ASSESSMENT OF SELECTED PERSISTENT ORGANIC POLLUTANTS IN HUMAN MILK FROM NAIROBI, NANDI, AND NYERI COUNTIES, KENYA

A thesis submitted in partial fulfillment of requirements for Masters Degree of University of Nairobi (Pharmacology and Toxicology)

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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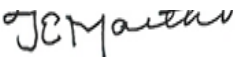
Signed:  Date: 24/08/2023

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DEDICATION

This thesis is dedicated to my creator the Most Gracious God. A special feeling of sincere gratitude goes to my late loving Father Joseph Kibirgen Lagat and Mother Christine Chesanga Lagat; my brothers and sisters; and my friends for their words of encouragement throughout the study.

I momentously dedicate this work to my wife Beatrice Chebet and my wonderful daughter Cloa Sarah Cherono for being there for me throughout the Master's program. They have been my greatest cheerleaders.

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ABBREVIATIONS AND ACRONYMS

α	*Alpha*
β	*Beta*
δ	*Delta*
γ	*Gamma*
µ	Micro
µg	Microgram
%	Percentage
°C	Degree Celsius
Σ	Summation
No.	Number
ADI	Acceptable Daily Intake
ANOVA	Analysis of Variance
ATSDR	Agency for Toxic Substances and Disease Registry
CAS	Chemical Abstracts Service
COP	Conference of the Parties
DDD	Metabolite of DDT
DDE	Metabolite of DDT
DDT	Dichlorodiphenyltrichloroethane
Codex	Collection of standards, guidelines and codes of practice to protect the health of consumers and ensure fair practices in the food trade.
EPA	Environmental Protection Agency
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
g	Gram
GC	Gas chromatography
GCMS	Gas chromatograph mass spectrometer
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane
HM	Human Milk
KBS	Kenya Bureau of Statistics
kg	Kilogram

KFIP and R	Kenya Feed Industry Policy and Regulations Issues
KNIP	Kenya National Implementation Plan for the Stockholm Convention on Persistent Organic Pollutants in Kenya
KNH-UON	Kenyatta National Hospital and University of Nairobi research ethics review committee
LARMAT	Land Resource Management and Agricultural Technology
lw	Lipid weight
Max	Maximum
MCHC	Maternal and Child Health Clinic
MEWNR	Ministry of Environment, Water and Natural Resources Kenya
Min	Minimum
mg	Milligram
MRL	Minimum Residue Level
MS	Mass selective detector
NACOSTI	National Commission for Science, Technology and Innovation
NCIDP	Nandi or Nyeri County Integrated Development Plan
ng	Nano gram
OC	Organochlorine
OCPs	Organochlorine Pesticides
OECD	Organization for Economic Co-operation and Development
iPCBs	Indicator Polychlorinated biphenyls (PCBs; 28, 52, 101, 138, 153 and 180)
PCPB	Pest Control Products Board
PHH	Public Health Hospital
POP	Persistent organic pollutant
SC	Stockholm Convention
UNEP	United Nations Environment Programme
US-EPA	United States Environmental Protection Agency
WHO	World Health Organization
WFPHA	World Federation of Public Health Associations'

ABSTRACT

Persistent organic pollutants (POPs) are a group of chemicals that persist for long periods in environment without being degraded by natural means. They also have the tendency to build up in fat-containing foods as well as in human bodies; therefore, traces can be exposed in human milk (HM). POPs consist of organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), among other halogenated organic compounds. Even though the bulk of POPs were prohibited or limited in Kenya in 1986, 1989, 2004, 2009, and 2011, reports continue to show their residues in the environment and human tissues. The aim of this study was to determine the levels of selected OCPs and PCBs in human milk (HM) samples, assess the health risks to exposed breastfed infants, and evaluation the possible sources of POPs in Nairobi, Nandi, and Nyeri Counties. HM samples were obtained from volunteer primiparas mothers in 11 maternal and child health clinics (MCHC) across Nairobi (3), Nandi (6), and Nyeri (2) Counties, Kenya. Study participants met selection criteria that included pre-sampling five years of continuous residency in the study location, be aged between 16 and 30 years old, be of apparent good health, be first-time mothers (primiparous), be a two- to eight-week lactation period, and be exclusively breastfeeding a single infant of apparent good health. A structured questionnaire was administered to each of the selected 116 mothers who subsequently offered HM samples. A replica of five grams of each of the individual HM samples was pulverized to a free-flowing powder with 25 grams of anhydrous sodium sulphate followed by Soxhlet extraction. The lipid content of each HM sample was determined gravimetrically using an aliquot of Soxhlet extract equivalent to 20%, while the 80% portion was cleaned up using aluminum oxide chromatography for GCMS/MS analysis. All HM (100%) samples from Nairobi and Nyeri counties, and 96.7% from Nandi County had quantifiable level(s) of one or more of; *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, *trans*-nonachlor, *p,p'*-DDD, *p,p'*-DDE, *o,p'*-DDT, *p,p'*-DDT, endosulfan ether, α-endosulfan, β-endosulfan, endosulfan sulphate, α-HCH, β-HCH,

γ-HCH, *δ*-HCH, HCB, PCB#28, PCB#52, PCB#101, PCB#138, PCB#153 and PCB#180 compounds. The least occurring compound quantified in 1.69% of HM samples was endosulfan ether, whereas the most occurring compound quantified in 72.88% of HM samples was *p,p'*-DDT. The mean occurrence levels of the compounds ranged from 0.152±0.044 ng/g lw for *trans*-chlordane to 5.426±12.237 ng/g lw for *p,p'*-DDE. Aldrin, *oxy*-chlordane, *o,p'*-DDD, *o,p'*-DDE, dieldrin, endrin, endrin aldehyde, endrin ketone, heptachlor, *cis(endo)*-heptachlor epoxide, *trans(exo)*-heptachlor epoxide, mirex, pentachlorobenzene, and methoxychlor were below detection levels. The ratio of *p,p'*-DDE and *p,p'*-DDT (*p,p'*-DDE/*p,p'*-DDT) was 7.82, indicating a possible past exposure to the DDT group of compounds. DDTs and iPCBs were the major contaminants quantified in 86.44% and 55.08% of HM samples, respectively. Significant differences in mean levels were recorded between Nairobi, Nandi, and Nyeri counties, for chlordane ($p = 0.04$) and DDT ($p = 0.03$). The infants' mean estimated daily intakes, ng/kg/bw/day, of $\sum_4$chlordane, $\sum_4$DDT, $\sum_4$endofulfan, $\sum_4$HCH, HCB, and $\sum_6$iPCB were 1,506, 11.886, 4.990, 3.170, 4.820, and 3.505, respectively. Consequently, 2.54% and 3.39% of infants ingested $\sum_4$DDTs and $\sum_6$iPCBs respectively, above the accepted daily intake (ADI) proposed by the Agency for Toxic Substances and Disease Registry (ATSDR). But all exposed infants ingested POPs below the ADI values proposed jointly by the Codex Alimentarius Commission of the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) or those proposed by the European Union Council (EU Council). 100% of the participants surveyed from all counties used a mixed diet, with 78% to 96% consumption of fish. This study concludes that humans are exposed to the banned POPs in Nairobi, Nandi, and Nyeri counties and that more than 70% of the participants consumed fish and other animal products, which normally bioaccumulate the studied POPs. Also infants are exposed to studied POPs, in some cases at levels that are toxicologically risky. Continuous mothers' and infants' exposure to the compounds, though in small doses, poses a

risk of an increased likelihood of lifetime chronic effects. Therefore, there is a need for frequent monitoring of POP levels in food consumed across Kenya, specifically those with high-fat content, along with water and animal feeds to control of human and environmental exposures.

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CHAPTER ONE

INTRODUCTION

1.1 Background information

The abiotic and biotic ecological units get exposed to thousands of compounds. These include organochlorine (OC) groups such as pesticides (OCPs) and polychlorinated biphenyls (PCBs) that have since been classified as Persistent Organic Pollutants (POPs) (Buccini, 2006; Kuvarega and Taru, 2007; Oluoch-Otiego *et al.,* 2016). Several OCs were intentionally produced for agriculture, home lawn, public health and garden applications, as well as components in electrical equipment and paints, in the advent of industrial revolution Table 1.1 (Massart *et al.,* 2008; Schmidt and Rodrick, 2003; Stillerman and Ditz, 2005; Wandiga, 2001). POPs are extremely stable cyclical ring (aromatic or aliphatic) carbon compounds with one or more covalently bonded chlorine (most often), bromine, or fluorine atoms without polar functional groups (Massart *et al.,* 2008; Ritter *et al.,* 2013). These compounds share hazardous properties such as high halogenation, 200–500 molecular weights, vapor pressures below 1000 Pa, semi-volatility with the capacity to migrate long distances, long half-lives, and lipophilicity (Ritter *et al.,* 1995). Therefore, POPs get widely distributed, highly accumulate in fat-containing organisms as well as in the human body, and biomagnify along the food chain, leading to tissue injuries (Stow, 2005; Tsygankov *et al.,* 2019; Wang *et al.,* 2011).

Studies traced back to six decades ago have categorized POPs as environmental and human health high-risk compounds (Bates *et al.,* 1994; Lee *et al.,* 2013; Li *et al.,* 2006; Moon *et al.,* 2009; WFPHA, 2000). At appropriate conditions, definite exposure of a POP compound, causes various human health hazards and environmental effects ranging from subtle biological changes and birth-defects to death (Alharbi *et al.,* 2018; Borchers *et al.,* 2010; Mostafalou and Abdollahi, 2013). The elderly, pregnant women and children are vulnerable and may be unsafe even at extremely low POPs exposure levels (European Commission, 2017).

Table 1.1: Economic and public health benefits of studied persistent organic pollutants

Compound	Economic and public health benefits	CAS No.
Aldrin	Pesticide: - sprayed on soils to eliminate corn rootworm, grasshoppers, local ectoparasitic, and termites among other insect pests.	309-00-2
Chlordane	Pesticide: - applied to agricultural crops to control a range of insects including termites.	57-74-9
DDT	Pesticide: - used to get rid of malaria and typhus spreading insects such as mosquitos.	50-29-3
Dieldrin	Pesticide: - used to regulate termites and textile pests among other insects transmitting diseases.	60-57-1
Endosulfan	Pesticide: - used on vegetables to reduce aphids, cabbage worms, Colorado potato beetles, leafhoppers and whiteflies.	115-29-7
Endrin	Pesticide: - used to control pests on agricultural crops leaves.	72-20-8
Heptachlor	Pesticide: - used to control insects on leaves and in soil i.e., termites of agricultural crops and malaria transmitting insects.	76-44-8
Hexachlorobenzene (HCB)	Pesticide: - used to kill fungi which affected food crops. HCB is also an industrial compound used in production of rubber, aluminium, munitions, and dyes and in wood preservation.	118-74-1
Mirex	Pesticide: - used to scuffle ants such as fire ants and termites. Industrial compound: - used as a fire retardant in plastics, rubber, transformers and capacitors.	2385-85-5
Polychlorinated Biphenyls (PCBs)	Industrial compound: - fluids majorly used in electric transformers and capacitors as heat exchangers and as additives in carbonless copy paper, paints, plastics and sealants. Also used as dielectric isolators in electrical equipment.	1336-36-3

Note: Adapted from "U.S. States and the Global POPs Treaty*"* by Stillerman and Ditz, (2005). CAS is a universally used number in the registry to provide unique and unmistakable identifier of a compound.

Rachel Carson's vivid imaginary of a world without bird songs because of highly toxic DDT in her 1962 book "silent springs" prompted the United States and some European (EU) Countries to ban DDT in the 1970s and 1980s (Longnecker and Rogan, 2001; Stillerman and Ditz, 2005). To mitigate the emerging global effects of POPs, the international community adopted the

Stockholm Convention (SC) on POPs on May 22, 2001, which entered into force on May 17, 2004 (UNEP, 2019). Kenya ratified SC text in 2004 (KNIP, 2007). According to Stockholm Convention Secretariat (2001), the SC's main objective is to control or totally eradicate POPs and therefore safeguard human health and that of the environment.

Initially, in 2001, SC conference listed twelve compounds "the dirty twelve" for immediate action, followed by nine more in 2009 (Stockholm Convention Secretariat, 2011). These compounds were classified into three actionable categories; A, B and C: i) A: subject to stoppage of manufacture and usage; ii) B: controlled manufacture and usage; and iii) C: controlled accidental release (Stockholm Convention Secretariat, 2013). In 2011 the conference added endosulfan and its related isomers, followed by hexabromocyclododecane in 2013. In 2015, the conference added three more compounds and their salts and esters, which were hexachlorobutadiene, pentachlorophenol and polychlorinated naphthalene (UNEP, 2019). And according to Stockholm Convention Secretariat (2013), the conference of state parties put decabromodiphenyl ether, dicofol, methoxychlor, pentadecafluorooctanoic acid, perfluorohexane sulfonic acid and its salts, short-chain chlorinated paraffins, substituted phenolic benzotriazole, among others under review. Table 1.2 presents the listing periods of the studied POP compounds by the Stockholm Convention and the years Kenya banned or restricted their release into her environment (PCPB, 2018; UNEP, 2019).

Trends in the levels of Stockholm Convention-listed POPs in the human population have been monitored using human milk since the 1980s (Berg *et al.,* 2017). WHO/UNEP systematized global HM monitoring programmes for POPs starting with 1987–1988 Round Table 1.3 (Malisch *et al.,* 2017). Kenya participated in rounds five and seven of 2008–2011 and 2016–2022, respectively, through the Department of Public Health, Pharmacology, and Toxicology (PHPT) of the University of Nairobi (KNIP, 2014). Composite (pooled) HM samples were sent to Baden-Württemberg Chemisches Laboratory, Germany, for analysis, whereas this study aims

to analyse the 2018 HM samples from the 2016–2022 round of the WHO/UNEP global HM monitoring program for selected basic POP residues. The selected POP compounds are aldrin, chlordane, DDT, dieldrin, endosulfan, endrin, heptachlor, HCB, and HCH (*δ, β, δ*, and *γ* also referred to as lindane), also named benzene hexachloride (BHC; a misnomer), heptachlor, methoxychlor, mirex, pentachlorobenzene, and iPCBs (28, 52, 101, 138, 153, and 180).

Table 1.2: Stockholm Convention Persistent Organic Pollutants Listing Amendments and the Years Kenya Banned or Controlled the Compounds

Compound	Isomers, metabolites, or congeners	Year of ban in Kenya
POPs listed at COP-4 (Initial 2004)		
Aldrin	Aldrin	2004
Chlordane	*cis*-chlordane, *trans*-chlordane; and *cis*-nonachlor, *trans*-nonachlor, and *oxy*-chlordane	1986
DDT	*p,p'*-DDT, *o,p'*-DDT and *p,p'*-DDE, *o,p'*-DDE, *p,p'*-DDD, and *o,p'*-DDD	1986 restricted to public health use
Dieldrin	Dieldrin	2004
Endrin	Endrin, endrin aldehyde, and endrin ketone	1986
HCB	Hexachlorobenzene	2004
Heptachlor	Heptachlor, heptachlor-exo-epoxide, and heptachlor-endo-epoxide	1989
Mirex	Mirex	Not imported
PCB	PCB (6 congeners): 28, 52, 101, 138, 153, and 180	2004
POPs listed at COP-4 (2009)		
Hexachlorocyclohexane	*α*-HCH, *β*-HCH, *γ*-HCH and *δ*-HCH	1986
Pentachlorobenzene	Pentachlorobenzene	2004
POPs listed at COP-5 (2011)		
Endosulfan	Endosulfan; *α*-endosulfan, *β*-endosulfan; endosulfan-ether; and endosulfan sulphate	2011
Lindane (*γ*-HCH)	Lindane	2011
POPs listed at COP-6 (2013 and 2015)		
Pentachlorophenol	Pentachlorophenol and its salts and esters;	2004

Note: Adapted from "Guidance on the Global Monitoring Plan for Persistent Organic Pollutants" by Stockholm Convention Secretariat, *2019*. COP stands for Conference of Parties.

Table 1.3: Rounds of WHO/UNEP global human milk monitoring for Persistent Organic Pollutants

Round	Period	Organizer	Countries	Parameters
1	1987 - 1988	WHO/EURO	12	Dioxins and PCBs.
2	1992 - 1993	WHO/EURO	19	Dioxins and PCBs.
3	2000 - 2003	WHO/EURO	26	Dioxins and PCBs. Later Stockholm Convention Initial POPs.
4	2004 - 2007	WHO/UNEP	13	Stockholm Convention POPs.
5	2008 - 2011	WHO/UNEP	45*	Stockholm Convention POPs.
6	2012 - 2015	UNEP	17	Stockholm Convention POPs.
7	2016 - 2022	UNEP	42*	Stockholm Convention POPs.

Note: Adopted from Malisch *et al.,* (2017). Asterisk (*) indicates Kenya participated.

1.2 Statement of the problem

In Kenya, traces of studied POPs have frequently been detected in environmental samples (air, sea, and land biota, soil, and water) and in human body tissue as years pass since their ban (Abong'o *et al.,* 2015; Barasa *et al.,* 2008; Gitahi *et al.,* 2002; Kairu, 1994; Kanja, 1988; Kinyamu *et al.,* 1998; Lalah *et al.,* 2003; Madadi *et al.,* 2002; Makokha *et al.*, 2018; Mungai and Wang, 2019; Ndunda *et al.,* 2018; Oluoch-Otiego *et al.,* 2016; Omwenga *et al.,* 2016; Osoro *et al.,* 2016). The Stockholm Convention state parties are obligated to regularly evaluate the occurrence and levels of Stockholm Convention-listed POPs in the environment and in human tissues within their jurisdictions. This is to monitor the effectiveness of compounds' elimination or control strategies employed (Stockholm Convention secretrariat, 2017).

Further, a mother's prior lifetime POP exposure poses a risk that a breast-feeding mother cannot avoid during lactation (Malcolm and John, 1992). Also, POP levels, once accepted as safe, have exhibited abilities to effect subtle but vital health effects, as just about one-million-fold lower PCB levels are proven to affect human brain development (Solomon and Weiss, 2002). Besides, data on human body burdens of Stockholm Convention-listed POPs in Kenya are scanty (MEWNR, 2014).

1.3 Objective of the study

1.3.1 General objective

The general objective of this study was to determine the human body burdens of selected POPs and associated risks to breastfeeding infants using HM samples provided by primiparous mothers living in Nairobi, Nandi, and Nyeri Counties, Kenya.

1.3.2 Specific objectives

The specific objectives of the study were:

i) To determine the occurrence and levels of selected POPs in HM from primiparous mothers living in Nairobi, Nandi, and Nyeri Counties, Kenya.

ii) To determine if the levels of selected POPs in HM causes any risks to the health of breastfeeding infants.

iii) To determine factors that influence the occurrence and levels of POPs in HM in Nairobi, Nandi, and Nyeri Counties, Kenya.

1.4 Hypothesis

Null Hypotheses (H_o):

1) The primiparous mothers living in Nairobi, Nandi, and Nyeri Counties, Kenya are not exposed to selected POP residues.
2) Breastfeeding infants of primiparous mothers living in Nairobi, Nandi, and Nyeri Counties, Kenya, are not exposed to selected POPs above established health risks levels.

1.5 Justification of the study

This study's motivation lies in the critical need to document information on the occurrence and levels of POPs accumulated over time in the human population and in breastfeeding mothers that could result in hazardous toxicological consequences for infants across Kenya. Also, HM samples collected for the 2016–2022 round of the WHO/UNEP global survey for Stockholm Convention-listed POPs have not been analyzed locally as was required of each participating

Country (Stockholm Convention secretrariat, 2017).

There is presently a scarcity of information on the magnitude and demographic distribution of POPs in the human population across Kenya, with regions such as Nandi County having no data available. Nandi County and others yet to be surveyed have the prospect of continuing agricultural activities that involve extensive use of pesticides. Therefore, the findings from this study will provide a very important reference to the scientific community and body of knowledge at large as far as trends in distribution and levels of POPs are concerned, hence contributing to the knowledge gap.

Also, the findings from this study will provide a critical appraisal of Kenya's current POP status and the effectiveness of the legal, administrative, and operational measures the country has put in place to eliminate or control the release of the compounds into the environment (KNIP, 2007; Wöhrnschimmel *et al.,* 2016). Also, the findings are expected to form the basis for National studies such as epidemiological follow-ups risk assessments and management by sectors such as Public Health, the environment, agriculture, and fishing. Undeniably, the generated data will contribute to historical trends in Kenya and be part of the global inventory (Pronczuk *et al.,* 2004; Schmidt and Rodrick, 2003).

1.6 Limitations of the study

This study faced limitations due to: the period the studied POPs have been in the mother's body, which can be several years in the past, immediately before HM collection, or within the HM sampling period; the compounds' exposure dosage forms, either single large exposure, multiple smaller exposures, or both combine; time; the cost of sampling and laboratory analysis; instruments/method of data collection/access of data; Sample and selection of sample (Small, limited to breastfeeding mothers, unwilling hospital offers); and generalizability. However, despite these limitations, the generated results meet the expectations of this study and can be referred to as the true reflection of POP distribution and level of status in the study Counties.

CHAPTER TWO

LITERATURE REVIEW

2.1 Persistent organic pollutant

The behaviour, effects, and fate of environmental compounds are determined by a variety of their physical and chemical (physicochemical) characteristics, which are depended on the compounds' structural atomic nature, Appendix 6, and their environmental conditions (Appendix 7). As a result, about 30% of limited compounds have crucial combined properties, such as degree of persistence, mobility, and toxicity at appropriate environmental conditions, to be classified as POPs, as illustrated in Figure 2. And according to Massey and Jacobs (2012), "Of the 5.7 million tons of pollutants released by 2006, 1.8 million tons are compounds considered persistent, bioaccumulative, or toxic; 970,000 tons are known or suspected carcinogens; and 857,000 tons are compounds that are considered reproductive or developmental toxicants." POPs are compounds recognised as of grave, global threat to human health and to environment, with effects felt away from their point of production, release, or use (Ritter *et al.,* 1995).

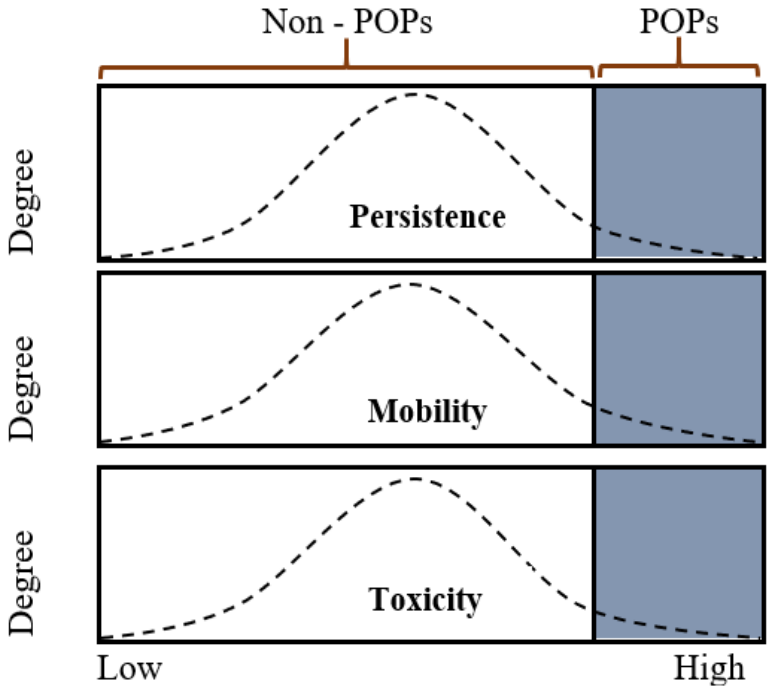

Figure 2.1: Persistent organic pollutant qualifying combined characteristics Adapted from "a review of selected persistent organic pollutants" by Ritter *et al* (1995)

POPs like dioxins and dibenzofurans can be of natural origin or inadvertently produced via industrial or certain materials combustion processes (Buccini, 2006; El-Shahawi *et al.,* 2010; Michaelidou *et al.,* 2008) or of anthropogenic origin, such as scores of earlier effective OCPs, industrial organochlorine fluids (PCBs), Brominated compounds, and perfluorinated compounds (D'Hollander *et al.,* 2010; Kodavanti *et al.,* 2018).

2.1.1 Organochlorine pesticides

Organochlorine pesticides (OCPs) consist of chlorinated benzenes or chlorinated cyclohexane, cyclodienes/diene, and DDT groups (Massart *et al.,* 2008; Sonawane, 1995).

2.1.1.1 Chlorinated benzenes/cyclohexane

Chlorinated benzenes, or cyclohexane, are halogenated cyclic compounds with a typical benzene ring structure Figure 2.2. They include hexachlorobenzene (HCB), benzene hexachloride (BHC), also known as hexachlorocyclohexane (HCH), and pentachlorobenzene. Technical HCH has eight different isomers, of which four, *alpha*-HCH, *beta*-HCH, *gamma*-HCH or lindane, and *delta*-HCH, are commonly encountered. Lindane or γ-HCH, which has been used in place of DDT, is the most toxic (acute), while β- HCH owing to its high stability or environmental persistence, exhibits high chronic toxicity (Nayyar *et al.,* 2014). Both β-HCH and γ-HCH were frequently found in high levels in HM, and β-HCH has the highest ability to accumulate in fat tissues (Nayyar *et al.,* 2014). α-HCH and δ-HCH isomers are seldom identified in human samples such as HM.

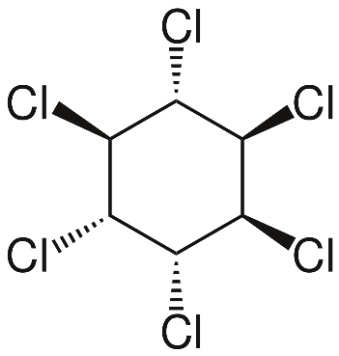

Figure 2.2: Halogenated cyclohexane structure

2.1.1.2 Cyclodienes

Cyclodienes are cyclic chemicals with a typical "endo-methylene bridged" structure (Figure 2.3). These include aldrin, chlordane, dieldrin, endosulfan, endrin and heptachlor, among others. Dieldrin is a more stable, oxygenated metabolite of aldrin. While heptachlor and

chlordane are closely associated compounds. *Oxy*-chlordane and heptachlor epoxide are the most persistent epoxy metabolites of chlordane and heptachlor respectively. Technical grade chlordane contains as impurities heptachlor epoxide and transnonachlor. Cyclodienes pesticides were not used extensively, yet their traces in HM, adipose tissue, and food have not been uncommon (Kannan *et al.,* 1992; Pirsaheb *et al.,* 2015; Sharaf *et al.,* 2014).

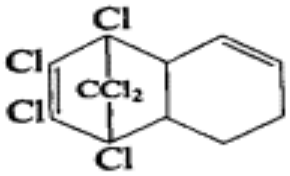

Figure 2.3: Cyclodienes structure

2.1.1.3 Dichlorodiphenyltrichloroethane and its analogues

Dichlorodiphenyltrichloroethane (DDT) and its analogs (DDD, DDE and methoxychlor) are cyclic compounds with two aromatics rings Figure 2.4. Zeidler was the first to synthesized DDT in 1874 and Miiller discovered its insecticidal properties in 1939 (Kanja, 1988). As reported by Kanja, (1988) commercial DDT consisted of "*p,p'*-DDT, 77.1%; *o,p'*-DDT, 14.9%; *p,p'*-DDD, 0.3%; *o,p'*-DDD, 0.1%; *p,p'*-DDE, 4.0%; *o,p'*-DDE, 0.1%; and 3.5% unidentified compounds". In the 1960s DDT was associated with environmental and human hazards leading to its restriction or ban starting in the 1970s (Longnecker and Rogan, 2001).

