92.0%). Only 4.1% used tobacco while 51.35% reported recent possible exposure to pesticides used on either mosquitos, bedbugs, cockroaches, flies, or lice in their homes. Additionally, their residential background was 100% rural.

Table 1: Characteristics of study participants

Participants quantitative characteristics		
Parameter	**Mean±SD**	**Range**
Mothers' age (years)	29.5±4.64	17.0 - 40.0
Mothers' weight (kg)	59.7±7.0	48.0 – 80.0
Mothers' height (cm)	160.1±6.33	144.0 – 175.0
Mothers' body mass index (BMI)	23.3±2.24	18.7 – 29.5
Infant's age (weeks)	6.78 ± 2.19	2.1 - 10.4
Infant's weight (kgs)	4.31± 0.80	2.6 - 5.8
Participants qualitative characteristics		
Parameter	**Frequency (n)**	**Percentage (%)**
Mothers' occupation		
Housewife	32	43.24
Schooling or with parents	20	27.03
Business	16	21.62
Employed	6	8.11
Mothers' parity		
Mothers breastfeeding first child	61	82.43
Mothers breastfeeding second child	7	9.46
Mothers breastfeeding third child	3	4.05
Mothers breastfeeding fourth child	3	4.05
Mothers' dietary habits		
Eating mixed diet	74	100
Eating fish	68	91.89
Eating meat and meat products	72	97.30
Drinking cow's milk	73	8.65
Eating poultry products	71	95.95
Eating eggs	70	94.59
Eating pork	7	9.46
Mothers exposed to pesticides	38	51.35
Mothers using tobacco	3	4.1
Infant's sex and feeding habits		
Male	34	45.95
Female	40	54.05
Breastfed exclusively	70	94.95

Human Milk Samples Lipid Content

Table 2 presents descriptive statistics of extractable lipid content and OCs residue of human milk samples. The average lipid content was 3.93±1.59% w/w whole milk and ranged from 0.23 to 9.45% w/w. Human milk from Aldai sub-county had the highest mean lipid content of 4.77±1.75% and that from Emgwen sub-country had the lowest mean content of 3.23±1.33%. Statistically, the extractable lipid content of the human milk samples when stratified into sampling location demonstrated significant difference, $p<0.05$, which can be attributed to variations in eating frequencies, food type consumed and/or sampling protocols.

Table 2: Descriptive statistics of lipid contents and OCs'

Chemical (OC)	Mean	Positive samples n (%)	Range	Median
Lipid Content (%)	3.93	-	0.23 – 9.45	3.75
∑Chlordane	0.26±0.12	12 (16.2)	0.07 - 0.62	0.17
Cis-Chlordane	0.14±0.06	8 (10.8)	0.09 - 0.40	0.12
Trans-Chlordane	0.15±0.05	7 (9.5)	0.07 - 0.37	0.11
Cis-Nonachlor	0.16±0.02	1 (1.4)	0.16 - 0.16	0.16
Trans-Nonachlor	0.27±0.06	3 (4.1)	0.16 - 0.34	0.32
∑DDTs	1.13±0.62	52 (70.3)	0.08 - 3.03	0.36
p,p'-DDD	0.20±0.02	1 (1.4)	0.20 - 0.20	0.20
p,p'-DDE	0.17±0.16	28 (37.8)	0.06 - 1.29	0.09
o,p'-DDT	0.13±0.05	11 (14.9)	0.08 - 0.18	0.12
p,p'-DDT	0.64±0.58	39 (52.7)	0.10 - 2.88	0.37
∑Endosulfan	4.39±1.04	30 (40.5)	0.04 – 9.30	0.30
α-Endosulfan	1.19±0.42	6 (8.1)	0.09 - 2.75	1.06
β-Endosulfan	2.80±0.70	3 (4.1)	0.70 - 5.71	1.98
Endosulfan sulphate	0.41±0.38	26 (35.1)	0.04 - 2.35	0.22
∑HCHs	2.32±0.91	27 (36.5)	0.09 - 4.81	0.37
α-HCH	0.21±0.04	2 (2.7)	0.09 - 0.32	0.21
β-HCH	0.56±0.28	13 (17.6)	0.14 - 1.55	0.45
γ-HCH	1.28±0.82	13 (17.6)	0.08 - 4.81	0.62
δ-HCH	0.27±0.08	5 (6.8)	0.16 - 0.51	0.22
∑PCBs	2.52±1.23	51 (68.9)	0.07 - 7.38	0.30
PCB#28	0.29±0.26	27 (36.5)	0.07 - 1.90	0.20
PCB#52	0.44±0.55	35 (47.3)	0.07 - 4.08	0.16
PCB#101	1.10±0.96	13 (17.6)	0.09 - 7.30	0.19
PCB#138	0.18±0.06	6 (8.1)	0.10 - 0.47	0.11
PCB#153	0.34±0.14	5 (6.8)	0.08 - 1.18	0.12
PCB#180	0.15±0.04	5 (6.8)	0.08 - 0.20	0.18