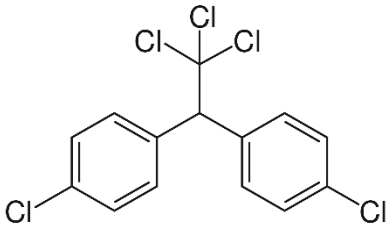

Figure 2.4: DDT structure

2.1.2 Polychlorinated Biphenyls

The structure of PCB compound consists of two benzene rings with certain or all substitutable hydrogen atoms replaced by chlorine atoms at positions that are conventionally numbered $C_{12}H_{10-n}Cl_n$, where n ranges from 1 to 10 Figure 2.5 (Ahlborg *et al.,* 1992; Andersson *et al.,* 1997). Theoretically, 209 PCB congeners are possible each with different physicochemical properties. Congener's lipophilic characteristics increase with increasing degree of chlorination, whereas volatility digresses. The

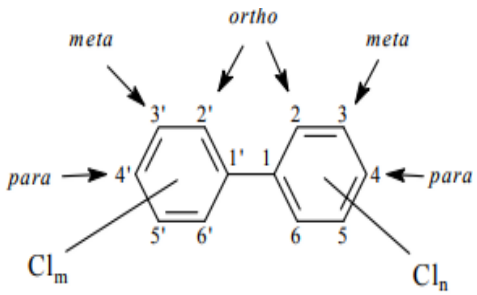

Figure 2.5: Polychlorinated Biphenyl structure

flash points of PCBs range from 170 to 380 ℃, therefore, their vapours are heavier than air and non-explosive, and as a result, they are practically fire-resistant. Other critical properties of PCBs are high thermal conductivity, low electrical conductivity, and high resistance to thermal degradation (Ahlborg *et al.,* 1992). And Naturvårdsverk (1998), and Erickson and Kaley (2011) reported that PCBs products were extremely valued for their notable insulating and plasticizing capabilities in addition to their extraordinary high temperature resistances.

According to Naturvårdsverk (1998) and Erickson and Kaley (2011), the industrial production of PCBs started in the late 1920s with diverse trade names, which included Clophen and Aroclor, among others. PCBs are classified into three groups characterized by non-ortho, *mono-ortho,* and *poly-ortho*, substituted biphenyl positions. And according to Ulaszewska (2011), *mono-ortho,* and *non-ortho* substituted compounds are more toxic. Due to PCBs environmental and human health hazards, their production and use were restricted globally in the 1970s (Jepson and Law, 2016).

2.2 Toxicokinetic of Persistent Organic Pollutants

Human gets exposed to POPs through dermal, inhalation, and ingestion routes (Dahl *et al.,* 1995). The exposed POPs are then absorbed, distributed, or tissue bound, metabolized, and excreted at different rates within the human body. These activities depend on the compound's physicochemical properties, individual susceptibility, and contact period (Dahl *et al.,* 1995). Dermal absorption is favoured by skin humidity and temperature, whereas inhalation and absorption depend on the compound's concentration in the inhaled air and their faster absorption via the alveolar epithelium. Ingested POPs are absorbed along the alimentary canal according to the compound's lipophilic characteristics, molecular size, membrane transport mechanisms, and actions of intestinal microflora and microvillus projections (Dahl *et al.,* 1995). The absorbed POPs can remain bound to plasma proteins or diffuse through lipid membranes into high-fat body tissues. Once in the human body, a POP compound may be excreted either

without transformation, metabolized and excreted, stored and slowly excreted, or undergo a combination of all these processes (La Merrill *et al.,* 2013; Needham *et al.,* 2005). POPs with high lipophilic characteristics and a slow rate of metabolism remain longer in the adipose tissue (Chen, 2016; La Merrill *et al.,* 2013). The time a compound takes to reduce to half its original amount is used to estimate the total time it will persist in the same environment (Konishi *et al.,* 2001; Zeilmaker *et al.,* 2020). Exposed compounds such as POPs in human bodies are mainly excreted through faeces, urine, breast milk, the biliary, and saliva (Tsygankov *et al.,* 2019).

2.2.1 Aldrin/Dieldrin

In the human body, aldrin/dieldrin distributes favourably into the adipose tissues, where they can remain for years or released gradually, and aldrin is rapidly converted to dieldrin (ATSDR, 2002a). The estimated biological half-lives in humans are 266 days for aldrin and 369 days for dieldrin. Results from studies in monkeys indicates that unchanged dieldrin and conjugated metabolites are excreted mainly in the faeces via the bile (ATSDR, 2002a). Substantial amounts of aldrin and dieldrin are also excreted through breast milk (ATSDR, 2002a).

2.2.2 Chlordane

Chlordane accumulates initially in the liver and kidneys, after which it relocates to high lipids tissues, where it persist for several years (ATSDR, 2018). Chlordane is oxidatively metabolized into *oxy*-chlordane, *cis*-nonachlor and *trans*-nonachlor which are then eliminated very slowly as compared with *cis*-chlordane and *trans*-chlordane in an animal's body fat. Body tissues eliminate *cis* isomers more quickly than *trans* isomers, yet both isomers exhibit no difference in their biological half-lives (ATSDR, 2018). The general lack of polarity and high lipophilicity of the terminal metabolites leads to more readily excretion of chlordane and its metabolites in the bile than in the urine. Substantial amounts of chlordane and/or its metabolites are also excreted via lactation and biliary via faecal excreta (ATSDR, 2018).

2.2.3 Dichlorodiphenyltrichloroethane

In the human body, DDT congeners are distributed into the lymph, blood, and the body lipid fatty tissues. DDT is metabolized in the human body into more stable metabolite *p,p'*-DDE, which is normally found at higher levels than DDT and DDD isomers. The adipose tissue storage affinity for DDT analogue increases from *p,p'*-DDD, *o,p,'*-DDT, and *p,p'*-DDT to *p,p'*-DDE reflecting the compound's lipophilicity (ATSDR, 2019a). Humans excretes DDT as DDA conjugates majorly through urine, and some excretion also occurs via faeces through biliary excretion and breast milk (ATSDR, 2019a). DDT isomers biological half-lives were ranked as DDE > DDT > DDD.

2.2.4 Endosulfan

Endosulfan accumulates in the brain, kidney and liver, amongst other human organs, for a short time and the α-isomer accumulates more than the β-isomer. In exposed individuals, endosulfan is readily metabolized into two stable stereoisomeric forms, the polar endosulfan sulphate and nonpolar endosulfan diol, by microsomal enzymes (ATSDR, 2015a). These can be further metabolized into endosulfan lactone, hydroxyl ether, and ether. However, almost half of the parent compounds in the tissues and excreta remain unchanged (Kuvarega and Taru, 2007). In humans and animals, endosulfan and its metabolites are eliminated mainly via faeces and urine, in addition to minor biliary and breast milk routes. Endosulfan biological half-lives are estimated to range from approximately 1 to 7 days in adult humans and animals.

2.2.5 Endrin

In the human body, endrin distributes into adipose tissues and skin. The major metabolic products of endrin are anti-12-hydroxyendrin and the corresponding sulphate and glucuronide metabolites. Endrin and metabolites such as endrin aldehyde, and endrin ketone are excreted in humans via sweat (ATSDR, 2019b; Iglesias-González *et al.,* 2020). Endrin is not persistent in human tissues and blood, therefore exhibiting a short half-life of about 2 days (ATSDR, 2019b).

2.2.6 Hexachlorocyclohexane

Hexachlorocyclohexane (HCH) isomers are absorbed by humans and distributed into adipose tissue, the brain, kidney, muscle, blood, and other tissues (ATSDR, 2005). β-HCH which has an estimated half-life of between 7.2 and 7.6 years in the human body, is the most stable isomer. γ-HCH may be transformed into hexachlorobenzene pentachlorophenol and pentachlorobenzene in the human body (ATSDR, 2005). HCH isomers are excreted primarily through urine, milk, and semen (ATSDR, 2005).

2.2.7 Heptachlor

Heptachlor is absorbed and distributed into human adipose tissues and skin, where it is stored or metabolized into heptachlor epoxide (ATSDR, 2007). Increased levels of heptachlor epoxide in adipose tissues (fat, serum, liver, and brain) are due to exposure to heptachlor or chlordane. Heptachlor is a metabolic product of chlordane, and heptachlor epoxide is also a minor metabolite of chlordane (ATSDR, 2007).

2.2.8 Hexachlorobenzene

Hexachlorobenzene (HCB) is moderately absorbed and distributed into human adipose tissues with high lipid content (ATSDR, 2015b). There is a differential release of HCB from adipose tissues, possibly as a function of lipophilicity (ATSDR, 2015b). In the human body, HCB is metabolized to pentachlorophenol and pentachlorothiophenol. HCB is mainly excreted unmetabolized through breast milk, faeces and urine (ATSDR, 2015b).

2.2.9 Methoxychlor

Once in the human gastrointestinal tract, methoxychlor is absorbed and circulated to body tissues, with high levels typically found in fat. In the liver, methoxychlor is metabolized rapidly; therefore, the parent methoxychlor compound and metabolites do not accumulate in fat or other tissue but are excreted rapidly in the urine and faeces via biliary excretion (ATSDR, 2002b).

2.2.10 Mirex

Mirex is absorbed and distributed widely in the human body and is sequestered in the lipids of the adipose tissues. Mirex is not metabolized in humans and is mainly excreted unchanged through faeces and human milk (ATSDR, 2019c).

2.2.11 Polychlorinated Biphenyls

Polychlorinated biphenyls (PCBs) are absorbed and distributed readily into human adipose tissues according to their lipophilic nature (Faroon, 2011). Polar metabolites of PCBs can undergo conjugation with glutathione and glucuronic acid (Faroon, 2011). Some PCB analogous have been found to persist in the human body for months or years due to their poor metabolism (Faroon, 2011). With long-term occupational and/or environmental exposure, an enzyme is induced and can make some PCBs less persistent in exposed humans than in the general population (Faroon, 2011). PCBs congeners and their metabolites are excreted slowly through breast milk, faeces, and urine.

2.3 Human health effects of persistent organic pollutants

Toxicological research have revealed that POPs adversely affect human health (Çok *et al.,* 2012; Mansour, 2009). Human health effects (Table 2.1) caused by POPs include defects in neurodevelopment, immune system development, haematological disorders, thyroid and estrogen dysfunctions, malformed genitalia, abnormal mating behaviour, decrease in testosterone level in males, stimulation of cancer, infants born with retarded growth, delayed cognitive development, induction of diabetes mellitus, insulin resistance, increased otitis media, Alzheimer's and amyotrophic lateral sclerosis, and death (Jayaraj *et al.,* 2016).

According to Jayaraj *et al.* (2016), POP exposure causes changes in low-density lipoprotein-cholesterol over time and increases oxidative stress markers in plasma, influencing the complement system and therefore, stimulating the immune system. According to Jayaraj *et al*

(2016) and ATSDR, (2019a), there is a decrease in birth weights of infants associated with prenatal exposure to POPs, which include *β*-BHC, DDT, HCB and mirex.

Jayaraj *et al* (2016) reported hypertensive disorders in pregnancy and gestational diabetes mellitus related to chronic chlordecone exposure. Also Jayaraj *et al* (2016) found an increase in the levels of fasting glucose and insulin, both markers of diabetics, as well as body mass index, cholesterol, and triglyceride, which are the indicators of obesity. Likewise Jayaraj *et al* (2016) linked heptachlor exposure to electron transport chain complex III impairment, resulting in mitochondria-mediated cell death. This, therefore, makes heptachlor act as a neurotoxicant with associated Parkinson's disease.

Table 2.1: Biochemical effects of studied Persistent Organic Pollutants

Compound	Biochemical effects	References
Aldrin and Dieldrin	Neurotoxic, tumerigenic effects, nausea, reproductive, genotoxic, developmental, immunological, vomiting, Parkinson's disease, muscle twitching, and aplastic anaemia.	(ATSDR, 2002a) and Jayaraj *et al.,* 2016
Chlordane	Convulsions, tremors, mental confusion, and incoordination	(ATSDR, 2018)
BHC/HCH	Cysts in hands; itching; psoriasis; eczema; leukoderma; skin rashes.	Jayaraj *et al.,* 2016
DDT	Prickling sensation of the mouth, confusion, headache, dizziness, incoordination, lethargy, nausea, vomiting, also anaemia, anorexia, anxiety, fatigue, hyperexcitability, muscular weakness, nervous tension, and tremors in the extremities,	(ATSDR, 2019a) and Jayaraj *et al.,* 2016
Endosulfan	Reduces the amount of white blood cell and macrophage movement and impairs the immune system due to their effects on humoral and cell immune mediators. Distresses sperm morphology, damages sex hormones, DNA and mutation, semen quality, sperm count, and spermatogonial cells,	Kuvarega and Taru, 2007 and Jayaraj *et al.,* 2016
Lindane	Induces birth defects, cancer, reproductive toxicity, causes hepatotoxicity and neurotoxicity, and damages the human body organs (liver and kidney) and systems (i.e., immune and neural).	(ATSDR, 2005)
PCB	Cause short-term memory and neurological disorders.	Faroon, 2011

According to ATSDR (2019b), endrin activate in men anti-androgenic effects and in women estrogenic effects. Jayaraj *et al* (2016) relates low exposure doses of organochlorine to neurotoxic effects on early psychomotor development and occurrences of gallstone disease. Likewise, Jayaraj *et al* (2016) reported an interference with thyroid hormone status due to neonatal exposure to endocrine-disrupting OCPs and vitamin D deficiency due to exposure to some OCPs in humans.

2.4 Sources and routes of human exposure to persistent organic pollutants

The studied POPs were extensively used globally in agriculture and public health for the control of pests and vectors and in the industry as components in electrical equipment and paints (El-Shahawi *et al.,* 2010; Loha *et al.,* 2018). Despite a global ban on the release of some POPs into the environment, which Kenya implemented more than three decades ago (Table 1.2), the compounds and their metabolites are still found in foods like fish and human subjects, even in remote instances of use (Table 2.2), and globally in human subjects (Table 2.3), an indication of the compounds' presence in the environment. This could mainly be due to the compounds persistence and leakages from stockpiles, illegal use, or the importation of contaminated food and/or feed products from regions where POPs are still in use (UNEP, 2013).

The US. EPA (2002) and Du (2016) reported that POPs are transported a great distance in the atmosphere when they volatilize from plant and soil surfaces. Also, POPs captured in the soil can be available for ultimate volatilization virtually at infinitum or, in other respects, chemically, thermally, or microbially degraded (Bruce-Vanderpuije *et al.,* 2019). This way, wildlife, humans, and other organisms are exposed to POPs on several occasions over protracted periods or through generations. Table 2.4 summarizes possible pathways (i.e., ingestion, inhalation, or dermal contact) through which humans get exposed to POPs from conception, through the placenta, and during breastfeeding, contingent upon the parent's previous or current exposures (FAO-WHO, 2019). However, humans are largely exposed to

POPs through ingested foods, mainly those with high fat content such as fish, beef, dairy, and poultry. Fruits, vegetables, and grains, due to their low-fat content, accumulate limited amounts of POPs (Noren, 1983). Also, considerable dermal, inhalation, or accidental ingestion exposure happens in or around residential places (Damalas and Eleftherohorinos, 2011).

Table 2.2: Studies on Stockholm Convention-Listed Persistent Organic Pollutants in Kenya

Matrix (Sampling Site)	Compounds (Result)	Sampling Year	Reference
Soil (Kapsabet, Nyeri and Voi)	*α*-HCH (ND - 7.52), *γ*-HCH (ND-1.32), *β*-HCH (ND-37.8), *δ*-HCH (ND-2.25), HCHs (0.03-48.1), *p,p'*-DDE (ND-3.3), *o,p'*-DDT (ND-8.75), *p,p'*-DDD (ND-3.46), *p,p'*-DDT (ND-11.6) and DDTs (ND-19.6) ug/g lipid.	Not Indicated	(Mungai and Wang, 2019)
Soil (Kiambu to Mombasa 449 km stretch)	*α*-HCH (0.3-0.62), *β*-HCH (2.51-7.66), *γ*-HCH (0.50-0.87), δ-HCH (1.17-3.01), HCHs (4.48-12.16), PCB28 (1.14-1.95), PCB52 (4.66-10.38), PCB101 (0.88-1.29), PCB118 (0.92-1.27), PCB138 (0.81-1.57), PCB153 (0.77-2.81), PCB180 (0.73-1.52), PCBs (9.90-20.80), *p,p'*-DDE (ND-1.99), *p,p'*-DDD (0.17-0.67), *p,p'*-DDT (0.68-4.29), DDTs (0.85-6.96), Heptachlor (0.92-22.22), Heptachlor-epoxide (0.39-3.22), Heptachlors (1.31-25.44), *α*-EDS (0.14-0.61), *β*-EDS (0.07-0.71), Endosulfan-sulphate (0.79-2.57), Endosulfans (1.00-3.89), Aldrin (0.31-2.73), Endrin (0.24-5.98), Enldrin-aldehyde (0.05-0.23), Methoxychlor (0.23-18.70), others (0.83-27.64), OCPs (8.47-76.1) ng/g.	Not Indicated	(Makokha *et al.,* 2018)
Soil (Mai Mahiu, Narok, Mount Suswa Conservancy, Juja and Limuru)	*α*-HCH (ND-1.62), *β*-HCH (ND), *γ*-HCH (ND-1.5), *δ*-HCH (ND-4.26), ∑HCHs (ND-7.38), p,p'-DDE (ND-1.84), *p,p'*-DDD (ND-4.87), *p,p'*-DDT (ND-23.43) and DDTs (ND-30.04), Heptachlor (ND-7.3), Heptachlor-epoxide (ND-1.31), Heptachlors (ND-8.61), *α*-EDS (ND-1.91), *β*-EDS (ND-1.86), Endosulfan sulphate (ND-12.18), Endosulfans (ND-15.95) others (ND-17.17), PCB28 (ND-9.83), PCB52 (ND-7.86), PCB101 (ND-13.83), PCB138 (ND-2.77), PCB153 (ND-21.95), PCB180 (ND-8.13), PCB118 (ND-9.72), PCBs (ND-55.49), ug kg^{-1} dw	January, 2015	(Sun *et al.,* 2016)
Fish (Lake Victoria)	PCBs: 28, 52, 101, 118, 138, 153 and 180 PCBs = 300-3,000 ug/kg lw	2014	(Oluoch-Otiego *et al.,* 2016)
Fish (Kiambu)	*α*-HCH (0.025-0.383), *γ*-HCH (0.013-0.169), *β*-HCH (ND), Heptachlor (ND-0.080), Aldrin (ND-0.241), Heptachlor epoxide (ND-0.068), *p,p'*-DDE (ND-0.034), Dieldrin (0.028-0.086), *o,p'*-DDD (ND-0.032), Endrin (ND-0.04), *o,p'*-DDT (ND-2.098), *p,p'*-DDD (0.116-1.684), *p,p'*-DDT (0.122-0.916) μg kg^{-1}.	September, 2011	(Omwenga *et al.,* 2016)
Fish (Machakos)	*α*-HCH (ND-0.013), *γ*-HCH (0.013-0.073), *β*-HCH (ND), Heptachlor (ND-0.014), Aldrin (ND-0.035), Heptachlor epoxide (ND-0.05), *p,p'*-DDE (0.004-0.037), Dieldrin (ND-0.057), *o,p'*-DDD (ND-132), Endrin (ND), *o,p'*-DDT (ND-0.033), *p,p'*-DDD (0.003-0.097), *p,p'*-DDT (0.005-0.158) μg kg^{-1}.	September, 2011	(Omwenga *et al.,* 2016)

Matrix (Sampling Site)	Compounds (Result)	Sampling Year	Reference
Water and Sediment (Lake Victoria)	*p,p'*-DDT, *γ*-HCH, *α*-HCH, *β*-HCH, *δ*-HCH, aldrin, dieldrin, heptachlor, heptachlor epoxide, *p,p'*-DDE, *p,p'*-DDD, endrin, endrin, aldehyde, Endosulfan sulphate, methoxychlor and Endosulfan (Water µg/L: - dry season ND-9.84±1.20; wet season ND-15.53±0.20, Sediment µg/Kg: - dry season ND-32.91±3.84; wet season ND-24.84 ± 2.65.	September, 2012 to May, 2013.	(Osoro *et al*., 2016)
Fish and Invertebrate (Lake Naivasha)	*α*-BHC (0-15), *β*-BHC (0-287), Endosulfan sulphate (0.002-0.09), dieldrin (0.002-0.01), Endrin aldehyde (ND-0.05), *p,p'*-DDT (0.002-0.01), *p,p'*-DDE (0.002-0.01) and *p,p'*-DDD (ND-0.015); DDTs (0.002-0.03) ppm dw.	Not Indicated	(Chelsea, 2012)
Fish (Indian Ocean Kenya)	aldrin (1.55-323), *p,p'*-DDD (1.68-98.9), *p,p'*-DDE (1.0-97.5), *p,p'*-DDT (9.11-29.3), dieldrin (4.81-109), Endosulfan (5.91-54.6) and lindane (16.1-1445). mg kg^{-1} wet weight.	January-May 2001	(Barasa *et al.,* 2008)
Fish – Nile tilapia and Perch (Lake Victoria)	Lindane (0.74-0.87), aldrin (0.28-0.48), *α*-endosulfan (1.70-1.45), dieldrin (0.18-0.30), *p,p'*-DDE (0.8-0.86), *p,p'*-DDT (0.59-0.81) µg kg^{-1}	1998	(Kasozi *et al.,* 2006)
Water and Fish (River Tana)	Aldrin (0.037: <0.007); Endosulfan (0.037: <0.007); Dieldrin (0.037: <0.007); Endrin (0.037: <0.007); DDT (0.037: <0.007); DDE (0.037: <0.007); DDD (0.037: <0.007) and Lindane (0.037: <0.007) µg l^{-1} water: ng g^{-1} Fish.	January and July, 1998	(Lalah *et al.,* 2003)
Fish and Bassa (Lake Naivasha)	DDTs 9.2 and 78.6, Lindane 100.5 and 2, Endosulfans 21.6 and 2 Dieldrin 34.6 and 2 µg kg^{-1}.		(Gitahi *et al.,* 2002)
Human Milk (Nairobi)	*p,p'*-DDT (2-258); *p,p'*-DDE (3-4818); *o,p'*-DDD (125-273); *o,p'*-DDT (2-443); *p,p'*-DDD (3-209); DDTs (4 6321); *p,p'*-DDT/*p,p'*-DDE (13-11991); *α*-HCH (2-38); *β*-HCH (3-6); Lindane (2-134) and Dieldrin (4273) ng g^{-1} wet weight.	1991	(Kinyamu *et al.,* 1998)
Human Milk (Across Kenya)	*p,p'*-DDT (0.06-44.53), *p,p'*-DDE (0.02-32.981); DDTs (0.02-69.87), *p,p'*-DDT/*p,p'*-DDE (0.2-20.7) Aldrin (0.006-0.388), Dieldrin (0.008-1.685), *β*-HCH (0.002-0.502), *γ*-HCH (0.002-0.502) and HCB (0.001-0.049). µg kg^{-1} lipid.	1983 - 1985	(Kanja, 1988)
Birds (Nakuru)	*α*-BHC (0-15), *β*-BHC (0-287), *γ*-BHC (0-45), DDD (0-194), DDE (10-3672), DDT (63-2541), heptachlor (0-6), heptachlor-epoxide (0-12) and lindane (0-3) mg kg^{-1} wet weight.	1/9/1990 to 31/9/1990	(Kairu, 1994)
Birds Eggs (Lakes)	Naivasha; DDE (Trace-107), Nakuru; DDE (Trace-350), Baringo; DDE (Trace-143) µg l^{-1}.	1970 - 1972	(Lincer *et al.,* 1981)

Table 2.3: Global studies on Stockholm Convention-Listed Persistent Organic Pollutant

Country (Sample Size)	Compounds and Results (ng/g lw)	Sampling Year	Reference
Australia (40)	DDTs (79.8)	2013 -2015	(Du *et al.,* 2017)
Belgium (206)	DDTs (56.63), HCB (5.57) and HCHs (2.92)	2019	(Aerts et al., 2019)
China (1760)	Chlordane (3.38), DDTs (1105), endosulfan (1.90), HCB (35.1), HCHs (638) and 6iPCBs (9.63)	2019	(Hu *et al.,* 2021)
Ghana (128)	6iPCBs (3.64)	2014-2016	(Asamoah *et al.,* 2018)
India (150)	DDTs (519.2), endosulfan (6.4), HCHs (46.6) and 6iPCBs (33.7)	-	(Bawa *et al.,* 2018)
Iran (50)	DDT (2345), HCB (570) and HCH (2617),	2017	(Shahmoradi *et al.,* 2019)
Jordan (120)	DDT (0.421) and HCH (0.19)	2014-2015	(Alawi *et al.,* 2017)
Mexico (50)	Chlordane (1.82), DDT (622.23), HCH (6.14) and 6iPCB (4.46)	2018	(Martínez *et al.,* 2022)
Taiwan (55)	Chlordane (0.244), DDT (12.015), endosulfan) (1.492) and HCH (0.72)	2007-2010	(Kao *et al.,* 2019)
Tanzania (47)	DDT (135), HCB (1.41), HCH (0.9) and 6iPCBs (4.45)	2012	(Müllera *et al.,* 2019)
Turkey (100)	DDT (333.53), HCB (12.23), HCH (196.52) and 6iPCBs (1874)	2013	Erog˘lu, *et al.,* 2018

Table 2.4: Known routes through which human are progressively expose t Persistent Organic Pollutants

Life Stage	BC	P	HM	IF	ISDM	Inhalation	Dermal
Ovum/sperm	√						
Fetus		√					
Infant			√	√	√	√	√
Toddler			√	√	√	√	√
Child				√	√	√	√
Adolescent				√		√	√
Adult				√		√	√

Notes: From (FAO-WHO, 2019). BC = Before Conception, P = Placenta, HM = Human milk, IF = Ingested food, ISDM = Ingested soil, dust, mouthing of objects.

POPs typically get into the food chain via watercourses, air transport, and persistent deposits in the soil (Table 2.5). Once in the watercourses, POPs gather in sediments, which invertebrates ingest and accumulate in their fatty tissues. Fish, especially mature ones, are to be expected to contain higher POP residues due to gradual accumulation after eating POP-exposed invertebrates (Agbohessi *et al.,* 2015). Other human food substances can be contaminated via the transfer of compounds from contaminated marine and/or agricultural food chains, during

food production or food processing, or as a result of leakage from food packaging (Connell *et al.,* 2007; Domingo, 2017; Thompson *et al.,* 2017; Thompson and Darwish, 2019). Because of biomagnification, humans at the top of the food web accumulate the highest POP concentrations (WHO, 2010). This means that human intake of POPs varies according to diet, nature of contact, and geographical area of residence.

Table 2.5: Sources of human exposure to Persistent Organic Pollutants compounds

Source	Means of POPs entry into the human body
Food	POPs deposits in waterways are taken up by invertebrates and accumulated by fish and other animals consumed by human.
Soil	POPs residues transported by air currents and storms systems deposits or precipitate on soil or solid surfaces where human can ingest or absorbed through the skin.
Indoor environment	POPs in materials such as building materials, furniture, textiles, carpets, and curtains, packing materials, electric and electronic appliances are inhaled, ingested, or absorbed by humans.
Air	POPs release from contaminated burning items or transformers are inhaled

Adopted from FAO-WHO, 2019.

Human dietary exposure to POPs is compelled primarily by the ingestion of foods of marine or terrestrial animal origin (Alharbi *et al.,* 2018; Buccini, 2006; Connell *et al.,* 2007; Darnerud *et al.,* 2006; Domingo, 2017; Mwakalapa *et al.,* 2018; Patton, 2009; Thompson *et al.,* 2017; Thompson and Darwish, 2019). POPs have been found in human biological samples across the globe, with positive associations between their occurrence levels and consumption of fish, meat, and dairy products (Aerts *et al.,* 2019; Grešner *et al.,* 2021; Hassan *et al.,* 2022; Matei *et al.,* 2023; Montano *et al.,* 2022; Zahira *et al.,* 2021).

2.5 Human milk and Persistent Organic Pollutants

Guided by medical and psychological studies that emphasized the considerable benefits of breast milk to infants, the WHO urged six months of postnatal exclusive breastfeeding (Pajewska-Szmyt *et al.,* 2019; WHO and UNICEF, 2003). Notable benefits of breast milk to infants include decreased rates of infectious disease (immunity), psychological benefits, and

increased rates of growth and development (Patton, 2009). There is an increasing interest in exclusive breastfeeding, just as there is a corresponding upsurge in apprehensions over the excretion of xenobiotic compounds with potential adverse health effects into breast milk (Solomon and Weiss, 2002). The familiar maxim "we are what we eat" illustrates the connection of human bodies with the technologically advanced environment from which food, drinks, and habitations are obtained. The mother's body becomes the environment for their children throughout pregnancy and through breastfeeding, owing to a maternal-infant dyad (Chemek and Nevoral, 2019; Kramer and Kakuma, 2012; Sarkar, 2009; UNEP, 2013). Therefore, assessing the risk-benefit ratio of breastfed infants represents one of the most puzzling facets of human Toxicology (Malisch *et al.,* 2017).

While many matrices can be used to evaluate human exposures to POPs, compound-specific factors limit the choices. The physicochemical properties of POPs (Appendix 7), which control their metabolism and excretion routes, influence the selection of the study matrix. The first study to evaluate POP residues in HM, Laug *et al,* (1951), reported substantial exposure of apparently healthy Black American women to DDT. Many other lipid-soluble POPs, which include chlordane, *oxy*-chlordane (a chlordane stable metabolite), *p,p'*-DDT, *p,p'*-DDE, *trans*-nonachlor, *cis*-nonachlor, HCB, -HCH, aldrin, dieldrin, mirex, and toxaphene, have been reported in HM globally (Jensen, 1983; Kanja, 1988; Kannan *et al.,* 1992; Mochungong and Zhu, 2015; Olisah *et al.,* 2020; Polder *et al.,* 2003; Salem and Ahmed, 2002). The majority of studies that examine one persistent contaminant usually examine other related compounds due to their characteristic similarities, such as lipophilicity (Aerts *et al.,* 2019; Luzardo *et al.,* 2014; Müller *et al.,* 2019).

Testing for POPs in HM epitomizes information on maternal body burden and the infant's exposed dose (Fång *et al.,* 2015). Lipid-adjusted POP levels in HM correlate well with those obtained from the blood of the same mother (Kanja *et al.,* 1992; Solomon and Weiss, 2002).

Also, HM offers a comparatively simple, non-invasive sampling procedure and integrates environmental or dietary exposures in relation to different consumption behaviours at high trophic levels (Bruce-Vanderpuije *et al.,* 2019). Certain cultures, though, could make it difficult to obtain HM samples as it may be considered taboo or stressful. An important consideration in the choice of HM as a biological indicator is that only a section of the population based on gender and age is covered.

Over the last seven decades, HM has been used as a biomonitoring medium with well-thought-out pointers of the actual human body burden of xenobiotics (Du *et al.,* 2016; Massart *et al.,* 2008). The collection of HM is considered non-invasive and appropriately accessible (GMP-TWG, 2004; Stockholm Convention Secretariat, 2007, 2011). Additionally, due to the high lipid component of HM, the extraction and purification of the xenobiotics are easy and highly accurate (Malisch *et al.,* 2017). More importantly, analytical techniques for most POPs listed in the Stockholm Convention in HM have evolved with time (Wang *et al.,* 2005).

2.5.1 Factors influencing POPs incorporation into human milk

Maternal lactation incorporates and retains POPs into HM when milk components pass from capillaries to the alveolar epithelial cells of the breast, fortified by biological activities, milk lipid content, and POPs lipophilic characteristics (Larry *et al.,* 2002). This progressively decreases maternal POP levels in a process known as ‘‘depuration’’. The pH and POP binding abilities of plasma and milk play a crucial role in modulating POP concentrations in plasma and milk (Needham *et al.,* 2002). Consequently, higher levels of weak alkaline POPs than weak acid POPs are transferred to HM from plasma (Needham *et al.,* 2002).

2.6 The determining factors for the maternal body burdens of POPs

The maternal body burdens of POPs are determined by factors that include occupation, household use of pesticides, age, body mass index, and parity (Dimitriadou *et al.,* 2016), diet (Malarvannan *et al.,* 2009), lactation period (Lovelady et al., 2002), tobacco use (Kanja, 1988),

and area of residences (Kanja, 1988).

Lignell *et al* (2011) found that older individuals have high body burdens of POPs, and (Albers *et al* (1996) reported lower POPs levels in nursing mothers and infants with increased body mass index (BMI) at sampling, whereas Mamontova *et al,* (2017) reported increased POPs blood levels when an individual losses weight. Alharbi *et al* (2018), Pohl *et al* (2007), and The National Academies Press (2006) studies found more POPs in blood of males than in females. LaKind *et al* (2004) found no association between the occurrence and quantities of POPs and smoking; however, Kanja *et al* (1988) stated on the contrary i.e., "non-smokers had lower levels of DDT in their milk than mothers who smoke".

Ingesting foodstuffs contaminated with POPs led to the proportional occurrence of POPs in human tissues. However, Kanja *et al* (1988) and Massart *et al* (2008) establish no correlations between the occurrence levels of organochlorines residues in human milk and those of the donor's favourite foods. Foods of animal origin exhibit high occurrence levels of POPs owing to biomagnification through the biotic food chain (Chung and Chen, 2011; La Merrill *et al.,* 2013; Muntean *et al.,* 2003; Noren, 1983; Stakeholders, 2014). According to Asamoah *et al* (2018) and Olisah *et al* (2020), HM from Lacto-vegetarian mothers has the lowest concentrations of dieldrin, dioxins, *p,p'*-DDE, *p,p'*-DDT, *β*-HCH, heptachlor epoxide, *oxy*-chlordane and PCBs when compared with HM from regular fish-eating mothers.

Schade and Heinzow (1998), Sarkar (2009), and Hardell *et al* (2010) studies inferred that with an increase in parity and breastfeeding there is a corresponding decrease in the occurrence levels of POPs in HM. In this respect, Lignell *et al* (2011) demonstrated that nursing affects blood levels of POPs.(WHO, 2015) reported an association between exposures from polluted indoor environments and HM.

2.7 Review of methodology

2.7.1 Study design

Realistic, transparent, credible, and scientifically robust study hypotheses and goals guide the design of a biomonitoring study (WHO., 2015). When the study involves human participants, their rights and well-being are to be protected (Schulz *et al.,* 2007). According to Bates (2005), a study design ought to guarantee proper long-term samples storage in order to permit future usefulness and access by researchers who did not participate in the study at the start. Biomonitoring biomarkers are carefully chosen based on their effects on human health, the need for their public health data, their ability to bioaccumulate with known exposure in human body tissues, the ability to collect and analyse the most appropriate matrices, and the availability of suitable analytical techniques (Bates *et al.,* 2005).

Matrix selection depends on knowledge of its applicability, biological significance, and complexity (Bates *et al.,* 2005). Therefore, matrix selection is a trade-off between scientific issues, ease of sampling, invasion and risks, and cost of sampling and laboratory analysis.

The degree to which a study findings are inferred to the broader population depends on the study's sampling frame (Bates *et al.,* 2005). The most ideal sampling frame for a biomonitoring study of a well-defined population is cross-sectional random selection, which ought to be accompanied by an adequate level of participation agreement. However, self-selection or volunteering, the use of specific characteristics or features, for example, breastfeeding women, and low degree of participation have been used, and results have been inferred for the general population (The National Academies Press, 2006).

2.7.2 Sample collection, transport, and storage

Validated protocols suitable for human milk and POPs are used in sample collection, labelling, transport, and storage (WHO/UNEP, 2007). Sample collection protocols greatly influence the lipid content and therefore POPs detection possibility of sampled HM, mainly due to maternal

factors during lactation (Lovelady *et al.,* 2002; Malisch *et al.,* 2017). For example, the highest HM production occurs early in the morning, and the lipid content reaches its peak at mid-morning, followed by a steady decline during the day (Lovelady *et al.,* 2002). In addition, HM obtained at the end of single breastfeeding has a higher lipid content than milk obtained immediately before breastfeeding (Kanja, 1988 and Lovelady *et al.,* 2002). Therefore, sampling protocols are to ensure that the components of all human milk samples are very similar. Table 2.6 presents some maternal factors and their effects on the human milk total lipid content.

Table 2.6: Factors which Causes Variations in the Total Lipid Content of Human Milk

Factor	Effect
Nursing or breastfeeding period	Increases
Lactation stage (postpartum age),	Increases
Diurnal rhythm	Depends on maternal meals at sampling time.
Breasts difference	Occurs
Full gestation or not	Occurs
Regional diet	Possible
State of nutritional.	Decrease in underfed mothers
Much carbohydrate, less-fat diet	Expected to decrease
Infections, metabolic disorders	Usually decreases
Medication	-
Mothers menstrual cycle or pregnancy	-
Parity	Decreases
Season	Related to diet and region
Age	-
Individuality	Adiposity increases
Miscellaneous	-

Note: Adapted from (Jensen**,** 1999). (-) Missing information.

Adhering to sampling protocol leads to accurate interpretation and comparison of findings within and between studies. Appropriate storage conditions are critical in the collection and analysis of human matrixes; therefore, adequate hygiene and care ought to be observed when handling, storing, preserving and analysing HM (Bates *et al.,* 2005). Plastic surfaces have the potential to contaminate the matrixes intended for POP analysis, causing analytical interferences brought about by plasticizers among other rubber and plastic components (WHO/UNEP, 2007).

2.7.3 Laboratory methods applicable for the analysis of selected POPs

Rigorous quality assurance and control procedures are required in the analysis of POPs residues in HM (Stockholm Convention Secretariat, 2013). These include the use of calibrated equipment and reference materials, the use of operational blanks, standard spike recoveries, establishing concentrations at which the equipment and method used are limited, and assigning values to analytical results (Leslie *et al.,* 2013; Muir and Sverko, 2006; Xu *et al.,* 2013).

Generally, the analysis of POP residues in HM involves extraction using suitable solvents, fat determination, extracts clean-up (and, occasionally, fractionation), and POP chromatographic separation, identification and quantification, mostly using flame ionization or mass spectroscopy (Stockholm Convention Secretariat, 2013).

2.7.3.1 **Samples preparations (POPs extraction) technique**

According to Muir and Sverko (2006), OCPs and PCBs residues have been extracted from HM using liquid-liquid extraction (LLE), solid-liquid extraction (SLE), solid-phase micro-extraction (SPME), solid-phase extraction (SPE), and QuEChERS techniques. These techniques employ Soxhlet, ultrasonicate, or microwave digester equipment. Extraction separates the lipid and lipophilic compounds from the entire milk matrix.

In Soxhlet extraction (SE), lipid and lipophilic compounds are repeatedly washed down (reflux) from dried milk samples held in Soxhlet apparatus using appropriate organic solvent(s) (Domingo, 2017; Tang, 2013). The technique, although time-consuming and less eco-friendly, is effective and uses simple apparatus.

Ultrasonic extraction (UE) appropriates lipophilic compounds from a permeable solid matrix by means of an energized organic solvent (Zhao *et al.,* 2005). The extraction process is normally repeated two or more times, therefore making it time-consuming and laborious (Ana-Andreea *et al.,* 2018; Muir and Sverko, 2006). The ultrasonic water baths are normally cheap

instruments; nonetheless, the extraction is less effective as compared to traditional Soxhlet extraction.

Microwave-assisted extraction (MAE) facilitates the extraction of POPs compounds by heating suitable organic solvent(s) from a permeable dried matrix (Henríquez-hernández, 2018). MAE and ultrasonic extraction have the same advantages and disadvantages, though the MAE technique exhibits limitations based on uneven dispersion of the microwaves, leading to moderate reproducibility (Henríquez-hernández, 2018).

Accelerated Solvent Extraction (ASE) operates under raised temperature and pressure, therefore allowing the use of organic solvent(s) as liquids above their boiling points and reducing both extraction time and solvent consumption (Sun *et al.,* 2005). A permeable freeze-dried matrix is treated with appropriate organic solvent(s) at suitable set ASE equipment temperature, pressure, and static cycles (Tang, 2013). ASE applies simple and rapid operation mechanisms and can be automated with the advantage of excellent recovery rates and the recovery of thermally labile compounds (LeDoux, 2011). However, equipment and associated maintenance costs are prohibitive.

Supercritical fluid extraction (SFE) separates compounds from the human matrix employing supercritical fluids such as carbon dioxide (Bavel *et al.,* 1995; García-Rodríguez *et al.,* 2008; Sánchez-Camargo *et al.,* 2019). SFE has the advantage of using lower critical temperatures, a short extraction period, and thus decreasing the quantity of organic solvents (Bavel *et al.,* 1995). SFE has an additional practical advantage of fractionating the extract. However, while equipment and associated maintenance costs are excessive, polar compounds are not extractable without polar modifiers (Sánchez-Camargo *et al.,* 2019).

QuEChERS constitute a custom-made sample preparation technique that is still under development (Bayat *et al.,* 2019; Luzardo *et al.,* 2013). This technique extracts POPs from milk samples in a Teflon centrifuge tube using the extracting solvent mixed with a dispersant to

obtained fairly pure extract. The use of small samples eliminates extract concentration steps because decreased amounts of sorbent and solvent are used, reducing extraction time.

2.7.3.2 **Samples preparations (POPs extract clean-up) technique**

Before POP compounds separation and determinations, HM extracts are cleaned up by separating lipids from POPs compounds. This is achieved by the use of chromatography i.e., solid-phase extraction and/or gel permeation chromatography (Needham and Wang, 2002). The chromatographic cleaning method is very selective with concentration effect, which contributes to high recoveries and reproducibility gives good results for diverse matrices. Chromatography uses either preparative column chromatography or commercially filled cartridges packed mostly with alumina, florisil or silica adsorbent (Lucci *et al.,* 2012).

Appropriate organic solvent is first used in order to wet and rinse the column, after which the extract is introduced, followed by elution solvent in small portions (Mamontova *et al.,* 2017). POPs elution solvents can be n-hexane, acetonitrile or combinations such as n-hexane and dichloromethane, and n-hexane and acetone (Pihlström *et al.,* 2019). Compounds partition in different ways into the two phases (the solid phase and the liquid phase), therefore flowing through the column at different rates.

Depending on the available instrumentation, POP compounds can be cleaned once or treated first with either alumina or Florisil in order to remove lipids, followed by fractionation using silica gel (Kuvarega and Taru, 2007). The principle of gel permeation chromatography based on molecular sizes is to remove the lipids from the extracts using hydrophobic gels so as to obtain only pure mixture of the eluting organic solvent and the compound residues of interest. An automated GPC system with a column has been developed. This system principally allows large molecules to elute faster through the gel, followed sequentially by smaller molecules, with the very smaller molecules eluting last. The solvents that have been used in this system include acetone mixed with either cyclohexane or dichloromethane, and ethyl acetate combined with

cyclohexane or hexane (Henríquez-hernández, 2018). The hydrophobic gels commonly used are Bio-Beads S-X3 and Envirobead S-X3, and the equipment operates at between 4 to 5 millimetres per minute (Mamontova *et al.,* 2017). Gel permeation chromatography produces the same high reproducibility as SPE.

The disadvantages of this cleaning system are the columns limited loading capacity, which can lead to false results if overloaded. Also, the equipment and its operational cost limit its frequent utilization. One of the most critical disadvantages of the system is the incomplete separation of closely similar lipophilic molecules due to their molecular sizes (Badawy *et al.,* 2022).

Automated high-pressure liquid chromatography (HPLC) has also been used to clean OCs analytes from lipid coextracts, and it has been found to reduce the process period and consumption of solvents (Lucci *et al.,* 2012). The efficiency of this cleaning method has been increased by undertaking extraction and purification in a single-step method for some OCs from fatty foods (Stoytcheva, 2011).

2.7.3.3 Separation and determination of POPs compounds

The most popular technique used to separate individual OCP and PCB compounds in a mixture is chromatographic analysis (Mamontova *et al.,* 2017). In the chromatographic system, the compounds interact with two phases and get separated from each other based on their distribution co-efficiencies (Ni *et al.,* 2011). One of the phases is stationary while the other is a mobile phase, which can either be a gas, which is used in gas chromatography (GC), or liquid, which is used in liquid chromatography (LC). The stationary phases are embedded in an appropriate tube, normally referred to as a column, using end capped silica gels or solids silica molecules. So far OCPs and PCBs have been separated using capillary columns or quartz packed with fused cross-linked methyl silicon, usually with nitrogen as mobile phase or carrier gas. Helium, nitrogen, and argon have been used as make-up gas either combined or alone. In LC mobile phases are usually mixtures of aqueous (water), organic solvents (methanol or

acetonitrile) and mobile phase modifiers which include acids (acetic, formic, oxalic, trifluoroacetic or phosphoric), ammonium hydroxide among others (Dong and Boyes, 2020).

The analysis of OCPs and PCBs using a GC or LC equipment utilizes coupled detectors for the compound's identification and quantifications. GC has been used in connection with Electron Capture, Flame Ionization, and Mass Spectrometer detectors, while LC has been coupled with Mass Spectrometer in the analysis of OCPs and PCBs (Guo and Kannan, 2015; Xu *et al.,* 2013). As reported by Muir and Sverko (2006) GC-ECD setup has comparatively better sensitivity for OCPs and PCBs; however, matrix interference in the case of HM at a low occurrence level may give false-positive results.

In the current technology, a group of very powerful equipment for the separation, identification and quantification of POP compounds have emerged. These, as reported by Ana-Andreea (2018) include: "gas chromatography low resolution mass spectrometry (GC/LRMS), gas chromatography high resolution mass spectrometry (GC/HRMS), comprehensive two-dimensional gas chromatography coupled to time of flight high resolution mass spectrometry, high resolution gas chromatography or high resolution mass spectrometry, liquid chromatography or high-resolution mass spectrometry, and ultra-high-performance liquid chromatography coupled with mass spectrometry.

2.7.3.4 Analytical integrity

Fundamental to the analysis of POPs residues in complex matrixes such as HM, is trace amounts of interfering contaminants which might cause erroneous positive results and/or considerable equipment sensitivity loss preventing residues from being detected "false negative" (Document Nº SANTE/12682/2019, 2019). Contamination may occur across the analytical stages, such as sample collection, transport, storage, preparations and equipment analysis (Martínez-Domínguez *et al.,* 2014). Also, critical are the factors that affect HM stability, such as storage temperature, freeze-thaw cycles, and processing time.

Within-laboratory, method verification should be carried out to provide fit for purpose evidence. The analytical protocols should be checked for sensitivity or recovery as measure of trueness or bias and precision as repeatability RSD_r% to meet performance criteria for environmental contaminants residues quantifications Table 2.7.

Table 2.7: Performance Criteria for Residues Quantitative Methods Applicable to Milk Analysis

Concentration range (mg/kg)	Repeatability		Mean recovery range (%)
	RSD_A%	RSD_L%	
0.001-0.01	30	32	70-120
>0.01-0.1	20	22	70-120
>0.1-1	15	18	70-120
>1	10	14	70-120

Notes: From Document Nº SANTE/12682/2019, 2019; RSD_A%: relative Deviation of analysis, excluding any contribution due to sample heterogeneity. RSD_L%: relative Deviation of the laboratory results, including 10% sub-sampling heterogeneity.

These criteria are applicable to methods used to analyse several food commodities including milk from cows, goat and buffalo among other matrixes. Also, identification parameters such as compound ion ratio and retention time should not deviate by more than particular unit.

2.7.4 Statistical analysis

Questionnaire and laboratory data related to POP residues are cleaned up, organized, analyzed, and then correlated to the primary sources (WHO., 2015). Databases for each variable are checked for accuracy (Kothari, 2004). The variables might include access privileges, description, location, collection method, names, formats, codes and null value acceptance. The elementary features of the data and reference ranges are normally analyzed using descriptive statistics (Stockholm Convention Secretariat, 2013). Factors that affect the toxicokinetic of OCPs and PCBs which include age, alcohol, body mass index, diet, disease, genetics, medication, sampled population and smoking, are clearly defined before sample collections (Batt *et al.,* 2017; Ni *et al.,* 2011). Also as reported by APHL, (2018) descriptive analysis

always incorporate findings related to each OCP and PCB measured by using the number of samples and evaluating the number of samples with non-detected results, those within relevant percentiles and overall occurrence means within set critical confidence intervals. Percentiles help to explain the shape of the distribution curve generated by the biomonitoring data; for instance, the 90th or 95th percentile indicates that the occurrence quantities are unusually high (Lee *et al.,* 2019).

To estimate the central tendency of the analytical results geometric means and medians have been established in order to provide better results than arithmetic mean due to skewed distribution of biomonitoring data which usually is longer at the upper tail end (The National Academies Press, 2006). However, for data with non-detected results higher than 40% the use of geometric means is not appropriate (APHL, 2018).

But, inferential analysis is used to extrapolate the findings to the sampled population, test the relationship of group and/or individual results and to estimate the associations between samples environmental variables and the occurrence levels of POPs (Gibson *et al.,* 2016; Hites, 2019). Due to the skewed nature of biomonitoring data, the descriptive or inferential analysis is mostly undertaken using nonparametric methods or data log transformed before subjecting to other statistical methods depending on data type, explanatory variables and study objectives (Stockholm Convention Secretariat, 2019). Subject to study design, the extent of the correlation between exposure and effect is enumerated using cumulative occurrence or occurrence rates in a cohort study, and odds ratio in the case of a controlled study (Chen *et al.,* 2018).

2.7.5 Determination of health risk to breastfeeding infants of POPs exposed mothers.

Lactating mothers steadily excretes exposed POPs (depletes body burden) while breastfeeding infants get exposed (Kuang *et al.,* 2020). This presents the infants with increased potential risks as a result of biomagnified POPs along the food chain up to HM. Therefore, the need for estimation of daily intake of POPs by breastfeeding infants.

Common steps in estimating potential risks to breastfeeding infants due to POPs excreted by exposed mothers are: 1) analysis of levels of POPs in mother's milk, 2) calculation of estimated daily intake (EDI) of POPs by infant's, and 3) comparison of each compound EDI value with set human allowable daily intake risk limits presented in Table 2.7 (Kuang *et al.,* 2020).

Table 2.8: Acceptable daily intake of studied Persistent Organic Pollutants by an adult human.

Compound	MRL (mg/Kg/day) Whole milk			MRL (ng/Kg/day) Whole milk		
	Codex	ATSDR	EU	Codex	ATSDR	EU
Aldrin	0.006	0.00003	0.0001	6000	30	100
CHL	0.01	0.0006	0.002	10000	600	2000
DDT	0.02	0.1 µg	0.01	20000	100	10000
Dieldrin	0.0060	0.00003	0.0001	6000	30	100
Endosulfan	0.006	0.005	0.01	6000	5000	10000
Endrin	0.0002	0.3 µg	0.01	200	30	10000
HCH	-	0.0006	-	-	600	-
Heptachlor	0.0001	0.0001	0.006	100	100	6000
HCB	0.01	0.00007	0.01	10000	70	10000
Methoxychlor	-	0.005	-	-	5000	-
Mirex	-	0.3 µg	-	-	5000	-
PCP	-	0.001	-	-	1000	-
PCB	-	0.02 µg	-	-	20	-

Notes: - Not available.

Different studies have adopted certain assumptions in order to calculate or estimate the daily intake of POPs by a breastfeeding infant (Kuang *et al.,* 2020). These include the average infant's body weight, the HM average lipid content, and the average HM consumed by the infant per day Table 2.8 (Kuang *et al.,* 2020). The occurrence levels of POPs in mothers' milk changes progressively with the lactation period, therefore, using data from a short period of lactation to calculate an infant's EDI may not represent the real amount ingested by the infant (Kuang *et al.,* 2020). Consequently, WHO (2007) recommended two to eight weeks postpartum lactation period as ideal to represent the whole lactation period.

Table 2.9: Established infant's weight, consumed milk and HM lipid content

Average infant weight (kg)	Average milk consumed by an infant per day (g)	Average human milk lipid content %	Reference
5	700	3.62	(Asamoah *et al.,* 2018)
5	700	-	(Tsygankov *et al.,* 2019)
7	600	3	(Paul *et al.,* 1988)
3.5	750	-	(Polder *et al.,* 2009)
5.8	800	3	(Malgorzata *et al.,* 2011)
5.3	800	-	(Song *et al.,* 2020)

- Not available.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study areas

This study was carried out in Nairobi, Nandi, and Nyeri Counties, Kenya (Figure 3.1). The place of residency, especially urban or rural, has effects on POP human exposure levels (WHO/UNEP, 2007). Therefore, the study counties were purposively selected based on agricultural and commercial activities, urban and rural status, and the absence of previous studies on POPs. Locations adjacent to Stockholm Convention-listed POP stockpile deposits, industrial plants, waste dumping, and incineration sites were avoided (Malisch *et al.,* 2017).

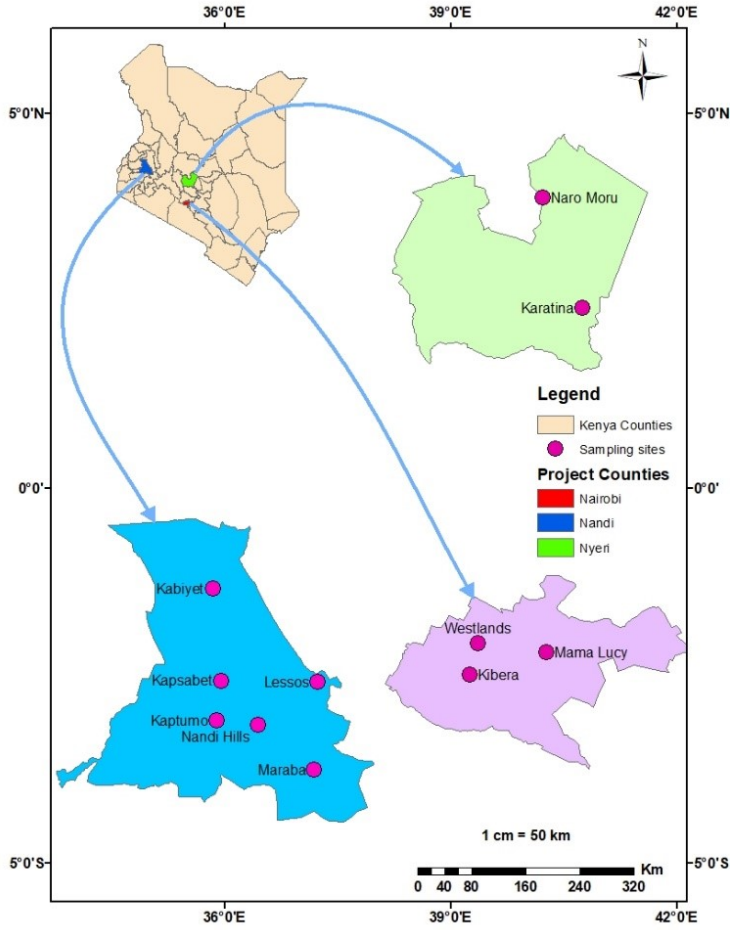

Figure 3.1: Maps of Nairobi, Nandi, and Nyeri Counties, Kenya

Within the selected counties, study subcounties were purposively picked cross-sectionally. The

main public health hospital (PHH) with a maternal and child health clinic (MCHC) was used as the sampling site within the subcounty.

3.1.1 Nairobi County

Nairobi County is an urban administrative, commercial, and industrial centre of Kenya. The County covers an area of 703.9 square kilometre and is between 1° 9’ and 1° 28’ S latitudes and 36° 4’ and 37° 10’ E longitudes, with an altitude of between 1,600 and 1,850 meters above sea level (Ndunda *et al.,* 2018). Nairobi County had 4,397,073 million inhabitants, or 6,247 persons per square kilometre, and an estimated growth rate of 3.43% per annum (KNBStats, 2019). Main activities include residential, commercial, industrial, automotive, open dumping of waste, and incineration of industrial and hospital waste. Human food comprises cereals, beans, milk, eggs, meat, chicken, fish, pork, fruits, and vegetables. Food sources are markets across the country including Lake Victoria, fishponds and the ocean for fish (Recha, 2018).

3.1.2 Nandi County

Nandi County is a predominantly agricultural rural area that is situated to the West of Nairobi within the Rift Valley and has an estimated 3.9% urban population that is distributed between Kapsabet and Nandi Hills urban centres (KNBStats, 2019). The County sits on a land mass of 2,856 square kilometres and is between 0° 4’ and 0° 38’ S latitudes and 36° 38’ and 37° 20’ E longitudes with an altitude of between 1,777 and 5,199 meters above sea level (NCIDP, 2018a). Nandi County has 885,711 inhabitants or 310 persons per square kilometres and an estimated annual population growth rate of 2.2% (NCIDP, 2018a). Main activities include subsistence agriculture, tea-growing estates, sugarcane, coffee, livestock keeping, tea factories, and human settlement. Human food comprises cereals, beans, milk, eggs, meat, chicken, fish, pork, fruits, and vegetables. Food sources are home and markets, including across the country, fishponds, and swamps for fish (Recha, 2018).