Organochlorines levels in human milk samples

In this study, 38 OCs (isomers, congeners, or metabolites) of 14 chemical groups were analysed and 21, equivalent to 55% of five groups were detected in various frequencies and quantities in 96% human milk samples. Only three samples were free from OCs chemicals, nine had between six and nine OCs and 62 had between one and five OCs (Figure 2). The OCs chemicals which were detected and quantified included *cis-/trans*-chlordane, *cis-/trans*-nonachlor, *p,p'*-DDT, *p,p'*-DDD, *o,p'*-DDT, *p,p'*-DDE, *α-/β*-endosulfan, endosulfan sulphate, *α-/β-/γ-/δ*-HCH, PCB#28, PCB#52, PCB#101, PCB#138, PCB#153 and PCB#180. The OCs groups findings were DDTs (70.3%, 1.13±0.62 ng/g lw), PCBs (68.9%, 2.52±1.23 ng/g lw), Endosulfans (40.5%, 4.39±1.04 ng/g lw), HCHs (36.5%, 2.32±0.91ng/g lw), and Chlordane (16.2%, 0.73±0.12ng/g lw). Aldrin, dieldrin, endrin, HCB, heptachlor, mirex and methoxychlor were not detected.

The four DDTs isomers (*p,p'*-DDT, *p,p'*-DDE, *o,p'*-DDT and *p,p'*-DDD) were individually detected in between 1.4% and 52.7% human milk samples. *p,p'*-DDT, and *p,p'*-DDE occurred in 52.7% and 37.8% milk samples respectively with levels ranging from 0.06 ng/g lw to 2.88 ng/g lw. *p,p'*-DDT mean level was high at 0.64±0.58 ng/g lw while that of *o,p'*-DDT was low at 0.13±0.05 ng/g lw. The six indicator PCBs analogs analysed were individually detected in between 6.8% and 47.3% human milk samples with levels ranging from 0.15±0.04 ng/g lw for PCB#180 to 1.10±0.96 ng/g lw for PCB#101. tri and tetra-chlorinated PCBs

(PCB#28 and PCB#52) occurred most frequently in 36.5% and 47.3% samples respectively and PCB#101 (penta-chlorinated PCBs) had the highest levels of 1.10±0.96 ng/g lw.