3.1.3 Nyeri County

Nyeri County is also a predominantly agricultural rural area that is situated in the Central Highlands of Kenya and has an estimated 12.9% urban population that is scattered across Nyeri and Karatina Towns, and Naromoru, Endarasha, Mweiga and Othaya urban Centres (NCIDP, 2018b). The County covers an area of 3,337.2 square kilometres and is between 0° 38’ and 0° 38’ S latitudes of equator and 36° 38’ and 37° 20’ E longitudes, with an altitude of between 1,777 and 5,199 meters above sea level (NCIDP, 2018b). Nyeri County has 759,164 inhabitants, or 228 persons per square kilometre with an estimated annual growth of 2.2% (KNBStats, 2019). Commercial activities include small-scale farming, tea, coffee, cereals and dairy farming. Human food comprises cereals, beans, milk, eggs, meat, chicken, fish, pork, fruits, and vegetables. Food sources are home and markets, including across the country and fishponds for fish (Recha, 2018).

3.2 Study Design

In addition to HM samples from Nandi County, samples collected for round seven of the UNEP/WHO global survey on Stockholm Convention-listed POPs of 2016 to 2022 period were analysed in this study. All the samples were conveniently collected across purposively selected PHHs. Breastfeeding mothers were accessed through MCHC where structured questionnaires were administered, followed by HM sample collection.

3.3 Sample size determination

According to the 2019 Kenya population and housing census (KNBStats, 2019), Nandi County had an estimated population of 885,711 people evenly distributed across the six subcounties. Therefore, based on Eq. 1. the estimated sample size for this study was 93 mothers.

$$n = \frac{(Z_{(1-\alpha)/2})^2 NP(1-P)}{\Delta^2(N-1) + (Z_{(1-\alpha)/2})^2 P(1-P)} \qquad \text{Eq. 1}$$

Where: N = Population size

n = Sample size

P = Population proportion

Z = Level of confidence

Δ = Degree of accuracy expressed as a proportion

α = Significance level

Therefore $$n = \frac{(1.96)^2 X 885{,}711 X 0.5 X 0.5}{0.01X(885{,}711-1) + (1.96)^2 X 0.5 X 0.5} = 93$$

3.4 Samples distribution among study locations

Nairobi and Nyeri samples, representing urban and rural locations, respectively, were distributed based on UNEP/WHO (2007) guidelines, which advised participating countries with up to 50 million people to collect 50 HM samples, 25 each from an urban and rural geographical location. Distribution of HM samples: Nairobi County; Eastland's 11 (Mama Lucy); Southlands 15 (Kibra); and Westlands 4 (Westland); Nyeri County; Mathira East 15 (Karatina); and Kieni East 15 (Naromuru). Nandi County sample distributions are presented in Table 3.1.

Table 3.1: Estimated target sample sizes in the six subcounties of Nandi County

Subcounty	Population	Target sample size
Chesumei	164,133	19
Nandi Central	147,553	17
Nandi East	119,173	13
Nandi North	166,171	19
Nandi South	172,750	20
Tinderet	115,931	13
Total County Population	**885,711**	**101**

3.5 Study participants' inclusion and exclusion criteria

This study's participants were breastfeeding mothers who satisfied the following selection criteria adopted from WHO/UNEP (2007) guidelines: pre-sampling five years of continuous residency in the sampling location; being below 30 years old; being apparently healthy; being

primiparas; being at two- to eight-week lactation period; and being exclusively breastfeeding an apparently healthy single infant.

3.6 Chemical reagents and materials

Chemicals and materials used were of analytical grade (Appendix 8).

3.7 Study ethics approval

This study was approved by the KNH-UON Ethics and Research Committee via letter reference number KNH-UON-ERC/A/348 and by the Biosafety, Animal Use, and Ethics Committee of the Faculty of Veterinary Medicine via FVM BAUEC/2019/197 letter (Appendix 1). Also, the National Commission for Science, Technology, and Innovation, via letter reference number NACOSTI/P/19/83781/27700, authorized this study (Appendix 2). All the participants demonstrated their acceptance to participate in the study by signing informed consent form (Appendix 4), and proper representation was arranged for those who were not able to assent. Demographic data and HM samples were collected, transported, stored, and analyzed in accordance with acquired ethical authorizations (Shrestha and Dunn 2020). Schematic representation of step-by-step activities undertaken in this study is presented in Appendix 9.

3.8 Demographic data collection

3.8.1 Pre-testing of the questionnaire

Pre-testing of the questionnaire was done by interviewing ten randomly selected mothers at Kapsabet County Referral Hospital, and necessary adjustments were instituted (Gekonde, 2014). The pre-testing participants were not included in the subsequent study.

Individual unique identification codes were generated using short form of the hospital's (PHH) name and a serial number, i.e., KCRH01, where KCRH refers to Kapsabet County Referral Hospital and 01 refers to a serial number. Both the filled-out questionnaire and the label on the HM sample vial obtained from one mother were coded with similar unique identification.

3.8.2 Administration of the questionnaire

A structured questionnaire was administered following the protocols outlined in Appendix 3 at a Maternal and Child Health Clinic (MCHC) of a selected PHH. Potential mothers were informed about the purpose of the study, and it was made clear to them that refusing to participate would not jeopardize their access to MCHC health services and that any information accessed would strictly be kept confidential. Those who satisfied the study inclusion criteria and were ready to participate were each guided by trained medical personnel at MCHC to register their acceptance by signing a consent form (Appendix 4). The consented participants were then guided to provide sociodemographic information for the effective completion of the questionnaire (Appendix 5).

Mother and infant anthropometric information sorted for included; health status, dietary and smoking habits, age (years or weeks), weight (kg), height (cm), residency period (years), lactation period (weeks), occupation, breastfeeding mode or frequency, use of pesticides (WHO/UNEP, 2007). In Nandi County, a total of 74 questionnaires were completed and 13 failed selection criteria and were not used in this study, whereas in Nairobi and Nyeri Counties, a total of 60 questionnaires were successfully completed and five were not used in this study.

3.9 Collection of human milk samples

Consented mothers were each given a clean, appropriately labelled vial with an identically labelled Teflon-lined cap. The mothers were then guided by trained medical personnel to directly and manually express a minimum of 15 mL of HM into the vial provided at the MCHC, following the protocols provided in Appendix 3. After an appropriate quantity of HM had been expressed, the vials were then tightly capped with the issued Teflon-lined cap and immediately stored in a freezer at negative 20 °C (-20 °C) awaiting shipment to the laboratory. HM samples were robustly packaged on dry ice in a clean, cool box and shipped overnight to the laboratories

at the departments of Chemistry (Chiromo) and Public Health, Pharmacology, and Toxicology (Kabete), University of Nairobi, for storage at -20 ℃ while awaiting analysis.

A total of 74 HM samples were collected across Nandi County in 2019, distributed as follows; 20 from Chesumei at Kapsabet County Referral Hospital (KCRH), 19 from Nandi Central at Nandi Hills County Hospital (NHCH), 10 from Nandi East at Lessos Health Centre (LHC), 4 from Nandi North at Kabiyet Sub County Hospital (KSCH-M), 10 from Nandi South at Kaptumo Sub County Hospital (KSCH-A), and 11 from Tindiret at Maraba Sub County Hospital (MTT). A total of 60 HM samples had earlier, in 2018, been collected, 30 each from Nairobi and Nyeri Counties, using similar sampling protocols.

3.10 Laboratory analysis

A total of 116 HM samples were successfully analysed.

3.10.1 Preparation of anhydrous sodium sulphate

Analytical-grade granular Na_2SO_4 was baked in a Memmert oven (model 600) set at 200 ℃ for 12 hours in order to remove contaminants (Institute of Environmental Studies, 2014). The chemical was cooled and stored in a desiccator, ready for use.

3.10.2 Sample pre-treatment and Soxhlet extraction

The Institute of Environmental Studies, 2014 method was used with slight modifications.

Figure 3.2: Dried free-flowing powder

Immediately before analysis, HM samples were pulled out of the storage freezer set at -20 ℃ and permitted to thaw at room temperature (25 ℃) for two hours. Then duplicate weights of about five grams of homogenized HM samples were accurately mixed with 25 g of previously activated Na_2SO_4 in an n-hexane-rinsed mortar and pulverized to powder. The powders were covered with aluminium foil and allowed to stand overnight in order to dry further. The

mixtures were further ground into free-flowing powder (Figure 3.2) and quantitatively loaded into Soxhlet thimbles (Figure 3.3), which were inserted into the Soxhlet extractors (Figure 3.4). A total of 200 mL of hexane: acetone (3:1, v/v) extraction mixture and two smooth boiling facilitating chips (anti-bumping granules) were added into 250 mL round-bottomed flasks.

The Soxhlet extractors were then fitted with 250-mL round-bottomed flasks, which were filled with extraction solvent at the lower end and with condensers at the upper end. Once completely assembled and the heating mantle switched on, the Soxhlet setup was allowed to reflux continuously for at least 16 hours at 8 cycles per hour. At the end of 16 hours, the extracts in each 250-mL flask were evaporated to about 5 mL by means of Büchi Rotavapor after adding 2 mL of isooctane.

Figure 3.3: ***Loaded Soxhlet thimble***

The final concentrate was quantitatively transferred into a pre-weighed and labelled 20 mL glass vial, followed by three rinses of 1 mL portions of HPLC-grade n-hexane flask rinses. Approximately 20% of the aliquot of concentrated extract was subjected to gravimetric analysis for the determination of HM lipid content, while the remaining 80% of the aliquot equivalent was evaporated further under a gentle flow of nitrogen gas to 1 mL followed by clean-up using aluminum oxide chromatography.

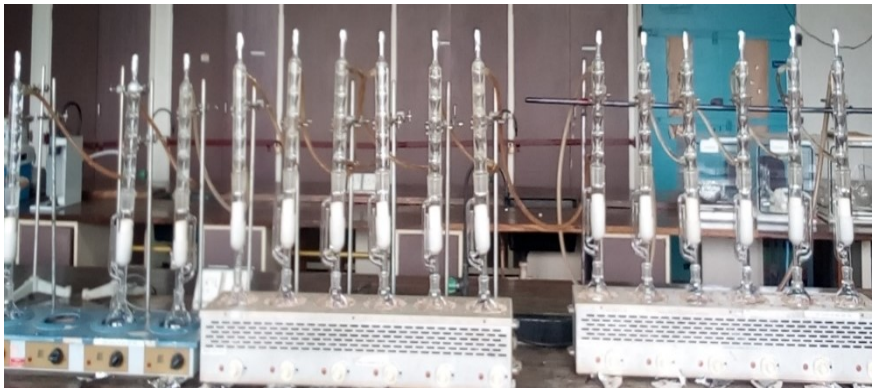

Figure 3.4: ***Soxhlet extraction apparatus***

3.10.3 Gravimetric lipid content determination

The gravimetric lipid determination method described by Linderholm *et al.* (2010) was used

with slight modifications. Dry-capped 20-mL glass vials were pre-weighed (W_1), loaded with 20% portions of the Soxhlet extracts. The glass vials were then heated at 105±0.5 °C for four hours and cooled in a desiccator to constant weights, which were recorded (W_{2i}). The procedure was then repeated twice with a reduced heating time of two hours (W_{2ii} and W_{2iii}). Finally, the three weights $W_{2i,}$ W_{2ii} and W_{2iii} were averaged to give W_2. The HM lipid contents were calculated using Eq. 2 and presented as percent weight/wet weight (% w/wet weight).

$$\text{Percentage Lipid Content} = \frac{(W_2\text{-}W_1) \text{ X WSE}}{\text{Mass of HM used } (\approx 5\text{g}) \text{ X WWE}} \text{ X } 100 \qquad \textbf{Eq. 2}$$

Where: W_1 = Capped, dry, empty 20 mL glass vial weight (g)

W_2 = Capped 20-mL glass vial heated to a constant weight (g)

WSE = Weight of stock extract (g)

WWE = Weight of extract used in lipid determination (g) (W_2-W_1)

3.10.4 Extract clean-up

3.10.4.1 Preparation of eight percent deactivated neutral alumina oxide

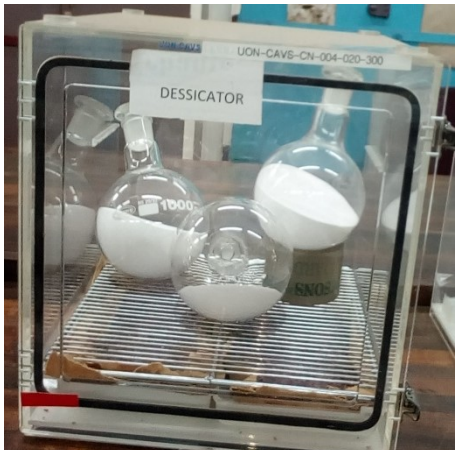

Figure 3.5: Eight percent deactivated AL_2O_3 in a desiccator

Analytical-grade neutral Aluminum oxide (AI_2O_3) was backed in a Memmert oven model 600 set at 200 °C for at least 12 hours in order to remove water and volatile contaminants, then cooled in a desiccator for four hours. 40 g of HPLC-grade demineralized water was added to 460 g of AI_2O_3 in a 1000 mL quick-fit round flask on a Shimadzu technical scale with an accuracy of 0.01 g and the highest weight of 300 g (Figure 3.5). The mixture was homogenized by shaking manually until no lumps were visible, followed by mechanical agitation for two hours (Institute of Environmental Studies, 2014). The mixture was then kept in a desiccator and allowed to stabilize for not less than 12 hours before use. The de-activated

alumina was used within 7 days after preparation.

3.10.4.2 Neutral alumina oxide column preparation

A 250 mL glass column with column 24/40 joint, ID 30 mm, column length 300 mm, and sintered glass frit was packed starting with one cm layer of baked Na_2SO_4, followed by 15 g of 8% deactivated neutral AI_2O_3, and lastly with one cm layer of baked Na_2SO_4 (Institute of Environmental Studies, 2014). The reagent additions were done while gently tapping the sides of the column in order to ensure uniform distribution of the packing in the column.

3.10.4.3 Ascertaining neutral alumina oxide column's optimal performance

The column was then conditioned with 15 mL of n-hexane and completely allowed to elute into a waste glass container. Immediately, without allowing the top Na_2SO_4 layer to dry up, 100 µL of a standard mixture of Studied POPs each at 50 ppb was introduced into the column, followed by 170 mL of n-hexane, and eluted into a 250 mL round-bottomed flask rinsed with n-hexane and labelled fraction 1. Finally, the column was eluted with 30 mL of n-hexane into a 100 mL round-bottomed flask rinsed with n-hexane and labelled fraction 2. The eluates were concentrated separately using a rotary evaporator to 2 mL after adding 1 mL of isooctane and transferred into a clean 10 mL glass vial, followed by three rinses of each flak with 1 mL of n-hexane. The eluates, after combining with the flasks' rinses, were evaporated further into dryness over a gentle flow of nitrogen gas reconstituted with 1000 µL of isooctane, vortexed for 30 seconds, and then loaded into auto-sampler vials. Not more than 2% of the analytes were allowed in fraction 2.

3.10.5 Sample extract clean-up using neutral alumina oxide column

The 80% extract, preserved after the removal of 20% portion for gravimetric lipid determination, was cleaned up using 8% deactivated neutral alumina oxide. The prepared alumina oxide column (Figure 3.6) was first conditioned with 20 mL of HPLC-grade n-hexane,

which was allowed to completely elute into a waste container. Immediately, without allowing the top Na_2SO_4 layer to dry up, HM extract with a lipid content equal to or less than 240 mg was gently introduced, followed by three times 1 mL of n-hexane vial rinses, and allowed to elute into n-hexane-rinsed 250 mL round-bottomed flasks.

Figure 3. 6: Extract clean-up

The column was further eluted with 165 mL of n-hexane. Extracts with a total lipid content of more than 240 mg were split into two equal portions, cleaned separately, and then combined. The cleaning elutants were concentrated to 1 mL using a BÜchi 320378 rotary evaporator after adding 1 mL of isooctane. Concentrated elutants were transferred quantitatively into a 10 mL glass vial and then concentrated to dryness over a gentle current of nitrogen gas. Finally, 1000 µL of isooctane was added, vortexed for 30 seconds, and transferred into a 2 mL auto-sampler glass vial ready for the GCMS/MS analysis (Institute of Environmental Studies, 2014).

3.10.6 Preparation of standard solutions

Stock standard solutions containing six PCB indicators (28, 52, 101, 138, 152, and 180), each at 1.25 µg/mL in toluene, were acquired through UNEP's, (2010) inter-laboratory testing capacity survey, and stock standard solution containing forty OCP compounds, each at 100 µg/mL in toluene, was obtained from Restek international. These standard mixtures were kept in amber glass vials at -20 ℃. From the original stock standard mixtures, a working standard (WS) solution with each compound at 200 ng/mL was prepared and kept in brown glass vials at -20 ℃. Calibration curve standards of 0.8, 2.5, 5, 10, 25, 50, 75, and 100 ng/mL for each compound were constituted using the 200 ng/mL WS solution.

The prepared calibration curve standards were kept refrigerated and removed a few minutes

before GCMS/MS analysis. Owing to solvent loss, the standards were often checked against the chromatograms obtained with the original standard solutions. A deviation of more than 5% was considered the highest, and new calibration curve standards would be prepared. Each glass vial carrying prepared standards was labelled to indicate the preparation date, initial weight, and dilution factors. (Institute of Environmental Studies, 2014).

3.10.7 Calibration Curve

Table 3.2 presents the amount of 200 ng/mL WS solution and isooctane that was used to prepare each level of the calibration standard (CS). Nine calibration levels or points, which include the blank, were adopted (Institute of Environmental Studies, 2014).

Table 3.2: Preparation of Calibration Standard Solutions

Calibration Level (ng/mL)	200 ng/mL WS aliquot (µL)	Isooctane (solvent) (µL)
0	0	1000
0.8	4	996
2.5	12.5	987.5
5	25	975
10	25	475
25	62.5	437.5
50	125	375
75	187.5	312.5
100	250	250

3.10.8 GCMS/MS Analysis Method Optimization

The triple quadruple GCMS-TQ8040 equipment with Multiple Reaction Monitoring (MRM) mode was used (Appendix 7). Shimadzu's multi-residue GCMS/MS-validated method for the separation and quantification of trace OCP and PCB compounds was used with slight modification (Prakash *et al.,* 2016). MRM transitions were monitored for each studied POP compound after optimizing their precursor ions, collision energies (CE), and quantitative and qualitative transitions.

3.10.9 Recovery Test Sample Preparation

The sensitivity and specificity of the method used were evaluated by including spiked bovine

milk in the test protocols (Polder *et al.,* 2003). The spiking levels for all the studied POP compounds were 4, 5, 10, 25, 50, and 100 ng/g lipid weight. Table 3.3 presents the amount of 200 ng/mL WS solution required to spike 25 mL of bovine milk in order to prepare the spike levels. Spiked bovine milk samples were mixed thoroughly and stored at -20 ℃ for `not less than 12 hours. The spiked samples were analyzed in triplicates using the adapted method as applied in the analysis of human milk samples. The percent recoveries of the compounds were used to measure the efficiency of the method. The method was accepted for use only when all the spiked amounts of studied POP compounds were recovered at between 60 and 130% (Sante, 2012).

Table 3.3: Bovine Milk Spiking Levels

Details	**Quantities used to spike 25 mL of bovine milk**					
Level.	1	2	3	4	5	6
Expected POPs ng/g lipid in each level	4	5	10	25	50	100
Volume in µL of 200 ng/mL WS	20	25	50	125	250	500

3.10.10 Separation, identification, and quantification of studied POPs

Calibration standards, spike samples, and test samples were analyzed using a Shimadzu TQ8040 GCMS system with a gas chromatograph GC-2010 plus model 225-2390011-58, S/N 021155400636, coupled to a triple quadruple mass spectrometer TQ8040NCI W10 RP 230V model 225-23911-58, and equipped with an AOC-20s autosampler model 221-72300-58 as well as an AOC-20i/2010 IVD R injector model 221-72315-59. The system was operated with a capillary column Phase ZB-5ms (5% phenyl and 95% dimethylpolysiloxane) with an internal diameter (i.d.) of 30 m × 0.25 mm (i.d..) and a film thickness of 0.25 µm. High-purity (99.999%) gases, helium as a carrier gas at 1.69 mL/minute and argon as a collision gas at 7.31-7.35 mTorr pressure range, were used.

The temperature of the GC-2010 plus, column oven was programmed starting at 50 ℃ and held

for 1.0 minute, then ramped to 125 ℃ at 25 ℃ per minute, then to 300 ℃ at 10 ℃ per minute, and finally held for 3 minutes, resulting in 24.5 minutes of stoppage time. The MS/MS transfer line was operated at 230 ℃, and that of the ion source was set at 290 ℃. The MS/MS electron ionization (EI) voltage was set at 70 eV, and the electron multiplier voltage was 1.99 kV + 0.9 kV offset above the auto-tuning process. There was a seven-minute delay in the filament multiplier. split-less mode was used to inject 1 µL of each solution at 275 ℃.

The equipment's daily performance was monitored using calibration standards levels two and six which were to compare well with previous injections. Batch analysis was done in sequence, starting with a solvent blank (isooctane), followed by calibration standards levels two and six respectively, then random injection of blank samples, spiked samples, 20 test samples, and the remaining calibration standards.

3.10.11 Integration and interpretation of chromatograms

Standards calibration curves were used to calculate the levels of POPs recovered in the spiked bovine milk samples, whereas spiked bovine milk calibration curves were used to calculate the occurrence levels of studied POPs in HM samples. System stability for the entire analysis was demonstrated by an overlay of the initial injection chromatograms of calibration standard levels two and six with all their subsequent re-injections. The responses of all calibration standard levels were within margins of 5%, and the linearity of each compound, given by squared regression, was greater than (≥) 0.950.

3.10.12 Validity check for the laboratory analytical procedures

As part of quality assurance, duplicate analysis, and regular checks on GCMS/MS analytic batches were adopted. Critical analysis steps, which include POPs extraction, clean-up and analysis, were evaluated for adequacy using bovine milk spiked with reference standards of the analytes. Bovine milk samples were analyzed before and after spikes at six concentration levels.

The spiking levels were chosen guided by the TQ8040 GCMS limit of quantifications (LOQ), which was used to estimate the sensitivity of the method, and the anticipated studied POPs occurrence levels in HM. Solvent blanks were also analyzed in order to monitor possible contaminations during sampling, extraction, and cleanup processes or interferences during instrumental analysis. The method detection limits (LOD), defined as three times the noise level for each compound, were calculated by reviewing the noises in the chromatograms next to the peak of interest using Eq. 3.

$$\text{LOD} = 3.3 \text{ X } \frac{\text{Response Standard Deviation}}{\text{Slope of the calibration curve}} \quad \textbf{Eq. 3}$$

The LOD was set as three times the noise divided by the response of the compound at the lowest calibration point multiplied by the concentration at that point (in ng injected). The instrument quantification limit (LOQ) was obtained in the same way, by multiplying the noise level by ten. For each studied POP compound, the LOQ obtained by the analytical procedures, which included extraction, clean-up and equipment measurement steps was used as the lowest level for reporting the POPs residue occurrence levels. Lower recoveries of spiked reference standards were used when rejecting the analytical method verification data, and each case had a different consideration from the other, using 60–130% as the reference recovery range.

3.10.13 Estimation of studied POPs daily intake by infants

Asamoah *et al* (2018) and Tsygankov *et al* (2019) reported 700 g as the approximate amount of HM consumed by a 5-kg infant between two and eight weeks old per day. Though the average body weight of the 116 infants involved in this study was 4.22 kg, 5 kg was adopted in the calculation of the study infants' daily intake (EDI) of studied POP compounds. Also, 700 g HM was adopted as that consumed by an infant per day for EDI calculations (Eq. 4).

$$\text{EDI} = \frac{C_{milk} \text{ X } 700 \text{ g milk/day X } C_{lipid}/100}{5 \text{ kg body weight}} \quad \textbf{Eq. 4.}$$

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

3.11 Data Analysis

3.11.1 Data Entry and Cleaning

Microsoft Excel 2016 (16.013530.20062 professional plus) was used in order to enter, clean, and prepare data for analysis. Numerical variables were described by their arithmetic means and medians. Descriptive statistics were done in order to get frequencies and pie charts. POP occurrence levels were transformed using logs before statistical testing in order to reduce skewed distributions and/or heterogeneity of variance between groups or counties.

3.11.2 Statistical Data Analysis

Statistical analysis of the data was performed using Excel 2016 and GraphPad Prism (viewer mode) Version 9.0.1 (151). The arithmetic means of the studied POP occurrence levels from the different study counties and when grouped as urban or rural were compared using one-way analysis of variance (Secretariat of the Stockholm Convention on Persistent Organic Pollutants, 2011; WHO/UNEP, 2007). The significance level was fixed at $\alpha = 0.05$, therefore giving a *p*-value of less than 0.05 (95% confidence limits). Chi-square tests were used to check whether respondents' characteristics were distributed evenly across the three study Counties. Linear regression was used to determine the association between different Counties' practices and the outcome of studied POPs exposure levels. The results obtained were presented using text, graphs, and statistical tables. Firstly, a univariate analysis was performed in order to detect the individual factors that may have an association with the levels of studied POPs in breast milk samples ($P < 0.10$). Then, the possible association among individually identified factors was determined by multivariate analyses. Single and subsequent multiple linear regression analyses

were used to identify determinants of OCP and PCB body burden among individual characteristics and environmental factors. The suspected outliers are reported and were included in the statistical calculations after log transformation.

CHAPTER FOUR

RESULTS

4.1 Study participants' demographic characteristics

The demographic characteristics of participants in this study (mothers and their infants) are presented in Table 4.1. All the mothers were apparently healthy, and 99% were born in Kenya. Likewise, all the mothers had resided continuously in the respective study counties for at least five years preceding the study sampling period. The mothers' average lactation period was six weeks and ranged from two to eight weeks.

Table 4.1: Participants' demographic characteristics of Mothers and Infants

Parameter	Nairobi	Nandi	Nyeri
Maternal participant (n)	**26**	**61**	**29**
Age (years)	22.5 (18-29)	22.4 (17-26)	22.5 (19-29)
Weight (kg)	59	58.87	59
Height (cm)	160	159.98	160
BMI (kg/m^2)	22.99	22.98	22.99
Diet	100% Mixed	100% Mixed	100% Mixed
Eat fish (% Yes)	96	90.32	78
Lactation period (Weeks)	6	6	6
Residence (Urban or Rural)	Urban	Rural	Rural
Uses tobacco (%)	1.5	0	0
Recent pesticide usage (%)	17.67	44.26	20.69
Infant participant (n)	**26**	**61**	**29**
Age (Weeks)	6	6	6
Weight (kg)	3.87	4.39	4.41

Note: (-) Missing information.

All mothers were primiparous and were aged between 16 and 30 years, with the majority (82.43%) in the 20 to 30 age brackets, each exclusively breastfeeding a singleton infant. The mothers mean weight was 58.94 kg (range: 48 to 80 kg) while their average height was 160 cm (range: 148 to 173.5 cm). The mothers' average body mass index (BMI) was 22.99 (range: 19.43 to 29.21). All the participants in the study consumed a mixed diet consisting of cereals and beans (100%), meat (96.61%), dairy products (95.76%), poultry products (94.07%) and fish (91.53%). Only 6.6% of the mothers used tobacco, while 24.58% reported recent usage of pesticides either against mosquitoes, bedbugs, cockroaches, flies or lice.

4.2 Lipid contents of the analysed human milk samples

The summary results of analysed HM samples extractable lipid contents (% w/w of wet weight) in descriptive mean, median, and range values and clustered column illustrations are presented in Table 4.2 and Figure 4.1. Nandi County had the highest mean percentage at 4.20±1.36%, followed by Nyeri County at 3.70±1.15%, and Nairobi County had the least at 3.09±1.25%. The mean lipid content of HM collected from rural dwellers in Nandi and Nyeri Counties was 3.95±1.33%, and that from urban residences in Nairobi County was 3.09±1.25%.

Table 4.2: ***Lipid Contents of Human Milk***

Sampling site	n	(% w/w of wet weight)				SD
		Mean	Median	Min	Max	
NW and NE	13	2.92	2.91	1.33	6.20	1.28
KBR	13	3.27	3.21	1.23	6.24	1.22
Summary of Nairobi County	**26**	**3.09**	**2.96**	**1.23**	**6.24**	**1.25**
K	15	3.49	3.70	1.69	5.77	0.98
NR	14	3.93	3.93	1.82	7.51	1.29
Summary of Nyeri County	**29**	**3.70**	**3.92**	**1.69**	**7.51**	**1.15**
KCRH	20	3.31	3.34	0.27	6.91	1.45
KSCH - A	10	4.82	4.09	1.79	7.71	1.78
KSCH - M	4	4.22	4.23	3.36	4.97	0.65
LHC	7	4.32	3.63	2.78	6.73	1.19
NHCH	19	4.24	4.09	1.16	7.59	1.59
MTT	1	4.30	4.30	4.30	4.30	0.00
Summary of Nandi County	**61**	**4.20**	**3.99**	**0.27**	**9.45**	**1.36**
All samples summary	**116**	**3.80**	**3.86**	**0.27**	**9.45**	**1.43**

Notes: n = Sample size, Min = List, Max = Highest, SD = Standard Deviation, NW = Nairobi West, NE = Nairobi East, KBR = Kibra, K = Karatina Subcounty Hospital, NR = Naromuru Subcounty Hospital, KCRH = Kapsabet County Referral Hospital, KSCH-A = Kaptumo Subcounty Hospital, KSCH-M = Kabiyet Subcounty Hospital, LCH = Lessos Health Centre, NHCH = Nandi Hills County Hospital, and MTT = Maraba Subcounty Hospital.

The different rates at which heterogeneously sized lipid globules disperse or rise in HM make it hard to aliquot samples with true lipid representation. Implying that milk samples should be thoroughly mixed at ≥ 38 °C in order to obtain a random distribution before resampling (Jensen, 1999). This was probably not achieved which led to wide variations in the HM lipid content observed in the study. Additionally, lactation processes and cyclic changes in lipid content

brought about by diurnal variation, diet fat and carbohydrate content relative to ingested meals, and amongst other factors, determine individual HM lipid content (Table 2.6).

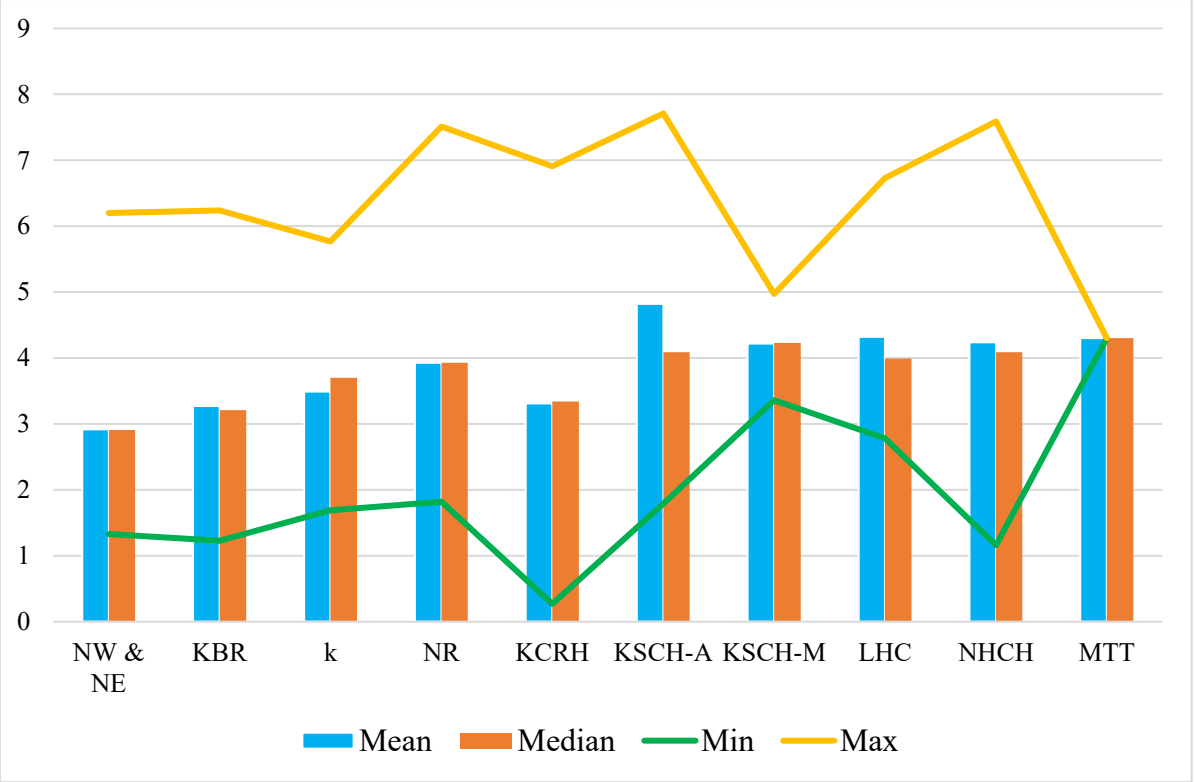

Figure 4.1: Clustered Column Illustrations of Lipid Contents of Analysed Human Milk

While a number of factors known to affect the HM total lipid content (Table 2.6) were controlled during sampling and the participants' dietary patterns were 100% mixed, the difference in HM lipid content observed can be attributed to regional diet patterns and activities. There was a significant difference, $P < 0.05$, between the percent mean lipid content of HM samples from rural (Nandi and Nyeri) Counties and those from urban (Nairobi) County. This can be attributed to the difference in the mothers' eating habits brought about by their accessibility to diet and the type of diet, presumably due to prevailing socioeconomic status (Lönnerdal, 1986). However, across the study Counties, the average HM lipid content was between 3% and 5% w/w, within the established range for human milk (Kanja *et al.,* 1988 and Jensen, 1999).

4.3 Analytical Quality Assurance (AQA) and Quality Control (QC)

Tables 4.3 and 4.4 present qualitative and quantitative ion pairs (m/z), collision energies (eV), LODs, LOQs, retention times (RTs), linear responses (r^2) and % RSD for studied POP compound obtained. LODs were between 0.010 ng/g lw given by *α*-endosulfan and 0.068 ng/g lw due to *trans(exo)*-heptachlor epoxide.

Table 4.4 present analytical method sensitive and specific results. The recovery mean results were between 71.44% for *δ*-HCH and 117.66% for *β*-endosulfan, and mostly met the 70% and 120% limits (Table 2.7). However, *δ*-HCH, aldrin, *p,p*-DDT, endrin aldehyde, heptachlor, and mirex were poorly recovered at 4 ng/g lw spiking level, which can be attributed either to compounds' insolubility in extraction solvent mixture (acetone/n-hexane), partial compound decomposition or leakage during extraction and/or clean-up procedures, or uneven compounds distribution in the fortified bovine milk.

Therefore, the analytical method used met good recoveries at between 41.94% and 151.10% across 4, 5, 10, 25, 50, and 100 ng/g lw spiking levels (Table 4.4). The best scores were achieved with 5, 25, 50, and 100 ng/g lw levels. Upon application of the exceptional case criteria of 30-140% (SANTE/12682/2019, 2019), the lower acceptable recovery limit of 30% was satisfied, but dieldrin, *o,p'*-DDD, and *β*-endosulfan failed the upper recovery limit of 140% in 4 and 10 ng/g lw spiking levels. However, precisions associated with recoveries of 31 studied POPs (81.58%) including dieldrin, *o,p'*-DDD and *β*-endosulfan were good with RSDs ≤ 20%. The instrumental regression coefficients (r^2) for all studied POP were > 0.990, with a good fit to linearity within the calibration range (Appendix 11).

Table 4.3: GCMS/MS Method Parameters; Retention Time, Ion Pairs, Collision energy, Limit of Detection and Limit of Quantification

Compound	RT	QnIP (m/z)	CE (eV)	QlIP (m/z)	LOD	LOQ	Slope	SD	% RSD
Pentachlorobenzene	9.197	249.90 > 214.90	20	249.90 > 178.90	0.016	0.055	12929.97	71.07	0.55
α-HCH	11.160	218.90 > 182.90	10	218.90 > 109.00	0.016	0.055	15106.29	83.40	0.55
HCB	11.229	283.80 > 248.80	24	283.80 > 213.90	0.016	0.056	22068.56	123.43	0.56
β-HCH	11.772	218.90 > 182.90	10	218.90 > 109.00	0.024	0.081	10024.33	80.80	0.81
γ-HCH	11.849	218.90 > 182.90	10	218.90 > 109.00	0.024	0.084	10561.77	88.36	0.84
δ-HCH	12.483	218.90 > 182.90	10	218.90 > 109.00	0.028	0.095	6331.09	65.76	1.04
Endosulfan-Ether	12.756	271.80 > 236.90	20	271.80 > 117.00	0.027	0.094	2895.10	27.00	0.93
PCB#28	13.025	255.90 > 186.00	30	257.90 > 186.00	0.019	0.064	19227.03	122.60	0.64
Heptachlor	13.286	271.80 > 236.90	20	271.80 > 117.00	0.026	0.088	6512.85	57.06	0.88
PCB#52	13.680	289.90 > 219.90	30	291.90 > 221.90	0.020	0.070	6255.90	43.93	0.70
Aldrin	13.996	262.90 > 193.00	28	262.90 > 203.00	0.023	0.080	15214.05	121.31	0.80
Isodrin	14.582	192.90 > 157.00	20	262.90 > 192.90	0.025	0.087	8921.01	77.32	0.87
Trans(exo)-heptachlor epoxide	14.765	352.80 > 262.90	14	352.80 > 281.90	0.028	0.096	3482.29	78.70	2.26
Cis(endo)-heptachlor epoxide	14.766	352.80 > 289.00	16	352.80 > 253.00	0.025	0.085	6367.01	54.33	0.85
Trans-CHL	15.232	372.80 > 336.80	10	372.80 > 263.90	0.017	0.058	12472.99	71.73	0.58
o,p'-DDE	15.259	246.00 > 176.00	30	246.00 > 211.00	0.018	0.063	52605.01	330.34	0.63
PCB#101	15.349	323.90 > 253.90	26	328.90 > 255.90	0.022	0.076	12570.26	95.92	0.76
Cis-CHL	15.478	372.80 > 336.80	10	372.80 > 263.90	0.022	0.074	10759.82	79.81	0.74
Dieldrin	15.488	276.90 > 241.00	8	262.90 > 193.00	0.027	0.092	2471.00	22.70	0.92
o,p'-DDD	15.481	235.00 > 165.00	24	235.00 > 199.00	0.010	0.034	637.36	2.18	0.34
α-END	15.237	194.90 > 160.00	8	338.90 > 266.90	0.010	0.033	898.80	2.99	0.33
β-END	15.487	194.90 > 160.00	8	338.90 > 266.90	0.012	0.041	4321.49	61.09	1.41
Trans-nonachlor	15.535	406.80 > 299.90	24	408.80 > 145.00	0.019	0.065	10822.29	69.86	0.65
Endrin	15.481	262.90 > 191.00	30	262.90 > 193.00	0.015	0.050	5607.47	83.91	1.50
Cis-nonachlor	15.535	406.80 > 299.90	24	408.80 > 145.00	0.026	0.088	10888.09	95.66	0.88
p,p'-DDD	16.021	235.00 > 165.00	24	235.00 > 199.00	0.017	0.059	89417.48	523.40	0.59

Compound	RT	QnIP (m/z)	CE (eV)	QlIP (m/z)	LOD	LOQ	Slope	SD	% RSD
p,p'-DDE	15.884	246.00 > 176.00	30	246.00 > 211.00	0.023	0.080	36534.65	290.67	0.80
o,p'-DDT	16.701	235.00 > 165.00	24	235.00 > 199.00	0.019	0.065	94714.52	615.90	0.65
Endrin aldehyde	16.903	249.80 > 214.90	26	249.90 > 179.00	0.011	0.039	1012.45	3.99	0.39
PCB#153	16.919	359.90 > 289.90	30	361.90 > 291.90	0.021	0.072	12140.99	87.90	0.72
END-Sulphate	17.352	271.80 > 236.90	18	386.80 > 252.90	0.011	0.038	1386.12	5.25	0.38
p,p'-DDT	17.402	235.00 > 165.00	24	235.00 > 199.00	0.027	0.093	10772.50	99.97	0.93
PCB#138	17.418	359.90 > 289.90	30	361.90 > 291.90	0.024	0.083	9542.67	79.50	0.83
Endrin ketone	17.422	180.90 > 145.00	15	114.90 > 51.10	0.010	0.036	1326.43	4.75	0.36
p,p'-Methoxychlor olefin	18.449	227.10 > 169.10	24	227.10 > 212.10	0.016	0.056	222.34	3.47	1.56
o,p'-Methoxychlor	18.829	227.10 > 169.10	24	227.10 > 212.10	0.022	0.077	129.96	1.00	0.77
PCB#180	18.638	393.80 > 323.90	30	395.80 > 325.90	0.017	0.060	6953.77	41.46	0.60
Mirex	19.388	271.80 > 236.80	18	273.80 > 238.80	0.026	0.091	7917.32	72.35	0.91

Notes: RT: Retention Time, QnIP: Quantitative ion pair (m/z), CE: Collision energy (eV), QlIP: Qualitative ion pair (m/z), LOD (ng/g lw): Limit of Detection, LOQ (ng/g lw): Limit of Quantification, SD: Standards Deviations and % RSD: Percent relative Deviation.

Table 4.4: ***Method recovery tests results***

Compound	Spiking levels (ng/g lipid weight (lw)) % recoveries						Mean	r^2	% RSD
	4	5	10	25	50	100			
Pentachlorobenzene	88.73	113.82	108.61	101.95	100.12	95.86	101.52	0.990	8.80
α-HCH	83.77	87.64	91.84	93.38	93.00	89.12	89.79	0.984	4.13
HCB	86.03	87.06	96.76	95.91	95.01	91.64	92.07	0.987	5.03
β-HCH	78.54	98.86	90.90	89.52	92.41	88.34	89.76	0.979	7.38
γ-HCH	80.06	86.12	83.57	87.22	89.52	86.32	85.47	0.980	3.82
δ-HCH	71.00	79.05	67.09	69.31	72.38	69.78	71.44	0.954	5.78
Endosulfan-ether	84.27	80.96	93.78	94.37	94.00	89.46	89.47	0.987	6.37
PCB#28	81.60	90.84	100.34	97.28	97.59	93.01	93.44	0.988	7.20
Heptachlor	73.04	75.96	82.04	85.88	92.03	90.56	83.25	0.986	9.25
PCB#52	73.68	81.51	97.41	92.86	94.74	90.25	88.41	0.986	10.23
Aldrin	126.73	119.40	110.63	96.78	95.78	90.23	106.59	0.985	13.70
Isodrin	82.38	95.23	103.77	97.72	97.30	92.04	94.74	0.986	7.57
Trans(exo)-heptachlor epoxide	90.39	127.69	119.16	88.98	84.50	78.27	98.16	0.987	20.58
Cis(endo)-heptachlor epoxide	83.50	107.71	100.96	98.39	98.73	94.32	97.27	0.987	8.28
Trans-CHL	97.39	97.17	102.57	98.21	98.75	95.03	98.19	0.987	2.54
o,p'-DDE	98.94	112.80	102.20	100.54	99.42	95.49	101.57	0.988	5.84
PCB#101	98.25	101.92	105.08	99.40	100.00	94.98	99.94	0.986	3.41
Cis-CHL	91.18	97.66	104.56	103.32	102.55	97.08	99.39	0.987	5.09
Dieldrin	96.97	113.20	149.80	112.57	106.44	99.07	113.01	0.984	17.02
o,p'-DDD	96.93	113.83	151.10	118.01	97.60	85.83	110.55	0.977	20.92
α-endosulfan	59.16	90.32	94.76	82.72	84.38	80.20	81.92	0.982	15.08
β-endosulfan	144.60	94.45	118.33	88.56	80.16	73.88	117.66	0.987	41.20
Trans-nonachlor	94.01	106.64	97.51	97.49	98.72	94.72	98.18	0.987	4.60
Endrin	109.33	-	-	118.65	106.95	96.46	107.85	0.977	8.45
Cis-nonachlor	88.91	96.48	106.10	99.80	102.12	96.98	98.40	0.986	5.94
p,p'-DDD	111.35	110.32	105.77	97.36	95.23	89.12	101.52	0.987	8.84

Compound	Spiking levels (ng/g lipid weight (lw)) % recoveries						Mean	r^2	% RSD
	4	5	10	25	50	100			
p,p'-DDE	101.59	107.10	107.11	102.68	103.03	98.14	103.27	0.990	3.33
o,p'-DDT	108.97	109.21	101.94	95.93	99.30	95.78	101.85	0.979	5.95
Endrin aldehyde	53.92	55.12	86.50	114.56	110.03	101.75	86.98	0.979	30.93
PCB#153	94.03	98.69	106.03	97.38	99.59	96.16	98.65	0.983	4.17
Endosulfan sulphate	99.98	41.94	68.58	75.49	86.52	84.11	76.10	0.940	26.06
p,p'-DDT	62.82	67.45	72.06	61.82	78.64	87.96	71.79	0.954	14.03
PCB#138	91.51	102.43	100.60	98.01	99.94	95.42	97.99	0.982	4.06
Endrin ketone	69.49	49.82	73.31	93.59	94.38	89.94	78.42	0.977	22.39
p,p'-Methoxychlor olefin	122.60	80.90	93.41	85.58	86.38	86.86	109.29	0.960	34.60
o,p'-Methoxychlor	78.72	97.91	94.68	90.25	88.93	84.54	89.17	0.981	7.75
PCB#180	83.24	93.88	100.50	100.79	102.50	97.71	96.44	0.981	7.40
Mirex	75.33	73.60	73.11	74.11	76.80	74.48	74.57	0.971	1.78

Notes: r^2 = Linear responses, % RSD = Percent Relative Deviation, (-) Missing data.

Appendix 10 presents a sample standard chromatogram showing the peaks of all the analytes. Appendix 11 present standards calibration curves used in the calculation of the occurrence levels of studied POPs compounds and Appendix 12 presents sample chromatogram of the analysed spiked bovine milk.

4.4 Occurrence and levels of studied POPs in the studied HM.

The summary results of pooled HM samples analysed by Baden-Württemberg Chemisches Laboratory, Germany, are presented in Table 4.5 (Madadi *et al.,* 2021). Whereas, Tables 4.6 to 4.9 present summary results on the occurrences (%) and levels (ng/g lw) of studied POPs in the analysed individual HM samples carried out by this study. Six groups of POP compounds occurred at variable frequencies and levels, either as parent compounds, metabolites, analogous, congeners, or blended. They included; *cis*- and *trans*-chlordane *cis*- and *trans*-nonachlor, *p,p′*-DDT, *p,p*-DDD, *o,p*-DDT, *p,p*-DDE, endosulfan ether, *α*- and *β*-endosulfan, endosulfan sulphate, HCB, *α-, β-, γ-,* and *δ*-HCH, PCB#28, PCB#52, PCB#101, PCB#138, PCB#153, and PCB#180.

The occurrences of the studied POP compounds were; DDTs (86.44%), indicator PCBs (55.08%), endosulfan (33.05%), HCHs (30.513%), HCB (25.42%) and chlordane (20.34%). And their mean occurrence levels were; DDTs (6.483±12.500 ng/g lw), indicator PCBs (2.925±1.095 ng/g lw), endosulfan (3.834±1.397 ng/g lw), HCHs (2.207±0.642 ng/g lw), chlordane (0.869±0.206n g/g lw), and HCB (0.216±0.152 ng/g lw).

Eight groups of studied POP compounds, which included aldrin, dieldrin, endrin, heptachlors, mirex, methoxychlor, and pentachlorobenzene did not occur above their quantifiable levels in any of the HM sample tested. However, the pooled sample had quantifiable levels of dieldrin, while, chlordane and endosulfan were below quantifiable levels contrary to results of individually analysed samples.

4.4.1 Comparisons of the results of pooled and individually analysed HM samples.

The two-analysis recorded non-detectable quantities of *cis*-chlordane, aldrin, *o,p*-DDE, and *δ*-HCH, and the detected compounds showed the same pattern. However, with the exception of endosulfan and dieldrin, which were only detected in individual samples and pooled samples respectively, all the other detected POPs were higher in the pooled samples. With the exception

of DDTs and HCB, which had a significant difference between the two analysis the rest of the compounds demonstrated credibility of the analysis process. The persistent low values for the individually analysed samples could be due to the inability to detect the majority that contributed to the pooled samples results. Therefore, the results of this study are validated.

Table 4.5: Results of pooled samples of 2009 and 2019 analysed by Baden-Württemberg Chemisches Laboratory, Germany in ng/g fat.

Compound	Individual Samples Results		Pooled Samples Results	
	2009	2019	2009	2019
Aldrin	0.134	<0.080	<0.080	<0.5
∑3chlordane	1.128	0.516	-	-
trans-chlordane	0.111	<0.058	<0.5	<0.5
cis-nonachlor	0.481	0.259	<0.5	<0.5
trans-nonachlor	0.481	0.256	<0.5	<0.5
∑5DDT	35.020	5.640	289.3	83.5
p,p'-DDD	11.034	0.137	1.8	<0.5
o,p'-DDE	7.60	<0.063	7.60	<0.5
p,p'-DDE	13.409	4.501	249.5	70.1
o,p'-DDT	2.832	0.242	1.4	0.69
p,p'-DDT	7.745	0.760	7.6	4.97
Dieldrin	4.700	<0.092	5.1	1.34
∑endosulfan	5.626	2.462	-	-
Endosulfan ether	0.860	0.579	<0.5	<0.5
α-endosulfan	0.177	0.113	<0.5	<0.5
β-endosulfan	0.474	0.162	<0.5	<0.5
Endosulfan sulphate	4.115	1.609	<0.5	<0.5
∑HCH	1.465	0.635	5.06	1.57
α-HCH	0.301	0.219	<0.5	<0.5
β-HCH	0.469	0.220	2.76	1.57
γ-HCH	0.386	0.197	2.3	<0.5
δ-HCH	0.309	<0.094	<0.5	<0.5
HCB	3.216	0.208	3.1	1.75
∑i6PCB	6.061	1.509	4.32	2.47
PCB#28	2.315	0.438	0.84	0.58
PCB#52	0.431	<0.070	0.43	0.065
PCB#101	0.302	0.079	0.17	0.056
PCB#138	1.024	0.294	0.99	0.61
PCB#153	0.934	0.351	1.27	0.72
PCB#180	1.054	0.346	0.60	0.43

4.4.2 Occurrence and levels of chlordane group

The occurrence frequencies of quantifiable chlordane and their mean occurrence levels,

respectively, are presented in Tables 4.6-4.9. In general, they are; trans-nonachlor 12.71%, 0.287±0.123 ng/g lw; *cis*-nonachlor 7.63%, 0.273±0.084 ng/g lw; *cis*-chlordane 5.93%, 0.157±0.045 ng/g lw; and *trans*-chlordane 5.93%, 0.152±0.044 ng/g lw. In Nairobi and Nyeri Counties, only *cis*-nonachlor and *trans*-nonachlor occurred in between 6.90% and 30.77% HM, whereas *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, and *trans*-nonachlor occurred in between 1.64% and 11.48% HM samples from Nandi County.

4.4.3 Occurrence and levels of dichlorodiphenyltrichloroethane group

Quantifiable levels of four DDTs congeners occurred in a number of studied HM samples as presented in Tables 4.5-4.8, and they included *p,p'*-DDT 72.88%, 0.694±1.101 ng/g lw; *p,p'*-DDD 44.07%, 0.159±0.157 ng/g lw; *o,p'*-DDT 34.75%, 0.204±0.174 ng/g lw; and *p,p'*-DDE 30.51%, 5.426±12.237 ng/g lw. The mean occurrence levels of DDT ranged from 0.065 ng/g lw to 128.979 ng/g lw, both extremes presented by HM samples donated by mothers living in Nairobi County. *p,p'*-DDE, the more persistent DDT metabolite, occurred in mean quantities of between 0.082 ng/g lw and 126.292 ng/g lw in milk donated by mothers living in Nyeri and Nairobi Counties, respectively. Similarly, the main component of the technical grade DDT, *p,p'*-DDT occurred at mean levels of between 0.092 ng/g lw and 11.086 ng/g lw in milk obtained from mothers living in Nyeri and Nairobi Counties respectively.

4.4.4 Occurrence and levels of endosulfan group

The endosulfan studied were quantified in a number of HM samples, and the following are the mean results endosulfan sulphate 22.88%, 0.812±1.172 ng/g lw; α-endosulfan 9.32%, 0.700±0.333 ng/g lw; β-endosulfan 4.24%, 1.743±0.558 ng/g lw; and endosulfan ether 1.69%, 0.579±0.085 ng/g lw. The mean occurrence levels of endosulfan ranged from 0.040 ng/g lw to 12.405 ng/g lw in HM from Nandi and Nairobi Counties, respectively. Quantifiable levels of endosulfan sulphate were found in 3.45% samples amongst samples from Nyeri County, with low levels of 0.047 ng/g lw. Three endosulfan occurred at sum quantifiable levels of 5.348 ng/g

lw in 40.98% of tested HM samples from Nandi County, and individual results were 0.659 ng/g lw for endosulfan sulphate, 1.163 ng/g lw for *α*-endosulfan, and 3.526 ng/g lw for *β*-endosulfan in 34.43%, 9.84%, and 4.92% tested HM samples, respectively. Mean quantifiable levels of 4.024 ng/g lw for four endosulfan occurred in 46.15% of HM samples from Nairobi and were distributed as follows: *α*-endosulfan 19.23%, 0.113 ng/g lw; endosulfan sulphate 15.38%, 3.170 ng/g lw; *β*-endosulfan 7.69%, 0.162 ng/g lw; and endosulfan ether 7.69%, 0.579 ng/g lw.

4.4.5 Occurrence and levels of hexachlorobenzene

Quantifiable mean levels of 0.216 ng/g lw of HCB occurred in 25.42% HM samples. HCB occurred in HM samples from Nairobi County (65.38%, 0.269 ng/g lw) and Nyeri County (44.83%, 0.146 ng/g lw).

4.4.6 Occurrence and levels of hexachlorocyclohexane group

Quantifiable levels of HCHs occurred in a number of analysed HM samples. The mean occurrences levels 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. The mean occurrence levels of HCH ranged from 0.094 ng/g lw to 4.814 ng/g lw in HM from mothers in Nandi County. A quantifiable mean level of 0.235 ng/g lw for γ-HCH was the only HCH analog that occurred in 10.34% HM samples from Nyeri County. Three HCH compounds occurred at sum quantifiable levels of 0.598 ng/g lw in 34.62% of analysed HM samples from Nairobi County, and individually *β*-HCH at 0.220 ng/g lw, *α*-HCH at 0.219 ng/g lw, and *γ*-HCH at 0.159 ng/g lw in 34.62%, 11.54%, and 7.69% of tested HM, respectively. Quantifiable levels 1.878 ng/g lw of HCH occurred in 39.34% of HM samples from Nandi County and were distributed as follows: *α*-HCH 3.28%, 0.209±0.043 ng/g lw; *β*-HCH 18.03%, 0.490±0.295 ng/g lw; *γ*-HCH 18.03%, 0.942±0.785 ng/g lw; and *δ*-HCH 8.20%, 0.237±0.084 ng/g lw.

4.4.7 Occurrence and levels of polychlorinated biphenyl group indicators

Quantifiable levels of iPCBs occurred in a number of HM samples. Their mean occurrence

levels were: PCB#28 (23.73%, 0.356±0.296 ng/g lw); PCB#52 (23.73%, 0.522±0.449 ng/g lw); PCB#101 (10.17%, 1.170±0.761 ng/g lw); PCB#138 (12.71%, 0.257±0.113 ng/g lw); PCB#153 (6.78%, 0.324±123 ng/g lw); and PCB#180 (16.10%, 0.296±0.184 ng/g lw). All iPCBs occurred in 70.49% of samples from Nandi County, whereas in Nyeri County PCB#52 and PCB#101 were not detected, and in Nairobi County PCB#52 was not detected. A sample from Nandi County had the highest occurrence levels of iPCB at 7.380 ng/g lw, and a sample from Nyeri County had the lowest at 0.061 ng/g lw. PCB#28 and PCB#52 were detected in the highest number of samples (23.73%), and PCB#153 occurred in the least number of samples (6.78%). PCB#101's occurrence level of 1.170±0.761 ng/g lw was highest, and that of PCB#180's of 0.296±0.184 ng/g lw was the lowest. iPCBs were relatively higher in samples from Nandi County (2.753±1.343 ng/g lw) than from Nairobi County (2.015±0.953 ng/g lw) and finally from Nyeri County (0.923±0.385 ng/g lw).