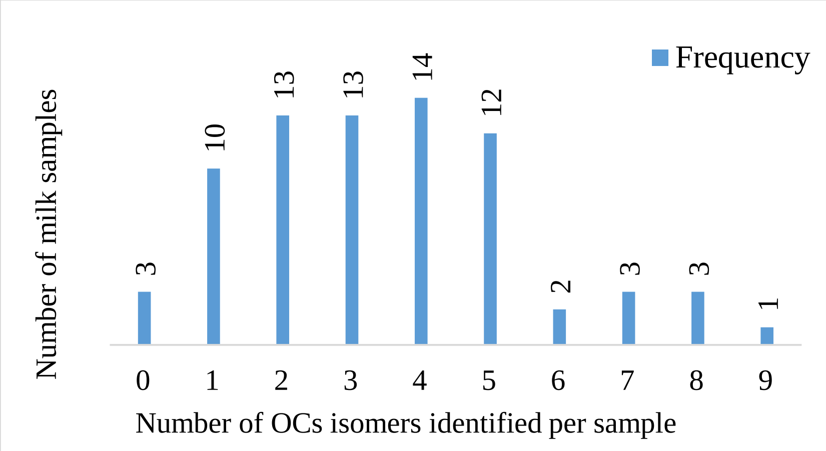


Figure 2: OCs detected per individual human milk sample

Three metabolites of endosulfan (alpha-, beta- and -sulphate) and four conformeric forms of HCHs (alpha-, beta-, delta- and gamma-) were individually detected in 30 to 50% human milk samples. 0.41±0.38 ng/g lw mean level of endosulfan-sulphate occurred in 35.1% human milk samples, while 1.19±0.42 ng/g lw mean level of *α*-endosulfan was detected in 8.1% samples and 2.80±0.70 ng/g lw mean level of *β*-endosulfan was quantified in 4.1% milk samples. The frequencies and levels of HCHs forms detected were *α*-HCH (4.24%, 0.215±0.053 ng/g lw), *β*-HCH (16.95%, 0.410±0.224 ng/g lw), *γ*-HCH (11.86%, 0.581±0.364 ng/g lw) and *δ*-HCH (5.93%, 1.001±0.452 ng/g lw).

Four metabolites of chlordane (*cis/trans*-chlordane and *cis/trans*-nonachlor) detected were *cis*-nonachlor (1.40%, 0.16±0.02 ng/g lw), *trans*-nonachlor (4.10% 0.27±0.06 ng/g lw), *trans*-chlordane (9.5%, 0.15±0.05 ng/g lw) and *cis*-chlordane (10.8%, 0.14±0.06 ng/g lw).

Estimated Daily Intake of Organochlorine Chemicals by Breastfed Infants

Table 3 presents a summary estimate of OCs exposed to exclusively breastfed infants daily. The results were compared with references values from FAO-WHO 2019 Codex Alimentarius Commission (WHO, 2010), 2021 edition of Agency for Toxic Substances and Disease Registry (ATSDR, 2021) and 2008 edition of European Union. The findings indicates that the EDIs of OCs by infants were mainly below ADI PCBs referenced values, however EDI for three infants (4.05%), exceeded 20 ng/Kg/ bw/day ADI value established by ATSDR.

Table 3: Estimated OCs (ng/Kg/day) exposed to infants daily

Organochlorine	Chlordane	DDTs	Endosulfans	HCHs	PCBs
Mean EDI (ng/Kg/day)	0.66	1.78	1.31	2.10	1.38
Range	0.14 - 3.32	0.11 - 25.05	0.16 - 25.01	0.10 - 23.05	0.16 - 26.35
TDI proposed by ATSDR (ng/kg/day)	600	100	6000	600	20
Infants exposed (%)	16.2	70.3	40.5	36.5	68.9
Infants exposed above TDI values (%)	0	0	0	0	4.05

Note: TDI = Tolerable Daily Intake, EDI = Estimated Daily Intake, Min = Minimum, Max = Maximum, ng/kg/day = nano gram per kilogram per day, ATSDR = Agency for Toxic Substances and Disease Registry.

DISCUSSION

Analysis of OCs in human milk has become critical in characterization of exposed lipophilic chemicals in a wide variety of contexts such as national population monitoring (Esteban & Castaño, 2009). However, an understanding of human factors which influence body burdens of OCs are imperative in the study design and data interpretation. These factors includes the OCs worldwide and residential usage patterns, individual: age, diet, occupation, parity and period of residency, and sampling and laboratory analysis protocols (Haddad et al., 2015). Therefore, the design of this study included human activities which contributes significantly to the background levels and body of OCs during samples and information collection (Berlin et al., 2002).

Distribution and Levels of OCs

In this study, there was no significant, $p>0.05$, difference in the occurrence levels of chlordane, DDTs, and HCHs across individual human milk samples, and between study locations, but there was, $p<0.05$, for endosulfan and PCBs. Emng'wen subcounty had significantly high mean occurrence levels of endosulfan and PCBs. The pattern of OCs occurrences was KSCH-M<LHC<KSCH-A<MTT<NHCH<KCRH, whereas that of their levels was chlordane<DDTs<HCHs<PCBs<endosulfan across the county. All detected OCs apart from HCHs, which was more abundant in Nandi Hills subcounty, were high in Emng'wen subcounty.

The observed trend of OCs residues corresponded with human activities which includes moderate consumption of fish around NHCH, KCRH, and MTT health centres (ASDSP, 2016; NCIDP, 2018a). Fish consumed across Nandi County comes from Lake Victoria which has been found to be polluted with OCs (Oluoch-Otiego et al., 2016; Osoro et al., 2016). Also this study is in agreement with other findings that have reported presents of chlordane, endosulfan, DDT, HCH, and PCBs around Lake Victoria catchment areas which include Nandi County (Abong'o et al., 2015; Kyalo, 2017; Mungai & Wang, 2019).

The *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, and *trans*-nonachlor isomers of chlordane were detected. *Cis and trans*-chlordane were quantified in 10.8% and 9.5% milk samples respectively giving a mean ratio of 1.1 for *trans-/cis*. This suggests a possible existence of fresh exposures of the chemicals, though the chemicals were not detected in earlier studies in Kenya (Kanja et al., 1986; Kinyamu et al., 1998; Madadi et al., 2021). The occurrence of oxidative persistent metabolites of chlordane (*cis*-nonachlor and *trans*-nonachlor) agrees with findings observed in biota such as fish, birds and mammals (Gibson et al., 2016; Kairu, 1994; Kasozi et al., 2006; Omwenga et al., 2016).

Among the four DDTs isomers detected, *p,p'*-DDT was high at 52.7% followed in order by *p,p'*-DDE, *o,p'*-DDT then *p,p'*-DDD (Table 2). However, there is a significant declined in the levels of DDTs in human milk in Kenya (Kanja et al., 1986; Kinyamu et al., 1998; Madadi et al., 2021). The high ration of the mean levels of *p,p'*-DDT over that of *p,p'*-DDE demonstrated recent exposure, despite restricted control in 1986 (PCPB, 2018). The widespread contamination levels of DDTs in the environment and food chains may explain the levels found in human milk from Nandi County (Abong'o et al., 2015; Mungai & Wang, 2019).

The occurrences of endosulfan were dominated by endosulfan sulphate at 35.1%, which had positive samples in each of the six study locations (Table 2). This was in agreement with the metabolic pathways of endosulfan in humans where it breaks down into bio-accumulative endosulfan sulphate and then further to endosulfan ether and diol (Stockholm Convention Secretariat, 2011). The findings show a continuous usage of endosulfan in the study locations. Also, the high frequency of α-endosulfan occurrence compared to that of β-endosulfan confirms reported accumulation of α-isomer than that of β-isomer in animal tissues (ATSDR, 2005), however, in KCRH the opposite was observed. This confirms the continuous usage of endosulfan in the areas surrounding KCRH.

Hexachlorocyclohexane (HCHs) contains a mixture of eight isomer forms with variable levels of bioaccumulation in human milk. HCHs occurred in low levels which were dominated by *β*-HCH (17.6%) and *γ*-HCH (17.6%), although mean *γ*-HCH residues levels were higher than that of *β*-HCH. This is because mixed HCH which include *β*-HCH was banned in 1986 and *γ*-HCH which is commonly known as Lindane was banned later in 2011 (PCPB, 2018). The findings from the study showed earlier exposure to HCHs since exposed HCHs degrade to more persistent *β*-HCH which has 10 to 30 times potential to accumulate in lipid than *γ*-HCH (Jayaraj et al., 2016; Phillips et al., 2005). In addition *α*-HCH, *β*-HCH, *γ*-HCH and *δ*-HCH varies along the food chains including excretion in human milk (Phillips et al., 2005).