Table 4.6: Overall Occurrence Frequencies (%) and Mean Levels (ng/g lw) of Studied Persistent Organic Pollutants in Studied Human Milk samples (n = 116)

Compound	Nairobi	Nyeri	Nandi	No. +	% No. +	Mean	Median	Min	Max	SD
$\sum_4$Chlordane	0.676	0.355	0.737	24	20.34	0.869	0.381	0.065	1.314	0.206
Cis-Chlordane	<0.074	<0.074	0.157	7	5.93	0.157	0.127	0.086	0.398	0.045
Trans-Chlordane	<0.058	<0.058	0.152	7	5.93	0.152	0.107	0.065	0.370	0.044
Cis-nonachlor	0.317	0.202	0.156	9	7.63	0.273	0.223	0.122	0.656	0.084
Trans-nonachlor	0.360	0.153	0.272	15	12.71	0.287	0.229	0.065	0.892	0.123
$\sum_4$ DDT	10.221	1.058	1.101	102	86.44	6.483	0.581	0.065	128.979	12.500
p,p'-DDD	0.172	0.102	0.166	52	44.07	0.159	0.088	0.059	1.292	0.157
p,p'-DDE	8.679	0.323	0.198	36	30.51	5.426	0.306	0.082	126.292	12.237
o,p'-DDT	0.311	0.173	0.126	41	34.75	0.204	0.144	0.065	1.458	0.174
p,p'-DDT	1.058	0.461	0.610	86	72.88	0.694	0.395	0.092	11.086	1.101
Ratio (DDT/DDE)	0.158	1.963	3.717			0.166				
$\sum_4$Endosulfan	4.023	0.047	4.405	39	33.05	3.834	0.254	0.040	12.405	1.397
Endosulfan ether	0.579	<0.094	<0.094	2	1.69	0.579	0.579	0.267	0.891	0.085
α-Endosulfan	0.113	<0.033	1.189	11	9.32	0.700	0.194	0.044	2.745	0.333
β- Endosulfan	0.162	<0.041	2.797	5	4.24	1.743	0.698	0.148	5.712	0.558
Endosulfan sulphate	3.170	0.047	0.418	27	22.88	0.812	0.181	0.040	12.405	1.172
$\sum_4$HCH	0.597	0.235	2.565	36	30.51	2.207	0.325	0.094	4.814	0.642
α-HCH	0.219	<0.055	0.209	5	4.24	0.215	0.156	0.056	0.444	0.053
β-HCH	0.220	<0.081	0.565	20	16.95	0.410	0.254	0.103	1.552	0.224
γ-HCH	0.159	0.235	0.790	14	11.86	0.581	0.268	0.081	3.670	0.364
δ-HCH	<0.094	<0.094	1.001	7	5.93	1.001	0.280	0.159	4.814	0.452
HCB	0.269	0.146	<0.056	30	25.42	0.216	0.158	0.054	1.307	0.152
$\sum_6$PCB	2.014	0.924	2.785	65	55.08	2.925	0.265	0.061	7.380	1.095
PCB#28	0.781	0.095	0.313	28	23.73	0.356	0.150	0.061	2.261	0.296
PCB#52	<0.070	<0.070	0.522	28	23.73	0.522	0.185	0.071	4.084	0.449
PCB#101	0.079	<0.076	1.269	12	10.17	1.170	0.170	0.079	7.303	0.761
PCB#138	0.328	0.261	0.195	15	12.71	0.257	0.184	0.080	0.846	0.113
PCB#153	0.527	0.175	0.343	8	6.78	0.324	0.177	0.079	1.180	0.123

Compound	Nairobi	Nyeri	Nandi	No. +	% No. +	Mean	Median	Min	Max	SD
PCB#180	0.300	0.392	0.142	19	16.10	0.296	0.131	0.064	1.427	0.184

Notes: *< = Less than Quantification Limit, No. + = Number Positive, % No. + = Percentage Number Positive, Min = List, Max = Highest and SD = Deviation.*

Table 4.7: Occurrence frequencies (%) and levels (ng/g lw) of Studied POPs in human milk samples from Nairobi County (n = 26)

Compound	KBR	NE/W	No. +	% No. +	Mean	Median	Min	Max	SD
$\sum_2$Chlordane	0.458	0.872	8	30.77	0.677	0.580	0.065	1.314	0.357
Cis-nonachlor	0.271	0.339	6	23.08	0.317	0.290	0.122	0.656	0.158
Trans-nonachlor	0.187	0.533	8	30.77	0.36	0.291	0.065	0.892	0.224
$\sum_4$ DDT	2.119	19.941	26	100.00	10.22	0.970	0.065	128.979	26.011
p,p'-DDD	0.114	0.246	18	69.23	0.172	0.086	0.063	0.751	0.179
p,p'-DDE	1.070	17.811	22	84.62	8.679	0.454	0.093	126.292	25.644
o,p'-DDT	0.408	0.250	13	50.00	0.311	0.168	0.074	1.458	0.32
p,p'-DDT	0.527	1.634	25	96.15	1.058	0.520	0.183	11.086	2.124
$\sum_4$Endosulfan	0.978	4.356	12	46.15	4.024	0.163	0.044	12.405	2.422
Endosulfan ether	0.579	<0.094	2	7.69	0.579	0.579	0.267	0.891	0.180
α-Endosulfan	0.113	<0.033	5	19.23	0.113	0.106	0.044	0.194	0.051
β- Endosulfan	0.148	0.175	2	7.69	0.162	0.162	0.148	0.175	0.044
Endosulfan sulphate	0.138	4.181	4	15.38	3.170	0.107	0.062	12.405	2.431
$\sum_4$HCH	0.501	0.497	9	34.62	0.598	0.188	0.105	0.966	0.223
α-HCH	0.156	0.250	3	11.54	0.219	0.156	0.056	0.444	0.091
β-HCH	0.186	0.247	9	34.62	0.22	0.176	0.103	0.522	0.131
γ-HCH	0.159	<0.084	2	7.69	0.159	0.159	0.085	0.233	0.048
HCB	0.249	0.287	17	65.38	0.269	0.162	0.091	1.307	0.274
$\sum_5$PCB	0.352	2.174	10	38.46	2.015	0.228	0.065	4.84	0.953
PCB#28	<0.064	0.781	4	15.38	0.781	0.386	0.089	2.261	0.455
PCB#101	<0.076	0.079	1	3.85	0.079	0.079	0.079	0.079	0.015
PCB#138	0.195	0.372	4	15.38	0.328	0.175	0.115	0.846	0.170
PCB#153	<0.072	0.527	1	3.85	0.527	0.527	0.527	0.527	0.103
PCB#180	0.157	0.415	9	34.62	0.300	0.131	0.065	1.127	0.250

Table 4.8: Occurrence frequencies (%) and levels (ng/g lw) of Studied POPs in human milk samples from Nandi County (n = 61)

Compound	KCRH	KSCH - A	KSCH - M	LHC	MTT	NHCH	NO. +	% No. +	Mean	Median	Min	Max	SD
$\sum_4$Chlordane	0.693	0.210	-	-	-	0.132	11	18.03	0.731	0.203	0.065	0.616	0.133
Cis-Chlordane	0.113	0.210	<0.074	<0.074	<0.074	0.132	7	11.48	0.151	0.127	0.086	0.398	0.061
Trans-Chlordane	0.152	<0.058	<0.058	<0.058	<0.058	<0.058	7	11.48	0.152	0.107	0.065	0.370	0.060
Cis-nonachlor	0.156	<0.088	<0.088	<0.088	<0.088	<0.088	1	1.64	0.156	0.156	0.156	0.156	0.020
Trans-nonachlor	0.272	<0.065	<0.065	<0.065	<0.065	<0.065	3	4.92	0.272	0.321	0.157	0.337	0.062
$\sum_4$ DDT	1.180	0.640	0.402	0.597	-	0.874	44	72.13	0.970	0.3595	0.075	3.032	0.573
p,p'-DDD	0.215	0.154	0.167	0.087	<0.059	0.100	26	42.62	0.145	0.088	0.064	1.292	0.177
p,p'-DDE	<0.088	<0.088	<0.088	<0.088	<0.088	0.198	1	1.64	0.198	0.198	0.198	0.198	0.025
o,p'-DDT	0.129	0.113	<0.065	<0.065	<0.065	<0.065	11	18.03	0.121	0.117	0.08	0.179	0.050
p,p'-DDT	0.835	0.373	0.235	0.511	<0.093	0.575	32	52.46	0.506	0.351	0.099	2.881	0.526
$\sum_4$Endosulfan	9.296	1.036	0.404	0.549	-	3.717	25	40.98	5.348	0.325	0.040	7.487	1.130
α-Endosulfan	1.775	0.381	<0.033	0.396	<0.033	2.102	6	9.84	1.163	1.0635	0.093	2.745	0.458
β- Endosulfan	5.712	<0.041	<0.041	<0.041	<0.041	1.340	3	4.92	3.526	1.982	0.698	5.712	0.773
Endosulfan sulphate	1.809	0.655	0.404	0.153	<0.038	0.275	21	34.43	0.659	0.213	0.040	2.347	0.398
$\sum$ HCH	1.494	0.855	0.306	0.580	-	4.117	24	39.34	1.878	0.364	0.094	4.814	0.861
α-HCH	0.209	<0.055	<0.055	<0.055	<0.055	<0.055	2	3.28	0.209	0.209	0.094	0.324	0.043
β-HCH	0.609	0.166	<0.081	0.356	<0.081	0.827	11	18.03	0.490	0.448	0.143	1.552	0.295
γ-HCH	0.384	0.689	0.306	0.224	<0.084	3.109	11	18.03	0.942	0.622	0.081	4.814	0.785
δ-HCH	0.292	<0.094	<0.094	<0.094	<0.094	0.181	5	8.20	0.237	0.222	0.159	0.508	0.084
$\sum$ PCB	7.951	0.830	0.340	0.393	0.204	0.911	43	70.49	2.753	0.295	0.071	7.380	1.343
PCB#28	1.371	0.233	0.189	0.069	0.204	0.189	22	36.07	0.376	0.1805	0.068	1.898	0.282
PCB#52	1.701	0.454	0.151	0.136	<0.070	0.230	26	42.62	0.534	0.1905	0.071	4.084	0.606
PCB#101	4.194	0.143	<0.076	0.188	<0.076	0.192	10	16.39	1.179	0.2265	0.106	7.303	1.050
PCB#138	0.219	<0.083	<0.083	<0.083	<0.083	0.102	5	8.20	0.160	0.121	0.098	0.472	0.068
PCB#153	0.343	<0.072	<0.072	<0.072	<0.072	<0.072	5	8.20	0.343	0.116	0.079	1.18	0.154
PCB#180	0.123	<0.060	<0.060	<0.060	<0.060	0.198	4	6.56	0.161	0.1465	0.077	0.198	0.038

Notes: *< = Less than Quantification Limit, No. + = Number Positive, % No. + = Percentage Number Positive, Min = List, Max = Highest and SD = Deviation.*

Table 4.9: Occurrence frequencies (%) and levels (ng/g lw) of Studied POPs in human milk samples from Nyeri County (n = 29)

Compound	K	NR	No. +	% No. +	Mean	Median	Min	Max	SD
$\sum_2$CHL	0.365	0.363	4	13.79	0.355	0.248	0.074	0.445	0.106
Cis-nonachlor	0.222	0.182	2	6.90	0.202	0.202	0.182	0.222	0.052
Trans-nonachlor	0.143	0.181	4	13.79	0.153	0.157	0.074	0.223	0.058
$\sum_4$DDT	1.093	1.026	29	100.00	1.059	0.513	0.148	2.733	0.551
p,p'-DDD	0.186	0.068	7	24.14	0.102	0.071	0.059	0.269	0.056
p,p'-DDE	0.307	0.337	13	44.83	0.323	0.225	0.082	0.814	0.226
o,p'-DDT	0.188	0.117	17	58.62	0.173	0.138	0.065	0.537	0.128
p,p'-DDT	0.412	0.504	27	93.10	0.461	0.375	0.092	1.378	0.322
$\sum_1$Endosulfan	0.047	-	1	3.45	0.047	0.047	0.047	0.047	0.009
Endosulfan sulphate	0.047	<0.038	1	3.45	0.047	0.047	0.047	0.047	0.009
$\sum_1$HCH	-	0.235	3	10.34	0.235	0.176	0.094	0.435	0.087
γ-HCH	<0.084	0.235	3	10.34	0.235	0.176	0.094	0.435	0.087
HCB	0.133	0.120	13	44.83	0.146	0.120	0.054	0.379	0.097
$\sum_4$PCB	1.028	0.681	12	41.38	0.923	0.125	0.061	1.918	0.385
PCB#28	0.102	0.092	3	10.34	0.095	0.102	0.061	0.123	0.031
PCB#138	0.491	0.189	6	20.69	0.261	0.286	0.080	0.491	0.127
PCB#153	<0.072	0.175	2	6.90	0.175	0.175	0.084	0.266	0.051
PCB#180	0.435	0.225	6	20.69	0.392	0.176	0.064	1.427	0.273

Notes: *< = Less than Quantification Limit, No. + = Number Positive, % No. + = Percentage Number Positive, Min = List, Max = Highest and SD = Deviation.*

4.5 Intake of studied POPs by study participants' breastfed infants

The participants exposed their infants to the studied POPs through breast milk (Appendix 13). The infant's exposure to studied POP compounds, assuming exclusive breastfeeding, were: $\sum_4$Chlordane 0.143 - 4.231 ng/g bw/day; $\sum_4$DDTs 0.112 - 538.127 ng/kg bw/day; $\sum_4$Endosulfans 0.140 - 73.521 ng/kg bw/day; $\sum_4$HCHs 0.096 - 23.048 ng/kg bw/day; HCB 0.301 - 21.791 ng/kg bw/day; and $\sum_6$iPCBs 0.169 - 26.347 ng/kg bw/day. Based on ADI proposed by ATSDR, 2021, 1.70% and 2.54% of infants were exposed to $\sum_4$DDTs and $\sum_6$iPCBs, respectively, at levels above ADIs of 100 ng/kg bw/day for $\sum_4$DDTs and 20 ng/kg bw/day for $\sum_6$iPCBs. However, based on ADI proposed by FAO-WHO in 2019 and European Commission in 2008, none of the participants' infants were exposed to levels above ADIs.

Table 4.10: Mean EDI of Studied POPs by breastfeeding infants in Nairobi, Nandi, and Nyeri Counties, Kenya (n = 116)

Compound	EDI (ng/kg/day)				Number Exposed	Exposed No. above ADI			% above ADI
	Mean	Median	Min	Max		Codex	ATSDR	EU	
Aldrin	<0.080	<0.080	<0.080	<0.080	0	0	0	0	0
$\sum_4$Chlordane	1,506	0.906	0.143	4.231	24	0	0	0	0
$\sum_4$DDT	11.886	2.752	0.112	538.127	102	0	2	0	1.70
Dieldrin	<0.092	<0.092	<0.092	<0.092	0	0	0	0	0
$\sum_4$Endosulfan	4.990	1.197	0.140	73.521	38	0	0	0	0
∑Endrin	<0.050	<0.050	<0.050	<0.050	0	0	0	0	0
$\sum_4$HCH	3.170	1.832	0.096	23.048	36	0	0	0	0
∑Heptachlor	<0.088	<0.088	<0.088	<0.088	0	0	0	0	0
HCB	4.820	1.475	0.301	21.791	30	0	0	0	0
∑MXC	<0.077	<0.077	<0.077	<0.077	0	0	0	0	0
Mirex	<0. 091	<0. 091	<0. 091	<0. 091	0	0	0	0	0
Pentachlorobenzene	<0.055	<0.055	<0.055	<0.055	0	0	0	0	0
$\sum_6$iPCB	3.505	1.130	0.169	26.347	65	0	3	0	2.54

Notes: ADI = Accepted Daily Intake, < = Less than Quantification Limit, EDI = Estimated Daily Intake, No. = Number, Min = List, Max = Highest, ng/kg/day = nanogram per Kilogram per Day, Codex = Codex Alimentarius Commission, ATSDR = Agency for Toxic Substances and Disease Registry, EU = European Union.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

This study is one of a few that have reported the occurrence and levels of POPs in HM across Kenya. Human milk has, with time since 1950s, become critical in the assessment of the distribution and levels of POPs in a wide variety of contexts, such as global population monitoring (Massart *et al.,* 2008). However, it is imperative to understand and control factors that influence the human body burdens of these persistent lipophilic compounds, their incorporation into human milk, and the ability to acquire suitable milk samples (Needham *et al.,* 2002; Needham and Wang, 2002; Ni *et al.,* 2011; Stockholm Convention Secretariat, 2013). Therefore, this cross-sectional study was based on primiparous mothers. Levels of POP in the milk of a primipara mother reflects that of the population in time and geographical space and can compare well with previous similar studies (Haddad, 2015).

Quality control blank samples had no responses at the analytes' elution time windows, while all reference standards spikes in bovine milk were recovered adequately, therefore ensuring the precision, sensitivity, and specificity of the laboratory protocols employed. Multiple Reaction Monitoring mode in GCMS/MS operations reduced the matrix interferences effect in identifications and quantifications of the analytes, therefore lowering detection levels.

5.1.1 Lipid content of HM from Nairobi, Nandi and Nyeri Counties

The mean lipid contents of studied human milk exhibited significant differences, $p < 0.05$, between the study locations. This might be explained by the variations in the type of food and eating frequency of the participants. Though all the study participants consumed mixed diet, rural dwellers, with mean HM lipid content of 3.95%, seemed better nourished than the urban dwellers, with mean HM lipid content of 3.09%. This might be explained by participants' food affordability and frequency, whereby rural dwellers could afford a balanced diet with frequent

animal products such as milk, eggs, et cetera. This is largely due to subsistence mixed farming, while urban dwellers who depend purely on purchased food might not (KNAP, 2018). Also, the difference could be due to cyclic diurnal variation in lipid content, which is excessive in milk from undernourished mothers who consume diets with low-fat and high-carbohydrate foods such as cereals (Jensen, 1999).

5.1.2 Factors that influence the occurrence of POPs in human milk in Nairobi, Nandi, and Nyeri Counties

All 116 participants who donated HM samples reported consumption of animal products such as fish, chicken, eggs, meat, and bovine milk, though the types and frequencies varied. And 114 HM, equivalent to 98.3%, had quantifiable levels of one or more POP compound(s). This points to a positive correlation between the occurrence of POPs in the HM and the consumption of these fat-containing foods by milk donors.

Mungai and Wang (2019) found quantifiable levels of POPs (DDTs and HCHs) in soil samples collected from Kapsabet and Nyeri towns, implying that earthworms in these areas could accumulate the compounds (Aerts *et al.,* 2019; Network *et al.,* 2021). Likewise, free range chickens in these areas could bioaccumulate DDTs and HCHs by consuming the exposed earthworms (Aerts *et al.,* 2019; WHO, 2010). Consequently, the biomagnified POPs are ingested by the humans through the consumption of exposed chicken and/or eggs (Aerts *et al.,* 2019; WHO, 2010). This, therefore, explains the positive correlations between the occurrence of DDTs and HCHs in human milk samples and consumption of free-range chicken and/or eggs by the milk donors from Nandi and Nyeri Counties. This is a classic case of POP bioaccumulation along the food chain i.e., soil-earthworm-chicken-egg-human-infant.

Consumption of fish by the milk donors correlate positively with the occurrence of POPs in the HM samples, with the exception of two samples that were in the contrary. This is in agreement with previous studies that have reported fish consumption as a probable source of POPs in the

human body (Aerts *et al.,* 2019; Chávez-Almazán *et al.,* 2020; Florence, 2022; Lee *et al.,* 2013; Solomon and Weiss, 2002; Tsygankov *et al.,* 2019; Wang *et al.,* 2019; Wenaty, 2019; Fång et al., 2015) However, the effects of consumption of bovine milk or meat on the occurrence of POPs in studied HM were not definitive. Nonetheless, earlier studies have reported positive correlations (Cao *et al.,* 2011; Chávez-Almazán *et al.,* 2020; Thompson *et al.,* 2017; Wang *et al.,* 2011).

Therefore, diet, which was mixed across the study locations, remains the most significant source of quantified POPs amongst the study participants. This is true because animal products consumed across Kenya, could somehow have been in contact with POP-contaminated environments such as Lake Victoria and Indian Ocean (Table 2.3). Similarly, animal feeds used across Kenya are mainly from the same source and from the same raw materials, so when contaminated with pollutants, the effects would spread across the country (KFIPR, 2013).

There was no significant difference ($p > 0.05$) in the mean ages and BMIs of the participants, and hence their effects on the levels of POPs in the HM samples could not be associated. However, a number of previous studies have reported a positive association between the levels of POPs in HM and age and body mass index and (ATSDR, 2019a; Dimitriadou *et al.,* 2016; Florence, 2022; Polder *et al.,* 2009). Advanced age has been associated with more accumulated POPs, whereas relative body fat content, which increases with BMI, dilutes POP concentrations. The age and body mass index of the milk donors contributed to some extent to the levels of quantified POPs in this study.

5.1.3 Occurrences and levels of studied POP compounds in human milk from Nairobi, Nandi, and Nyeri Counties

The sum of four DDT analogues (*p,p'*-DDD, *p,p'*-DDE, *o,p'*-DDT, and *p,p'*-DDT) and the sum of six indicator PCBs (28, 52, 101, 138, 153, and 180) were the most prevalent POP groups in 86.44% and 55.08% HM samples, respectively (Table 4.5). Whereas, chlordane, endosulfan,

HCB and HCHs groups as sum of appropriate metabolites *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, *trans*-nonachlor, endosulfan ether, *α*-endosulfan, *β*-endosulfan, endosulfan sulphate, *α*-HCH, *β*-HCH, *γ*-HCH or *δ*-HCH occurred moderately (Table 4.5). This is despite the chemicals being banned more than three decades ago in Kenya (PCPB, 2018). Therefore, the main explanation to this is the compounds' environmental persistence, transboundary spread, and/or long duration of high dose in the medium of exposure (El-Shahawi *et al.,* 2010). However, there was a general reduction in the occurrences and levels of studied POPs in Nairobi and Nyeri Counties as compared to earlier findings (Table 4.5).

Dichlorodiphenyltrichloroethane mostly *p,p'*-DDT, and *p,p'*-DDE metabolite occurred widely at the range of 0.059 ng/g lw to 126.292 ng/g lw (Table 4.6 and Appendix 13). Even though, the mean occurrence levels of *p,p'*-DDE were extremely higher than that of *p,p'*-DDT, more individual HMs samples (72.88%) had *p,p'*-DDT (Table 4.6 and Appendix 13). This was consistent with reported continual change of *p,p'*-DDT to *p,p'*-DDE through metabolism and their half-lives in the human body of 6 and 10 years respectively (EPA, 2008). The average of the ratio of *p,p'*-DDT to *p,p'*-DDE was 0.166 pointing to a historic exposure (Table 4.6).

Endosulfan occurrences were dominated by endosulfan sulphate which was quantified in 22.88% HM (Table 4.6). This was in agreement with the metabolic pathways of endosulfan in humans, where it breaks down into bio-accumulative endosulfan sulphate then further to endosulfan ether and diol (Kuvarega and Taru, 2007). The findings point to a possible recent usage of endosulfan in the study Counties, with Nairobi and Nandi Counties being the most polluted.

Hexachlorocyclohexane occurred in low levels dominated by *β*-HCH and *γ*-HCH. This indicates historic exposure of HCHs since, with time, exposed HCHs metabolized or isomerized to the more persistent *β*-HCH which has 10 to 30 times the potential to accumulate in lipids than *γ*-HCH (Phillips *et al.,* 2005; Rai *et al.,* 2012 and Jayaraj *et al.,* 2016). In addition,

accumulation of α-HCH, β-HCH, γ-HCH and δ-HCH varies along the food chains up to excretion in HM (Phillips *et al.,* 2005 and Rai *et al.,* 2012).

Chlordane occurred at low levels, with unexpected occurrences of *cis*-chlordane and *trans*-chlordane in samples from Nandi County. The mean ratio of *trans*- and *cis*-chlordane was 0.968, suggesting the possible existence of fresh exposures because the body excrete *cis* isomers more than *trans* (Bondy *et al.,* 2003). This was in contrast to earlier studies, which did not find detectible levels of these compounds in Kenya (Table 2.2). The occurrence and levels of persistent oxidative metabolites of chlordane (*trans*-nonachlor and *cis*-nonachlor) agrees well with the frequent findings in HM samples. Oxidative persistent *oxy*-chlordane was detected in some samples by GCMS/MS using MRM mode; however, the occurrence levels could not be tabulated due to lack of a reference standard.

Hexachlorobenzene occurred in low levels. Though prohibited for agricultural use, HCB could occur as a by-product in several chlorinated industrial chemicals and/or solvents (WHO, 2006). In the human body, HCB is metabolized to Pentachlorophenol and pentachlorothiophenol; however, HCB is mainly excreted unmetabolized via HM, faeces and urine (ATSDR, 2015b). Though traces of Pentachlorophenol occurred in some samples as detected by the sensitive MRM mode of GCMS/MS, their occurrence levels could not be tabulated due to a lack of reference standard.

Polychlorinated biphenyls occurred at low levels, with sum-6iPCBs being in the same order of magnitude as found earlier in Kenya (Kanja, 1988; Madadi *et al.,* 2021). Generally, the levels of sum-6iPCBs have decreased in Kenya. Across the studied HM samples, *tri* and *tetra*-chlorinated PCBs (PCB#28 and PCB#52) occurred most frequently, while PCB#101 (*penta*-chlorinated PCBs) had the highest occurrence level (Table 4.6 and Appendix 13). PCB#153 (*hexa*-chlorinated PCBs) was the dominant congener in HM samples and was widely reported in the least number of HM samples (Fång *et al.,* 2015; Müller *et al.,* 2017). Granting that

equipment containing PCBs were imported for used in the industry and in power stations in Kenya (Kanja *et al.,* 1992), the low trace levels in the HM and environment as has been observed in earlier studies (Table 2.2), points either to the rare application or proper disposal of PCB-containing equipment in Kenya.

5.1.4 Geographical distribution of the occurrences and levels of studied POPs in HM in Kenya.

There were slight variances, $p < 0.05$, in the occurrence levels of studied POPs among individual HM samples and between study locations. This indicates that, though with four exceptional outliers, primiparous mothers were exposed to similar background levels of POPs across the study Counties. The outliers were all from Nairobi County and had high levels of DDT of between 8.138 ng/g lw and 128.979 ng/g lw (Table A13.2). Apart from the four outliers, the levels of DDT quantified across Nairobi, Nandi, and Nyeri were significantly low, with no significant variations ($p > 0.05$) between the Counties. For chlordane, and based on Tukey's multiple comparisons test, Nairobi and Nandi Counties had significant variation, $P = 0.044$, while there was no significant variation in the occurrence levels in Nairobi and Nyeri Counties, $P = 0.999$, and in Nandi and Nyeri Counties, $P = 0.143$. Similarly, the occurrence levels of endosulfan, HCB, HCHs, and PCBs in HM samples across the study Counties were not different, $P > 0.05$. Therefore, the background sources of human exposure to POPs in Kenya are quite significant, $p < 0.05$. Generally, the geographical differences of the study Counties had no effects on the occurrence and levels of POPs in HM, which can be attributed to the entwined environmental components of the studied Counties.

5.1.5 Trends in the occurrences of studied POPs in human milk of primiparous mothers of Kenya.

Generally, there is a reduction in the occurrence and levels of studied POPs in HM from nursing primiparous mothers in Kenya (Figure 5.1); though, a slight increase of 24.14% occurred for

chlordane from 0.700 to 0.869 ng/g lw. In the 1980s, PCBS were below the detection limit of the equipment used at that time (Kanja *et al.,* 1986). But in the 2008–2011 and 2016–2022 global surveys, and this study, 4.32, 2.47 and 2.925 ng/g lw quantifiable levels of PCBs occurred respectively. This therefore implies that between 2009 and 2019 there has been a decrease of 32.29% in the occurrence levels of PCBs in HM (Table 4.5).