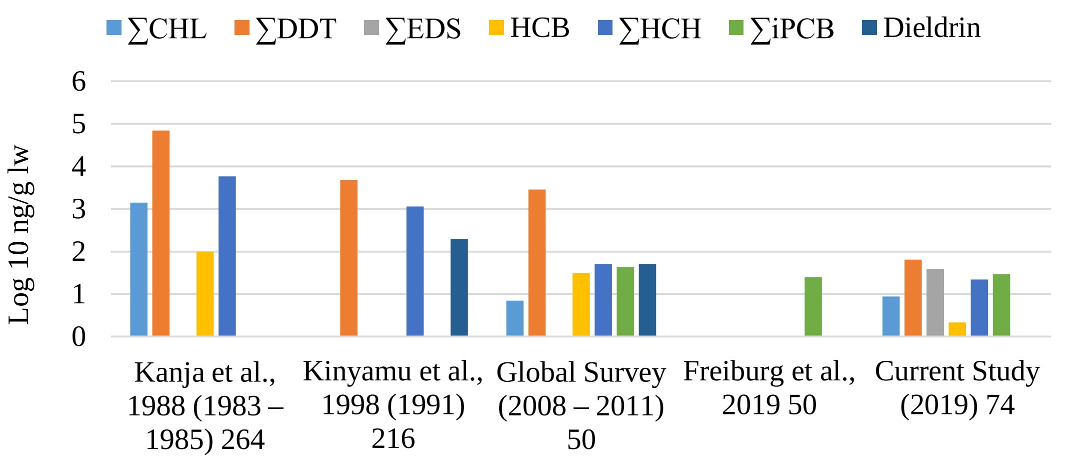
PCBs occurred in low levels with sum-iPCBs being in the same order of magnitude as found earlier in Kenya (Kanja et al., 1986; Madadi et al., 2021). Generally, the occurrence level of sum iPCBs have decreased in Kenya. Across the studied human milk samples, *tri*, and *tetra*-chlorinated PCBs (PCB#28 and PCB#52) occurred most frequently while PCB#101 (*penta*-chlorinated PCBs) had the highest occurrence levels (Table 3). PCB#153 (*hexa*-chlorinated PCBs) was the dominating congener in human milk samples which was widely reported in the least number of human milk samples (Fång et al., 2015; Müller et al., 2017). Equipment which contained PCBs used in Kenya were tightly sealed, however poor management of these equipment lead to chemical leakages (Kanja, 2010; Kinyamu et al., 1998). This may explain the lack of PCBs detection earlier (Kinyamu et al., 1998).

Organochlorines Trends in Human Milk in Kenyan

Generally, there was a reduction in the occurrences and levels of OCs chemicals in human milk in comparison to National and Global data (Figures 3 and 4). However, chlordane increased by 24.14% from 0.700 to 0.869ng/g lw. In 2008 - 2011 global survey, 4.32ng/g lw quantifiable levels of PCBs occurred (Madadi et al., 2021). This implies that between 2009 and 2019 there was a decrease of 32.29% in the levels of iPCBs in human milk. For endosulfan, it was not possible to observe the time trend since no earlier studies were found. Aldrin, dieldrin, endrin, and heptachlor were not detected, but they had been reported in quantifiable levels in earlier studies and this indicates reduction in the chemical's levels (Figure 3). Quantifiable levels of methoxychlor and mirex have not been reported in Kenyan human milk. Therefore, this study findings demonstrated that OCs elimination and control measures employed by Kenya are effective.