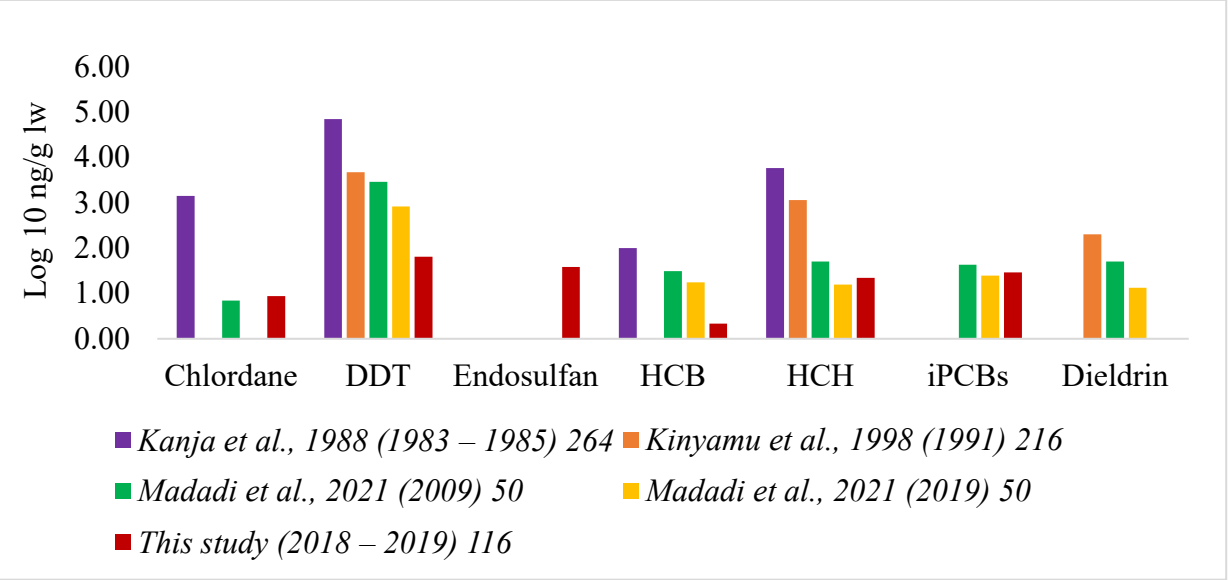

Figure 5.1: Clustered column illustrations of trends of log transform occurrence levels of Studied POPs in HM in Kenya.

For endosulfan, it was not possible to observe the time trend, since no earlier studies were found. Aldrin, dieldrin, endrin, heptachlor, and pentachlorobenzene were not found in this investigation, despite the fact that they had been quantified in past studies. This suggests that the levels of the compounds have decreased (Table 2.3). Pentachlorophenol was detected in this investigation in trace amounts. The presence of methoxychlor, mirex, or pentachlorophenol in human milk was not observed in this investigation, and no data were found indicative of their presence in Kenya. This study findings therefore, point to a possible effectiveness of Kenya's government's POPs restrictions or bans, which were enforced starting in 1986 in compliance with international proposals that were later made mandatory by the Stockholm Convention.

The mean occurrence levels of studied POPs in HM from Nairobi, Nandi and Nyeri Counties, Kenya were largely below most of the global findings (Figure 5.2).

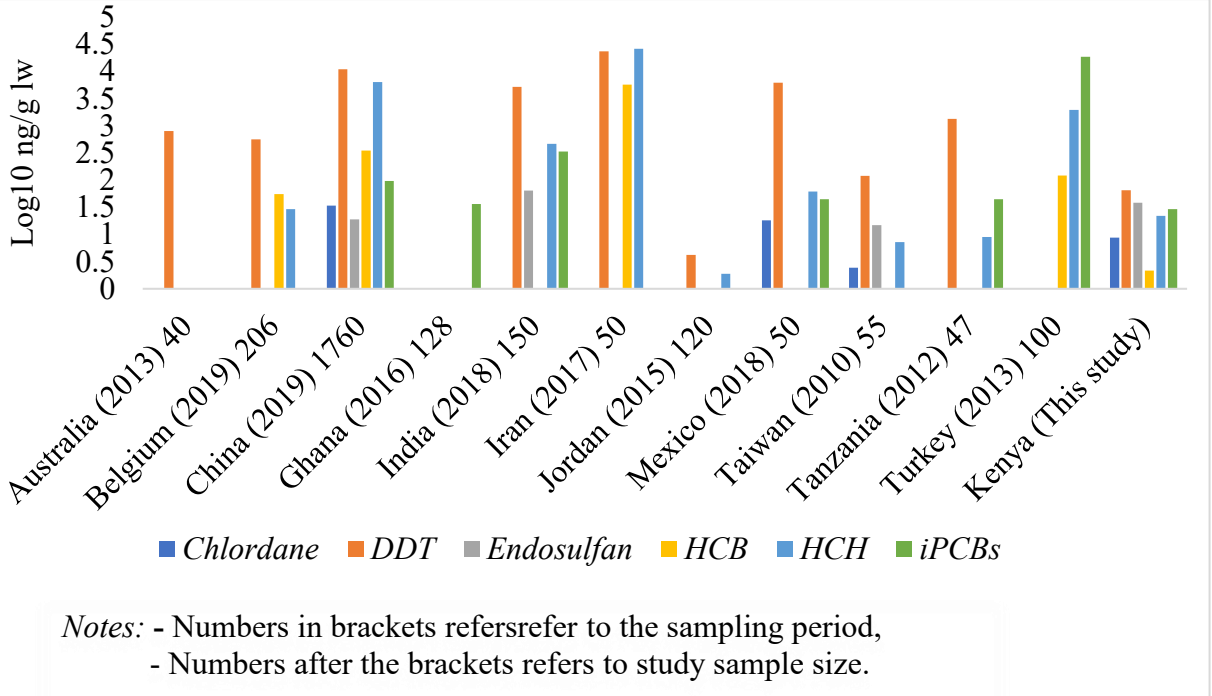

Figure 5.2: Clustered column illustrations of this study and global findings.

5.1.6 Health risks to infants of mothers who are exposed to the studied persistent organic pollutants in Nairobi, Nandi, and Nyeri Counties

Four infants two from Nairobi and two from Nandi Counties were exposed to either $\sum_4$DDTs or $\sum_6$iPCBs pollutants above the daily accepted risk levels (Appendix 14). However, of serious concerns is the exposure of one infant from Nairobi to the two groups of pollutants above their daily toxicological limits. While breastfeeding provides good nutrition and important psychological development for the infant together with helpful protection against infections, HM is the major excretion route of lipid-soluble contaminants such as studied POPs (Sarkar, 2009). An infant who consumes HM for three to six months takes in ≥ 20% of contaminants in the body of the mother (Rogan, 1996), and adverse health risks arising from contaminants are greatest during sensitive early life developmental stages.

According to this study, the estimated daily intake of the $\sum_4$DDTs analogues and $\sum_6$iPCBs was found to be higher than the Proposed Acceptable Daily Intakes (PADIs) based on ATSDR 2021 by two and three nursing infants, respectively (Table 4.9 and Appendix 14). One infant exceeded the PADIs (ATSDR, 2021) for both $\sum_4$DDTs and $\sum_6$iPCBs. None of the infants exceeded the PADIs (ATSDR, 2021) for $\sum_4$chlordane, $\sum_6$endosulfan, HCB and $\sum_4$HCH. Repeated exposure to $\sum_4$DDTs and/or $\sum_6$iPCBs at levels above the PADIs may result in neurological disorders, hepatic, developmental and immunotoxin effects (Jayaraj *et al.,* 2016). The risk of any such effect depends on the life stage, the dose and the duration to which exposure exceeded the ADIs.

WHO recommends exclusive breastfeeding of infants for six months after birth (Unicef *et al.,* 2014). However, in Kenya, it is common practice to breastfeed for up to 2 years. This means that Kenyan infants could be at eminent risk and the importance of positive health benefits due to HM versus high risk associated with POPs contamination calls for scrutiny. In particular, the four infants with whom their mother's milk had $\sum_4$DDTs and $\sum_6$iPCBs levels above PADI, may risk being exposed to sustained levels of health concern.

The use of PADI's established for adults, might 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 and Nougadère *et al.,* 2020). Based on Müller *et al* (2017) POPs such as OCPs and PCBs are reported to have common mechanisms of action and effects. When all the above postulates are taken into considerations, the infants' health concern increases significantly.

5.2 Conclusion

This study aimed to assess the occurrence and levels of POPs in HM, their toxicological risks to breastfeeding infants, and their possible sources. The study results affirm that primiparous mothers in Nairobi, Nandi, and Nyeri Counties are exposed to chlordane, DDT, endosulfan, HCB, hexachlorocyclohexane (δ, β, δ, and γ HCH which is referred to as Lindane), and indicator PCBs compounds. These were 23 POP compounds belonging to seven groups out of the 13 POP groups investigated. These POP compounds occurred differently across 98.3% of the tested HM samples and reflected mixed historical and recent exposures. DDTs and PCBs were the main groups and were each quantified in more than 50% of the studied HM samples across the study counties.

Generally, the occurrence levels of POPs across the studied HM samples were low compared with earlier similar studies in Kenya and around the globe. The decreased residue levels of the majority of POPs point to possible efficiency of the regulatory restrictions implemented through the Stockholm Convention and local initiatives. Except for four outliers from Nairobi, which had high levels of sum DDTs, there were no significant variations ($p > 0.05$) in the residue levels of the compounds between individual participants and the study Counties.

The results established that four infants suffered a high probable toxicological risk from the sum of four DDT analogues and the sum of six indicator PCBs through exclusive breastfeeding. The daily intake of the compounds by the infants, assuming exclusive breastfeeding, exceeded the accepted daily intake of an adult human based on risk limits proposed by ATSDR. Of significance is the daily exceedance of the risk levels of the two groups of compounds by an infant in Nairobi County. There could be high toxicological risks if the synergistic and potentiating effects of the compounds are considered. Also, if such a consideration is applied to all the quantified POPs, especially in situations of up to nine exposed compounds per individual, then all the exposed infants were faced with potential amplified toxicological risks.

These study participants consumed animal products such as fish, chicken, eggs, meat, and bovine milk, which correlate positively with quantified POPs in HM milk samples. All the study participants consumed a mixture of vegetables and animal products in their diet. Therefore, the results of this study, which concur with previous findings, deduce that quantified POPs in HM samples were most probably acquired through the diet consumed in Nairobi, Nandi, and Nyeri Counties.

5.3 Recommendations

The following recommendations were made from the study;

i. There is a need for continuous surveillance of POPs in human milk in order to map out and minimize or eradicate potential human exposure sources.
ii. There is a need for studies in prolong exposures to developing infants of multiple groups of compounds to ascertain the combined effects of the compounds, which cannot be avoided throughout breastfeeding.
iii. There is need for regular POPs residue monitoring strategies in foods of animal origin and animal feeds in order to generate Nationwide baseline data on status of POPs levels.
iv. Stringent controls on the use of pesticides on food meant for human consumption should be done in order to guarantee the safety of humans.

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APPENDICES

APPENDIX 1: ETHICAL APPROVALS OF THE RESEARCH

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
Tel:(254-020) 2726300 Ext 44355

KNH-UON ERC
Email: uonknh_erc@uonbi.ac.ke
Website: http://www.erc.uonbi.ac.ke
Facebook: https://www.facebook.com/uonknh.erc
Twitter: @UONKNH_ERC https://twitter.com/UONKNH_ERC

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

17 NOV 2017

Ref: KNH-ERC/A/348

17th November, 2017

Dr. Laetitia Kanja
National Study Coordinator
Department of Public Health, Pharmacology and Toxicology
College of Agriculture and Veterinary Sciences
University of Nairobi

Dear Dr. Kanja,

RESEARCH PROPOSAL – UNEP- COORDINATED SURVEY OF HUMAN MILK FOR PERSISTENT ORGANIC POLLUTANTS: GUIDELINES FOR ORGANIZATION, SAMPLING AND ANALYSIS {NATIONAL PROTOCOL 2017} (P579/10/2017)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH-UoN ERC) has reviewed and **approved** your above proposal. The approval period is from 17th November 2017 – 16th November 2018.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH-UoN ERC before implementation.
c) Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH- UoN ERC for each batch of shipment.
g) Submission of an *executive summary* report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

Protect to discover

For more details consult the KNH- UoN ERC website **http://www.erc.uonbi.ac.ke**

Yours sincerely,

PROF. A.N GUANTAI
CHAIR, KNH-UoN ERC

c.c. The Principal, College of Health Sciences, UoN
The Director, CS, KNH
The Assistant Director, Health Information, KNH

Figure A1.1. KNH-UON Ethics and Research Committee Approval

UNIVERSITY OF NAIROBI
FACULTY OF VETERINARY MEDICINE
DEPARTMENT OF VETERINARY ANATOMY AND PHYSIOLOGY

P.O. Box 30197,
00100 Nairobi,
Kenya.

Tel: 4449004/4442014/ 6
Ext. 2300
Direct Line. 4448648

Mr. Nehemiah Birgen
University of Nairobi
Dept of PHP&T

REF: FVM BAUEC/2019/197

27/02/2019

Dear Mr. Birgen,

RE: Approval of Proposal by Biosafety, Animal use and Ethics committee

Assessment of selected persistent organic pollutants in human milk from Nairobi, Nandi and Nyeri Counties, Kenya.

By Mr. Birgen (J56/7708/ 2017).

We refer to your MS.c proposal submitted to our committee for review and your application letter dated 13/02/2019.

We have reviewed your proposal, particularly section 3.1 that involves collection, transportation and processing of human milk samples from mothers in selected public health hospitals from the three counties.

We are satisfied that appropriate biosafety measures will be undertaken during the study as per ethical rules and regulations.

We hereby give approval for you to proceed with the experiments as outlined in the submitted proposal.

Yours sincerely

Kaluwa

Dr. Catherine Kaluwa, BVM, MSc, Ph.D
Chairperson,
Biosafety, Animal Use and Ethics Committee
Faculty of Veterinary Medicine.

Figure A1.2. Biosafety, Animal Use and Ethics Committee, Faculty Veterinary Medicine Approval

APPENDIX 2: RESEARCH AUTHORIZATION BY NACOSTI

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

Telephone:+254-20-2213471, 2241349,3310571,2219420
Fax:+254-20-318245,318249
Email: dg@nacosti.go.ke
Website : www.nacosti.go.ke
When replying please quote

NACOSTI, Upper Kabete
Off Waiyaki Way
P.O. Box 30623-00100
NAIROBI-KENYA

Ref: No. **NACOSTI/P/19/83781/27700**

Date: **29th January, 2019**

Nehemiah Kipngetich Birgen
University of Nairobi
P.O. Box 30197 - 00100
NAIROBI

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"Assessment of selected persistent organic pollutants in human breast milk from Nairobi, Nandi and Nyeri Counties, Kenya"*** I am pleased to inform you that you have been authorized to undertake research in **Nairobi, Nandi and Nyeri Counties** for the period ending **29th January, 2020.**

You are advised to report to **the County Commissioners, the County Directors of Education and the County Directors of Health Services, Nairobi, Nandi and Nyeri Counties** before embarking on the research project.

Kindly note that, as an applicant who has been licensed under the Science, Technology and Innovation Act, 2013 to conduct research in Kenya, you shall deposit **a copy** of the final research report to the Commission within **one year** of completion. The soft copy of the same should be submitted through the Online Research Information System.

GODFREY P. KALERWA MSc., MBA, MKIM
FOR: DIRECTOR-GENERAL/CEO

Copy to:

The County Commissioner
Nairobi County.

The County Director of Education
Nairobi County.

The County Director of Health Services
Nairobi County.

The County Commissioner
Nandi County.

The County Director of Education
Nandi County.

The County Director of Health Services
Nandi County.

The County Commissioner
Nyeri County.

The County Director of Education
Nyeri County.

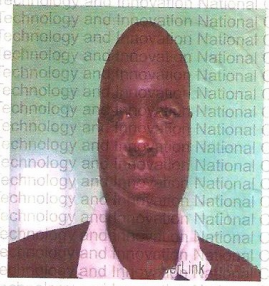
The County Director of Health Services
Nyeri County.

THIS IS TO CERTIFY THAT:
MR. NEHEMIAH KIPNGETICH BIRGEN
of UNIVERSITY OF NAIROBI, 9548-2002 Nairobi,has been permitted to conduct research in *Nairobi, Nandi , Nyeri Counties*

on the topic: *ASSESSMENT OF SELECTED PERSISTENT ORGANIC POLLUTANTS IN HUMAN BREAST MILK FROM NAIROBI, NANDI AND NYERI COUNTIES, KENYA.*

for the period ending:
29th January,2020

Permit No : NACOSTI/P/19/83781/27700
Date Of Issue : 29th January,2019
Fee Recieved :Ksh 1000



.....
Applicant's Signature

.....
Director General
National Commission for Science, Technology & Innovation

APPENDIX 3: SOCIODEMOGRAPHIC DATA AND HUMAN MILK SAMPLES COLLECTION PROTOCOLS

Research topic: ASSESSMENT OF SELECTED PERSISTENT ORGANIC POLLUTANTS (POPs) IN HUMAN MILK FROM NAIROBI, NANDI AND NYERI COUNTIES.

Introduction

Persistent organic pollutants (POPs) are organic compounds that are resistant to environmental degradation through chemical, biological, and photolytic processes. POPs persist in the environment for long periods, are capable of long-range transport by wind and water therefore, their effects spread to areas far from where they were exposed.

Persistent organic pollutants characteristic lipophilicity makes them bioaccumulate in human and animal tissue and biomagnify in the food chains, and have potentially significant impacts on human health and the environment. Exposure to POPs can cause serious health problems including certain cancers, birth defects, dysfunctional immune and reproductive systems, greater susceptibility to disease and even diminished intelligence. POPs include DDT and PCBs which were widely used in the past in agriculture and public health. Human milk is an important specimen used to evaluate occurrence levels of these compounds in the human body. Human milk has been used to test for POPs globally for several decades and has given good indication of their prevalence in the population.

Study participants (human milk donors) selection criteria

Collection of human milk will be conducted at Maternal and Child Health Clinics (MCHC) providing postnatal services. Mothers with markedly different exposure to POPs shall not be included in the study to avoid skewing the results. Therefore, selection criterion to be met by the mother before donating milk are:

1. Mother shall be primiparas and breastfeeding their first-born singlet child (i.e., no twins).
2. Mother shall be under 30 years of age.
3. Mother shall have resided in the represented area (County) for at least five years.
4. Both the mother and the infant shall be apparently healthy, including normal pregnancy.
5. Lactation period or infant age shall be between two and eight weeks.

Sampling standard operating procedure.

1. Sensitize the health care workers about the study, their critical role and persistent organic pollutants (by the principal researcher and the research assistant).
2. Sensitize potential milk donors about the study, persistent organic pollutants and the importance of their participation (by sensitized health care worker).

   NB. Notwithstanding the study concern and objectives, potential human milk donors shall be encouraged to breastfeed exclusively for the first six months and assured that refusal to participate shall not in any way affect their future access of clinical services at the hospital.

3. Uniquely identify consenting forms, survey questionnaires and human milk collecting glass vials for each individual donor (by the health care worker).

   NB: Use small square sticker to label the Teflon of the 50 mL glass vial and the rectangular sticker to label the glass vial (by the health care worker).
4. Provide a private room for consenting and expression of milk with access to clean water in order to upheld confidentiality (by the health care worker).
5. Guide the mother to understand the study consent requirements, sign the consent form and give information as per the questionnaire (by the health care worker).
6. Wash hands before handling milk sampling glass vials or expressing milk (by the health care worker and the milk donor).
7. After cleaning the hands and the breasts, express milk directly into the given clean 30 mL glass vial to between half full and full observing high hygiene (by the human milk donor).
8. Ensure sample glass vial is cap screwed tightly using aluminum foil with the dull side being in contact with the sample once enough milk is expressed (by the health care worker).
9. Immediately or before the expiry of 72 hours ensure the milk samples are stored in the freezer (by the health care worker).

APPENDIX 4: PARTICIPANT'S INFORMED CONSENT FORM

Certificate of Consent

I have been invited to take part in the study on Persistent Organic Pollutants (POPs) using Human Milk under the University of Nairobi. I have been told the purpose and procedures of this survey, in summary--

Purpose of the study

Persistent organic pollutants (POPs) mainly those of man-made origin can be found in the environment. These compounds are resistant to natural degradation and are often found in fat-containing foods, including human milk. The University of Nairobi has permitted NEHEMIAH KIPNGETICH BIRGEN, to undertake a study on selected POPs using human milk. The data obtained from this study will be use purely for educational purposes, however, National and County health sectors will have access for possible health risks interventions.

While concerns about POPs have been raised, the evidence for the health advantages of breastfeeding has continued to increase. On a population basis, exclusive breastfeeding for six months is the recommended feeding mode for the vast majority of infants, followed by continued breastfeeding with appropriate complementary foods for up to two years or beyond.

Procedures

The researcher is requesting you to offer information and approximately 30 mL of breast milk. You are to hand express the breast milk at your convenience. Collected milk will be analysed at the University of Nairobi.

Risks and discomforts

You may have some discomfort when you express your milk by hand. You will be train on how to express milk. None of the questions asked will be personal.

Confidentiality

The information collected from this study will be kept confidential. Information about you that will be collected from this study will be stored in a file that will not have your name on it, but a number assigned to it instead. The name associated with the number assigned to each file will be kept under lock and key and will not be divulged to anyone except NEHEMIAH KIPNGETICHBIRGEN.

Alternatives to participation

You do not have to take part in this study if you do not wish to do so, and refusing to participate will not affect your attendance in this clinic in any way. You will still have all the benefits that you would otherwise have at this clinic.

Contact information

If you have any questions, you may ask them now or later. If you wish to ask questions later, you may contact the following person: NEHEMIAH K. BIRGEN 0722621327.

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a subject in this study. I also consent that any excess breast milk sample may be kept for related study in the future.

Name of Participating Mother	**Date (dd/mm/yy)**	**Signature**
_____	___/___/____	_____

If illiterate an independent literate person shall witness.

(If possible, this person should be selected by the participant and should have no connection to the researcher)

Name of Independent Literate Witness	**Date (dd/mm/yy)**	**Signature**
_____	___/___/____	_____
Name of the clinic representative	**Date (dd/mm/yy)**	**Signature**
_____	___/___/____	_____
Name of the researcher	**Date (dd/mm/yy)**	**Signature**
_____	___/___/____	_____

APPENDIX 5: THE STUDY QUESTIONNAIRE

My name is NEHEMIAH KIPNGETICH BIRGEN, a Postgraduate Student at the University of Nairobi pursuing Master of Science degree in Pharmacology and Toxicology. I am currently doing research on selected persistent organic pollutants in human milk in partial fulfillment for the requirements for the award of the degree. You are therefore requested to take approximately five minutes of your time to kindly complete this questionnaire. Your genuine responses are greatly appreciated. This questionnaire has been designed to assist the researcher to collect data. The information which you will provide will be used only for the purpose of this study and will be held strictly confidentially.

Part I: General Information

1. Date (day/month/year): ____/____/____, Time: _____

2. Hospital Name/Address: _____

_____

3. County: _____ Sub County: _____

Part II: Mother's Information

1. Mother's code number: _____

2. Name (Optional): _____ Weight: ____ kg and height: _____ ft.

3. Age: _____, $15 \leq () \leq 30$ years.

4. Occupation i.e., house wife, farmer, business type, industry engagement etc., ___

_____Period: _____ years.

5. Earlier occupations/periods if different from the current one: _____

_____

6. Home area: _____ address: _____

7. How many years have you lived at your current home area: _____ years.

8. What is your main staple food: _____

9. Is your regular eating practices () totally vegetarian - eats no food of animal origin, () partially vegetarian - eats mainly vegetables, but also () milk, () chicken, () eggs, () fish or () almost exclusively foods of animal origin.

10. Which foods of animal origin do you take?

a) Fish from; () Lake () Pond () River () or () Market.

b) Chicken from: () Home () Local Market or () External Market

c) Eggs from: () Home () Local Market or () External Market

d) Meat from: () Home () Local Market or () External Market

e) Milk from: () Home () Local Market or () External Market

f) Pork from: () Home () Local Market or () External Market

12. Have you used tobacco? () Yes or () No. [**If yes**: () smoked, () chewed or () snuff. Approximate period of use in years: _____

Part III: Child information

1. What is the infant's date of birth? (Day/month/year): ____/____/____.

3. Infant sex: () female or () male.

4. What is the infant's body weight? _____ Kg

5. Do you breastfeed exclusively? () Yes or () No.

6. How many times do you breastfeed per day? _____

Part IV: Information on the use of pesticides

1. Has your household been treated with pesticides during the last one year i.e., 2018? () Yes or () No**. If yes**, against which pests? () mosquito, () flies, () cockroaches, () bedbugs, () lice, () others name: _____

2. Name pesticide(s) used if possible: _____

3. Who administered the pesticide(s) for the control pests? _____

And was it by () spraying or () dusting, frequency: _____dates: _____

4. What is the main agricultural/industrial activities around your home area?

_____

5. What are sources of water both for animal and domestic use: _____

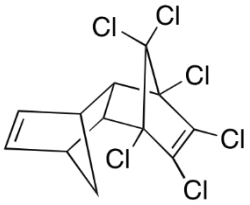
_____

6. What are the major sources of animal feeds: _____

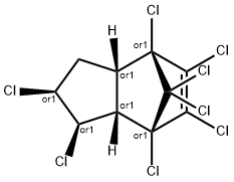
Part V: Human milk donation

1. 30 mL is the targeted volume of human milk. Actual quantity collected: ____ mL

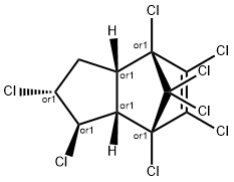
APPENDIX 6: CHEMICAL STRUCTURES OF STUDIED PERSISTENT ORGANIC POLLUTANTS



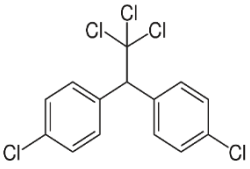
Aldrin



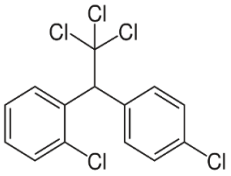
***cis*-chlordane (*alpha*-chlordane)**



***trans*-chlordane (*gamma*-chlordane, *beta*-Chlordane)**



***p,p′*-DDT**



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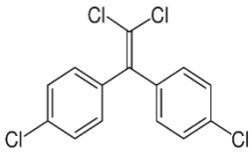

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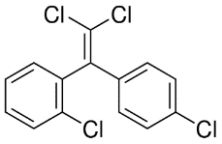

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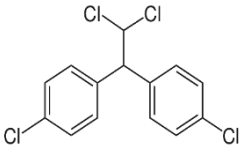
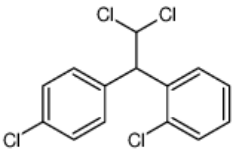


***p,p′*-DDD**



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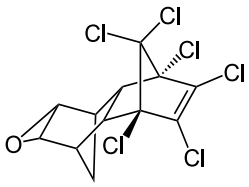
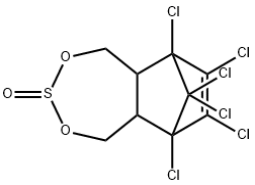
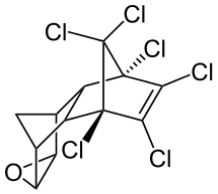


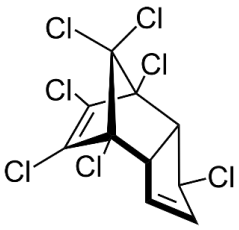
Dieldrin



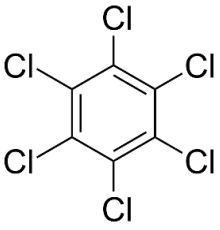
Endosulfan



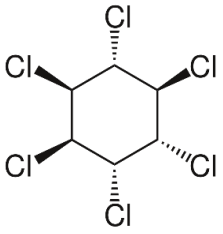
Endrin



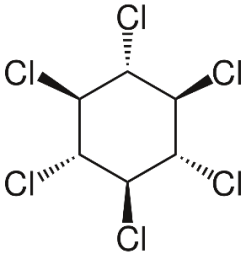
Heptachlor



Hexachlorobenzene



***α*-hexachlorocyclohexane**



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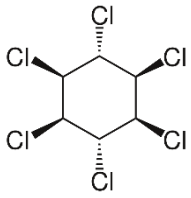
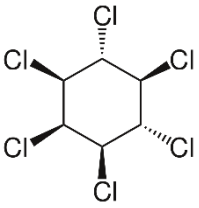
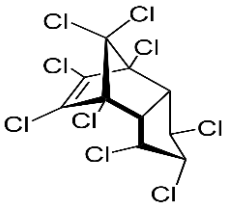