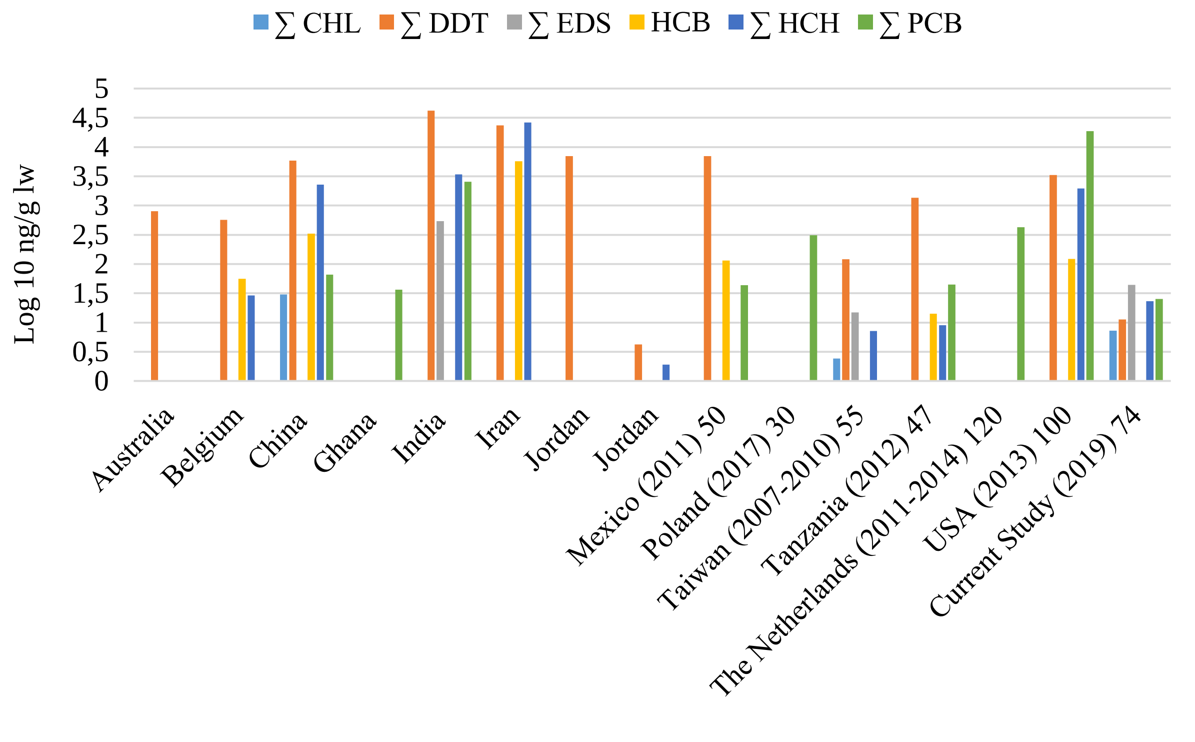
Estimated Daily Intake of OCs by Exclusively Breastfed Infants

In the current study, the EDI of $\sum_6$iPCBs for three breastfed infants exceeded 20ng/kg/bw/day the ADI proposed by ATSDR (Table 3). No infant exposed to DDTs, chlordane, endosulfan, and HCHs exceeded ADI levels proposed by ATSDR, codex and EU. Repeated exposure to PCBs at levels above ADIs given by the above authorities may result in neurological disorders, hepatic, developmental and immunotoxin effects (Jayaraj et al., 2016). The risk of such effects depends on the life stage, the dose and the duration to which exposure exceeds the ADIs.



Note 1). Numbers in parenticies refers to sampling period
2). Numbers after the parenticies refers to study sample size

Figure 3: Clustered column illustrations of trends of log transform occurrence levels of OCs in human milk in Kenya



Note 1). Numbers in brackets refers to sampling period
2). Numbers after the brackets refers to study sample size

Figure 4: Clustered column illustrations of this study and global findings

WHO recommends exclusive breastfeeding of infants for six months after birth (Ayisi & Wakoli, 2014). However, in Kenya, it is a common practice to breastfeed for up to 2 years. This implies that Kenyan infants could be exposed to Health effects if breast milk contains OCs residues, and therefore undermining the health benefits of breastfeeding. This might be the case for the three infants whose mother's milk had sum of indicator PCBs levels above ADI provided by ATSDR. However, the use of ADI's established for adults may not be appropriate for infants. This is because infants eat additional diet per kilogram of body weight with dynamic growth and developmental processes, and are therefore more vulnerable to compounds stress than adults (Müller et al., 2017). Also, risk assessments were based on the Toxicological Evaluation of each group of compounds independently with assumptions on the cumulative effects such as synergism, potentiation, antagonism and inhibition (Alvito et al., 2016; Nougadère et al., 2020). Based on Müller et al. (2017), OCs such as OCPs and PCBs are reported to have common mechanisms of action and effects. When the above postulates are taken into consideration, the infants' health concern increases significantly.

CONCLUSIONS

The findings of this study show that human populations living in Nandi County have OCPs and PCBs groups of OCs in their bodies which lactating mothers transmit to their infants during breastfeeding. This is the pioneering data in Nandi County, Kenya to demonstrate the exposure of lactating mothers and infants to residues of OCs. The information generated is only relevant to the study locations and time since the results of such a study are dependent on the types of human milk and the methodologies used. The occurrence values of OCs determined points to both past and current sources, despite their banned in Kenya more than three decades ago. Additionally, the detection and quantification of between one and nine chemicals per sample is an important aspect of the study. Even if the information obtained is not generalizable to the whole Country, the study points to the need for frequent monitoring for OCs residue in food, animal feeds, both abiotic and biotic environments, and human subjects. This is because the results of studies carried out in Kanya since the 1980s and 2019 show that a wide range of OCs are present across the country and across all possible analysis matrices. Real-time facts about the occurrence and levels of OCs in the above-mentioned matrices and with subsequent identification of their release sources and exposure routes will aid in the implementation of public health policies aimed at environmental and human health. In summary, despite the likelihood of transfer of OCs to infants through breastfeeding, the mothers should not be discouraged from the practice. Human milk remains to be a complete nourishment source for infants' needs in their first six months of life.

ABBREVIATIONS

ANOVA	Analysis of Variance
CAS	Chemical Abstracts Service
COP	Conference of the Parties
EPA	Environmental Protection Agency
FAO	Food and Agriculture Organization of the United Nations
GC-EI-MS/MS	Gas Chromatography – Electron Ionization Mass Spectrometry
HPLC	High Performance Liquid Chromatography
KCRH	Kapsabet County Referral Hospital (Emeng'wen Sub County)
KNH	Kenyatta National Hospital
KSCH – A	Kaptumo Sub County Hospital (Aldai Sub County)
KSCH – M	Kabiyet Sub County Hospital (Mosop Sub County)
LHC	Lessos Health Centre (Tindiret Sub County)
MTT	Maraba Sub County Hospital (Tindiret Sub County)
NHCH	Nandi Hills County Hospital (Nandi Hills Sub County)
UON	University of Nairobi
ZB	Zebron

DATA AVAILABILITY

The data used to support the findings of this study are available from the corresponding author upon request. Note also that the legal requirements of research clearance as per KNH-UON Ethics and Research Committee restrict public sharing and access of raw data for ethical concerns.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ETHICAL APPROVAL

The study protocols were approved by Kenyatta National Hospital-University of Nairobi Ethics and Research Committee and by the Faculty of Veterinary Medicine, Animal Use and Ethics Biosafety Committee, University of Nairobi. The National Commission for Science, Technology and Innovation of Kenya authorized this study. The participants' sociodemographic data and milk samples were collected, transported, stored, and analysed following ethical approvals as directed by Helsinki Declaration (Shrestha & Dunn, 2020) on Medical Research involving human subjects.

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