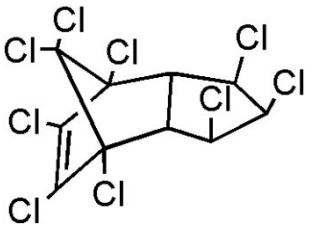
***γ*-hexachlorocyclohexane (Lindane)**



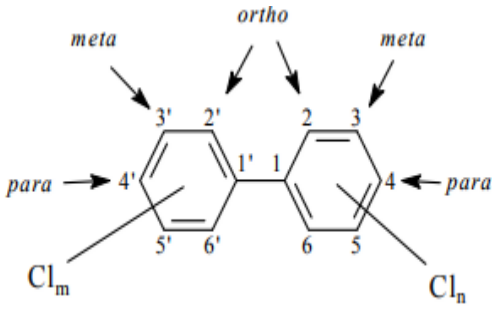
***δ*-hexachlorocyclohexane**



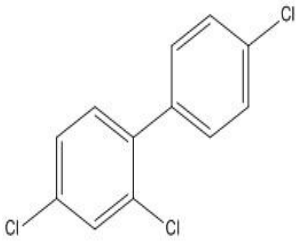
***trans*-nonachlor**



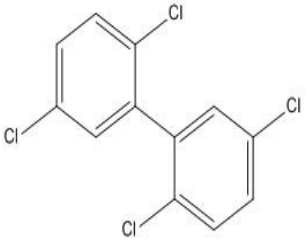
***cis*-nonachlor**



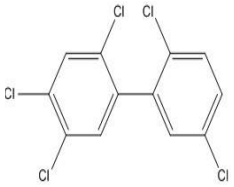
PCB Structure



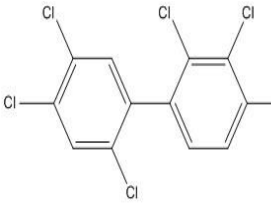
PCB 28



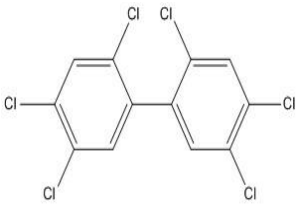
PCB 52



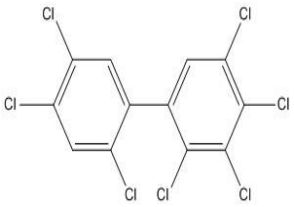
PCB 101



PCB 138



PCB 153



PCB 180

APPENDIX 7: PHYSICOCHEMICAL PROPERTIES OF STUDIED PERSISTENT ORGANIC POLLUTANTS

Compound	Melting point	Boiling point (at 2 mm Hg)	Vapour Pressure (P) (mm Hg at 20 ℃)	Henry's law constant (K_H) (atm/mol/m^3/ at 25 ℃)	Water solubility (µg/L at 25 ℃)	Octanol-water partition coefficient (log K_{ow})	Organic carbon-water partition coefficient (log K_{oc})
Aldrin	Pure-104 ℃, Technical – 49 – 60 ℃	145 ℃	2.31 x 10^{-5}	4,96 x 10^{-4}	17-180	5.17 - 7.4	2.61 - 4.69
Dieldrin	175-176 ℃	decomposes	1.78 x 10^{-7}	5.8 x 10^{-5}	140	3.692 - 6.2	4.08 - 4.55
Chlordane	<25 ℃	165 ℃		4.8 x 10^{-5}	56	6.00	4.58 - 5.57
DDT	108.5 ℃	185 ℃ (decomposes)		1.29 x 10^{-5}	1.2 - 5	4.89 - 6.914	5.146 - 6.26
Methoxychlor	89 ℃	decomposes	1.4x10^{-6}	1.6x10^{-5} atm-m^3/mol (estimated)	0.04 mg/L	4.68–5.08	4.9
Endosulfan	106 ℃	106 ℃	1.73×10^{-7}	6.5×10-5 atm m^3/mol	0.53 mg/L	3.83 (alpha) and 3.62 (beta)	4.03 (alpha) and 4.13 (beta)
Endrin	200 ℃	245 ℃ (decomposes)	7 x 10^{-7}	5.0 x 10^{-7}	220 - 260		3.209 - 5.339
Heptachlor	Pure: - 95 – 96 ℃. Technical: - 46 -74 ℃.	Range: - 135 – 145 ℃, at Pressure of 1-1.5 mm Hg decomposes at 760 mm Hg.	3 x 10^{-4}	2.3 x 10^{-3}	180	4.40-5.5	4.38
HCB	227 – 230 ℃	323-326 ℃ (sublimes)	1.089 x 10^{-5}	7.1 x 10^{-3}	40	3.03 - 6.4	2.56 - 4.54
α-HCH	159–160 ℃	288 ℃, at pressures of 760 mmHg	4.5x10^{-5} mmHg at 25 ℃	6.86x10^{-6}	10 ppm; 69.5 mg/L at 28 ℃	3.8	3.57
β-HCH	314–315 ℃	60 ℃, at pressures of 0.5 mmHg	3.6x10^{-7}	4.5x10^{-7}	5 ppm	3.78	3.57
γ-HCH	112.5 ℃	323.4 ℃ at	4.2x10^{-5}	3 x 10^{-7}	17 ppm;	3.72	3.0; 3.57

Compound	Melting point	Boiling point (at 2 mm Hg)	Vapour Pressure (P) (mm Hg at 20 ℃)	Henry's law constant (K_H) (atm/mol/m^3/ at 25 ℃)	Water solubility (µg/L at 25 ℃)	Octanol-water partition coefficient (log K_{ow})	Organic carbon-water partition coefficient (log K_{oc})
		760 mmHg			insoluble in water		
δ-HCH	141–142 ℃	60 ℃ at 0.36 mmHg	3.5x10-5 at 25 ℃	$2.1x10^{-7}$	10 ppm	4.14	3.8
Mirex	485 ℃	decomposes	$3x10^{-7}$ mm Hg	5.16x10-4 atm m^3/mole	0.60 mg/L	5.28	3.763
PCBs (Aroclor 1254)	No data	65–390	$7.71x10^{-5}$	$2.0x10^{-3}$	No data	6.5	No data
Pentachlorobenzene	86.0 ℃	277.0 ℃	0.002 mm Hg at 25 ℃	7.03e-04 atm-m^3/mole	0.18 to 1.34 mg/L	5.18	6.49

Sources:(ATSDR, 2000; Downie and Templeton, 2013 and Manzetti *et al.*, 2014)

APPENDIX 8: CHEMICALS AND MATERIALS

Chemicals used were: Analytical grade acetone, anhydrous sodium sulphate and alumina oxide; HPLC grade (99% purity) isooctane and general purpose n-hexane were used. General purpose n-hexane, Cas No: 110-54-3, manufactured by Shaanxi Shengren Petrochemical Trading Co., Ltd was triple distilled, Figure A8.1. All-glass fractionating column, a condenser glass, anti-bumping granules, and a two-litter distillation quick fit glass bottle were used. The rate of distillation was set appropriately in order to avoid co-distilled impurities. The initial 200 mL portion of the distillate, which served to rinse the column, and the solvent at about 400 mL level assumed to possibly have concentrated impurities, were discarded. 500 mL of the distillates were condensed and analyzed using GCMS/MS to ascertained batch purities. The distillates were accepted for use only when their analysis showed no response in the entire chromatogram.

Figure A8.1. ***Set up for solvent distillation process***

Glassware cleaning

Glassware used in the study were carefully washed in order to avoid introduction of targeted POP compounds or other contaminants to samples or extracts. The glassware were thoroughly rinsed with warm soapy water, scrubbed with a brush and then left to soak overnight in water with detergent. The glassware were then rinsed using distilled water, acetone followed by overnight drying in Memmert 600 oven at 105±0.5 °C. Immediately before use, each piece of glassware was rinsed with triple distilled n-hexane.

Table A8.1. Apparatus, equipment and glassware

Item	Description	Application
Apparatus		
Aluminium foil.	Thickness, Extra Thick (9 – 25 microns), Strong and Durable.	Covering mixed weight HM and Na_2SO_4 drying powder in a mortar.
Argon gas.	Ultra-High Purity Grade 99.999%, Compressed Gas Code: 262, G2-10.2 m^3 (20000kPa)	Aid in the generation of Product ions for Mass Spectrometry MRM method.
Cotton wool.	White, clean and soft non-absorbent.	Plugging test tubes and condensers.
Crucibles.	Smooth internal porcelain.	Reagents activation at 200 ℃
Desiccators	Heavy glass or Aluminium frame with panels made of acrylic glass.	Storage of hygroscopic reagents and test materials.
Extraction thimble.	Munktell cellulose thimbles grade 30, Art no.; 1201.030080, lot no.; 1221 Gmbh Niederschlog A D09471 Bazemtein/Germany.	Hold free-flowing milk and Na_2SO_4 mixture during Soxhlet extraction.
Filter papers	Whatman filter paper: 150 mm qualitative filter paper	Filtering solutions.
Glass wool.	Strong, soft, pliable glass wool ideal for general lab and industrial applications. Suitable for filtering.	Used to hold cleaning reagents in the column tubes.
Heating mantles	110 v, 200 Watts heating mantles that fit 250 mL, 500 mL and 2000 mL round bottom flasks.	Heating solvents at regulated temperatures during solvent distillations and Soxhlet extraction.
Helium gas.	Ultra-High Purity Grade 99.999% (N4.0), Compressed Gas Code: 262, G2-10.2 m^3 (20000 kPa)	GCMS/MS Carrier gas
Masking Tape.	Pressure-sensitive tape, made of thin and easy-to-tear paper.	Labelling and hold on support.
Mortar and pestle.	Unglazed porcelain or glass with integrated pouring spouts rims.	Mixing and crushing milk and Na_2SO_4 into free-flowing powder.
Nitrogen gas	Ultra-High Purity Grade 99.999%, Compressed Gas Code: 262, G2-10.2 m^3 (20000 kPa)	Extracts condensation.
Racks	Polypropylene, metallic and wooden.	Hold and transport vials.
Spatulas.	Different sizes of nickel Stainless Steel.	Scoping reagents and standards.
Stands and clamps.	Retort stand with a burette clamp	Holds chromatographic columns during Al_2O_3 extract cleanup.
Soxhlet	Assortment of a 250 mL glass flask, Soxhlet glass extractors and glass condenser.	Sample extract preparations.
Equipment		
Capillary column	Zebron™ Phenomenex Phase: ZB-5 ms, L = 30 m, ID = 0.25 mm, FT = 0.25µm. Serial No. 572067, Order No. 7HG-G010-11, Temp. Limit – 60 to 325 Isothermal programmes.	Separation of POPs compounds
Freezer.	Chest Freezer (W×D×H) | (mm)	Storage of samples and extracts.

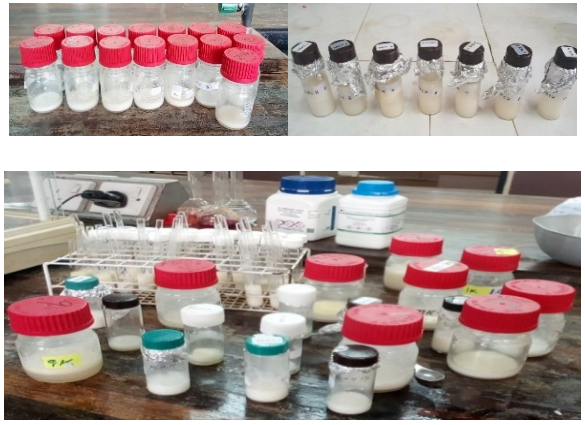
Item	Description	Application
Refrigerator.	1002x597x842 245L	The temporary storage of extracts and working standards.
GCMS/MS	GCMS TQ8040 with GC 2010 Plus, serial No.: 0211554 and Model No.: -225-2390011-58, 00636 SHIMADZU CORP	Separation and analysis of POPs compounds.
Mechanical shaker.	Lab-scale platform shaker Model SSL2 with a linear reciprocating shaking action, an amplitude of 20 mm, and speed range of 20 to 250 rpm with quick release handles and four horizontal securing bars cushioned with rubber with.	Mechanical mixing of preparation samples.
Nitrogen concentrator.	Techne, Model No.: DB 200/3, Serial No.: 001392 – A, V – 230, 50 – 60 HZ, F 3.15 A	Evaporation of solvents into small volumes i.e., 0.5 mL or to dryness.
Oven.	Joh achelis and johns GmbH and Co. Memmert Oven, model.: 600, Serial No.: D06062, Volts 240 v,	Drying glassware, activation of reagents, gravimetric lipid determination.
Rotary evaporator.	Rotavapor – R Model.: Büchi, Serial No.: 320378, Wat 200 Volts 220	Condense extracts after Soxhlet extraction and Al_2O_3 cleanup.
Ultrasonicator	Sonic 405, Model. LUC-405, Volts 240 v 50 Hz, watts 350 w, Serial No.: 09111755.	Dissolution and mixing of standard compounds and extracts.
Vortex mixer.	Digi System Model.: VM-2000C, Speed.: 3000 rpm, Serial No.: VMC – 17030181, Volts – 240 v, 50 Hz, 1 A	Mixing extracts in the vials before aliquoting or GCMS injection.
Weighing scales.	Sartorius analytical scales (MC1), analytic Ac 1203 an accuracy 0.0001 g, Highest weight 60 g. (Ohaus, China); Technical scale, an accuracy 0.01 g., Highest weight 300 g., (Shimadzu, Japan)	Taking the masses of HM samples, vials and their contents, standards and regents.
Glassware		
Bottles	Borosilicate Glass	Samples collection.
Condenser	Borosilicate Glass	Solvent distillation and Soxhlet sample extraction.
Flasks.	50 mL, 250 mL and 2000 mL Glass round bottom.	Solvent distillation and Soxhlet sample extractions.
	Glass 5000 mL Flat bottom	Solvent mixture preparations.
Chromatographic Columns	Borosilicate Glass 300 mm, ID 30 mm, Column 24/40 joint with 4 mm PTFE stopcock and glass frit.	Preparative columns for the cleanup of samples extracts
Pasture pipettes.	Borosilicate Glass	Dispensing solvents.
Soxhlet extractor.	Borosilicate Glass	Holds extraction thimble containing samples for reflux extraction.

APPENDIX 9: SCHEMATIC REPRESENTATION OF HUMAN MILK COLLECTION AND TESTING

Briefing of potential HM donors

HM Transport apparatus → Cool Box

Fredge 4°C – 8°C ← Storage → -20°C Freeze



HM samples thawing before analysis

Weighed HM samples

HM samples Mixed with Na_2SO_4

Mixed HM and Na_2SO_4 for overnight drying

Dried free flowing powder (HM and Na_2SO_4)

Thimble Used

Thimble Loaded with dried free flowing powder

Soxhlet extration apparatus

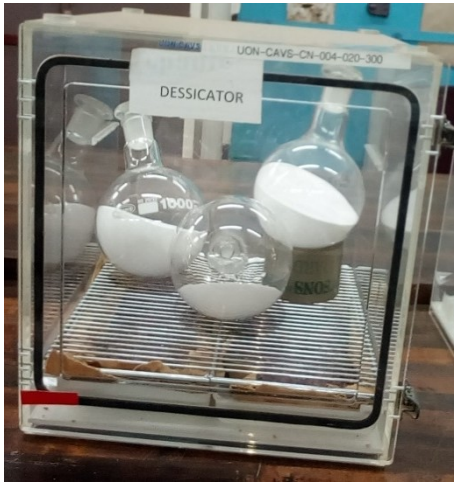
Supervision – Extract concentration

Gravimetric determination of lipids in Human Milk

Aliquot of HM extracts for lipid determination

Oven set at 105±o.5 ºC loaded with HM extract

HM extracts dried to a constant weight.

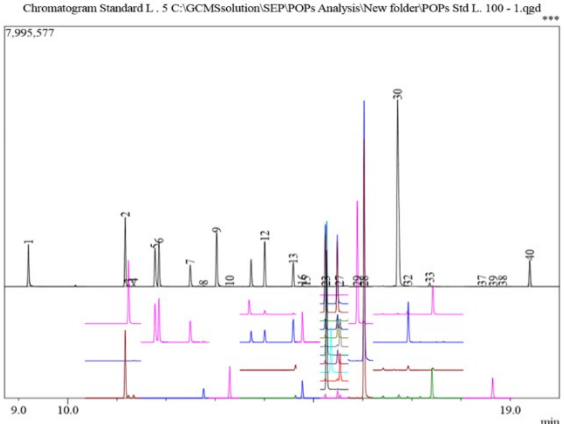


8% Diactivated ALO in a dessicant

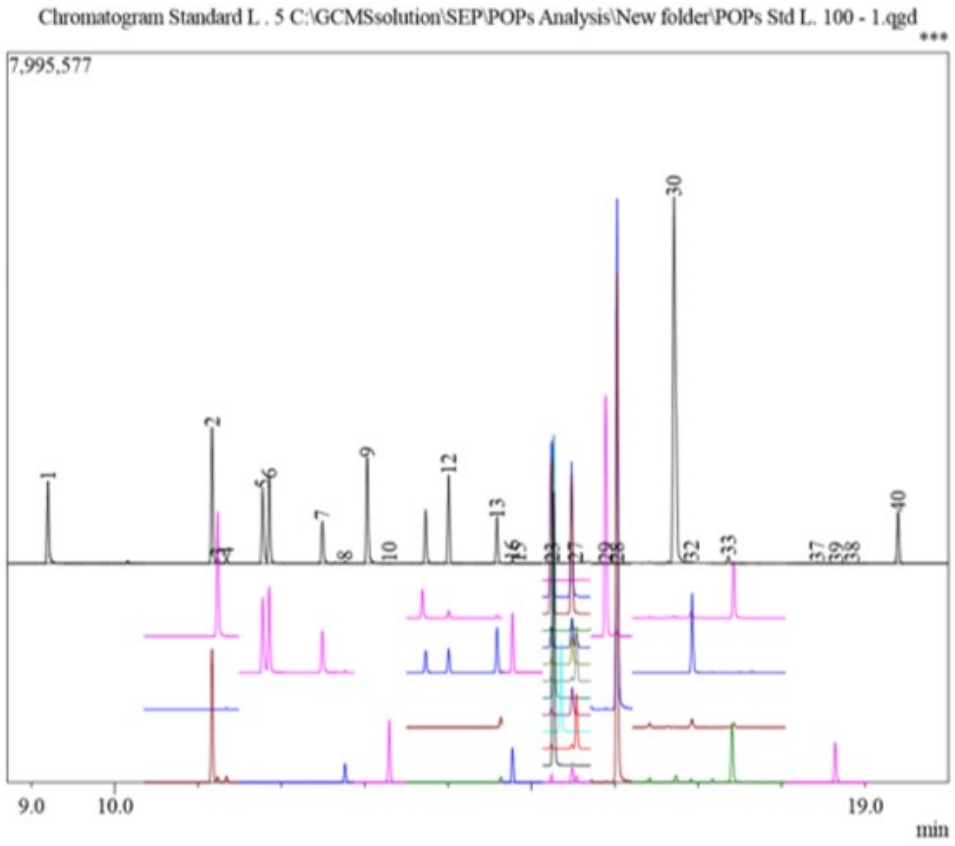
Extracts for column clean-up

Alumina column

GCMS/MS Analysis

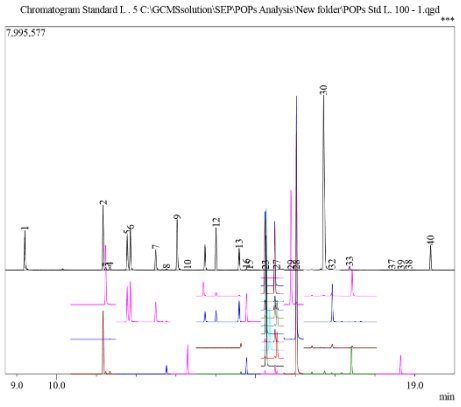


APPENDIX 10: A SAMPLE GCMS/MS CHROMATOGRAM OF REFERENCE STANDARDS



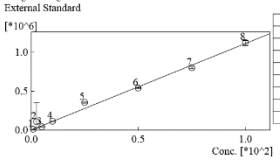
S/No	Compound	S/No	Compound
1.	Pentachlorobenzene	21.	Dieldrin
2.	Alpha-Hexachlorocyclohexane	22.	o,p'-DDD
3.	Hexachlorobenzene	23.	Alpha-Endosulfan (Endosulfan I)
4.	Pentachlorophenol	24.	Beta-Endosulfan (Endosulfan II)
5.	Beta- Hexachlorocyclohexane	25.	Trans-Nonachlor
6.	Gamma-Hexachlorocyclohexane (Lindane)	26.	Endrin
7.	Delta- Hexachlorocyclohexane	27.	Cis-Nonachlor
8.	Endosulfan Ether	28.	p,p'-DDD
9.	2,4,4'-Trichlorobiphenyl (*28)	29.	p,p'-DDE
10.	Heptachlor	30.	o,p'-DDT
11.	2,2,5,5'-Tetrachlorobiphenyl (*52)	31.	Endrin Aldehyde
12.	Aldrin	32.	2,2',4,4',5,5'-Hexachlorobiphenyl (*153)
13.	Isodrin	33.	Endosulfan Sulphate
14.	Heptachlor-exo-epoxide	34.	p,p'-DDT
15.	Oxy-Chlordane	35.	2,2',3,4,4'5'-Hexachlorobiphenyl (*138)
16.	Heptachlor-endo-epoxide	36.	Endrin Ketone
17.	Trans-Chlordane	37.	p,p'-Methoxychlor
18.	o,p'-DDE	38.	o,p'-Methoxychlor
19.	2,2',4,5,5'-Pentachlorobiphenyl (*101)	39.	2,2',3,4,4',5,5'Heptachlorobiphenyl (*180)
20.	Cis-Chlordane	40.	Mirex

APPENDIX 11: CALIBRATION CURVES OF STUDIED POP COMPOUNDS



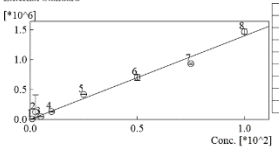
Calibration

ID#:1 Mass:249.90>178.90 Name:Pentachlorobenzene
f(x)=11125.094251*x+0.000000
rr1=0.995001 rr2=0.990027
MeanRF:15091.49 RFSD:11602.72 RFRSD:76.88
CurveType:Least Square Method
ZeroThrough:Through
WeightedRegression:None
External Standard



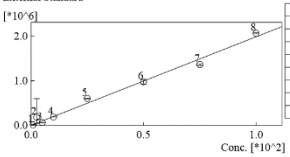
#	Conc. (ppb)	Mean Area
1	1.000	10324.25
2	2.500	108867.00
3	5.000	42940.50
4	10.000	113335.00
5	25.000	355571.00
6	50.000	539618.50
7	75.000	800130.67
8	100.000	1125562.67

ID#:2 Mass:218.90>182.90 Name:alpha - BHC
f(x)=13992.932951*x+0.000000
rr1=0.991874 rr2=0.983813
MeanRF:17574.11 RFSD:13307.77 RFRSD:75.72
CurveType:Least Square Method
ZeroThrough:Through
WeightedRegression:None
External Standard



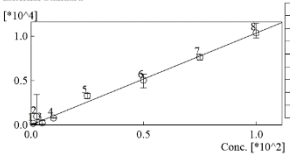
#	Conc. (ppb)	Mean Area
1	1.000	10155.50
2	2.500	124942.75
3	5.000	47049.00
4	10.000	130569.67
5	25.000	420011.33
6	50.000	704749.25
7	75.000	931955.00
8	100.000	1467202.33

ID#:3 Mass:283.80>248.80 Name:Hexachlorobenzene
f(x)=19882.021685*x+0.000000
rr1=0.993579 rr2=0.987200
MeanRF:25135.78 RFSD:19626.13 RFRSD:78.08
CurveType:Least Square Method
ZeroThrough:Through
WeightedRegression:None
External Standard



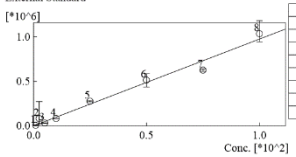
#	Conc. (ppb)	Mean Area
1	1.000	13881.25
2	2.500	182238.75
3	5.000	64688.00
4	10.000	188602.33
5	25.000	602806.00
6	50.000	978017.25
7	75.000	1364966.00
8	100.000	2063949.00

ID#:4 Mass:263.90>164.90 Name:Pentachlorophenol
f(x)=103.471225*x+0.000000
rr1=0.994848 rr2=0.989722
MeanRF:140.11 RFSD:104.26 RFRSD:74.41
CurveType:Least Square Method
ZeroThrough:Through
WeightedRegression:None
External Standard



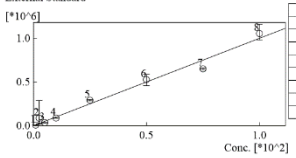
#	Conc. (ppb)	Mean Area
1	1.000	174.75
2	2.500	953.75
3	5.000	242.75
4	10.000	784.67
5	25.000	3290.33
6	50.000	5038.50
7	75.000	7614.67
8	100.000	10368.67

ID#:5 Mass:218.90>182.90 Name:beta-BHC
f(x)=9765.417083*x+0.000000
rr1=0.989306 rr2=0.978727
MeanRF:11838.73 RFSD:8678.82 RFRSD:73.31
CurveType:Least Square Method
ZeroThrough:Through
WeightedRegression:None
External Standard



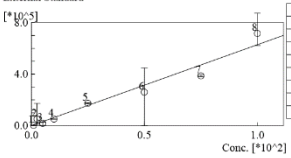
#	Conc. (ppb)	Mean Area
1	1.000	6826.50
2	2.500	82310.25
3	5.000	32813.25
4	10.000	83210.33
5	25.000	276982.67
6	50.000	514355.00
7	75.000	626569.67
8	100.000	1035492.33

ID#:6 Mass:218.90>182.90 Name:gamma-BHC (Lindane)
f(x)=10009.855967*x+0.000000
rr1=0.990177 rr2=0.980451
MeanRF:12245.50 RFSD:9153.74 RFRSD:74.75
CurveType:Least Square Method
ZeroThrough:Through
WeightedRegression:None
External Standard



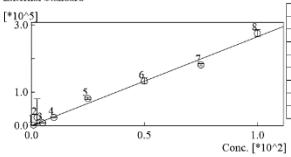
#	Conc. (ppb)	Mean Area
1	1.000	6650.75
2	2.500	86165.00
3	5.000	33873.75
4	10.000	87151.67
5	25.000	290648.00
6	50.000	526361.25
7	75.000	649384.33
8	100.000	1054575.67

ID#:7 Mass:218.90>182.90 Name:delta-BHC
f(x)=6286.537061*x+0.000000
rr1=0.976723 rr2=0.953988
MeanRF:7012.77 RFSD:5738.93 RFRSD:81.84
CurveType:Least Square Method
ZeroThrough:Through
WeightedRegression:None
External Standard



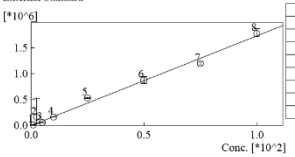
#	Conc. (ppb)	Mean Area
1	1.000	2107.75
2	2.500	51590.00
3	5.000	18831.50
4	10.000	51057.00
5	25.000	174043.00
6	50.000	260413.25
7	75.000	386875.33
8	100.000	715807.67

ID#:8 Mass:240.90>205.90 Name:Endosulfan Ether
f(x)=2651.007094*x+0.000000
rr1=0.993323 rr2=0.986690
MeanRF:3331.71 RFSD:2595.66 RFRSD:77.91
CurveType:Least Square Method
ZeroThrough:Through
WeightedRegression:None
External Standard



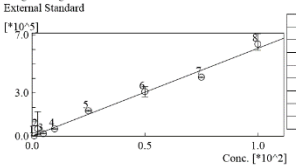
#	Conc. (ppb)	Mean Area
1	1.000	1851.00
2	2.500	24094.25
3	5.000	8348.25
4	10.000	24404.67
5	25.000	80879.33
6	50.000	133049.75
7	75.000	180890.67
8	100.000	274678.33

ID#:9 Mass:255.90>186.00 Name:2,4,4'-Trichlorobiphenyl (#28)
f(x)=17299.987089*x+0.000000
rr1=0.994153 rr2=0.988340
MeanRF:22384.04 RFSD:17109.50 RFRSD:76.44
CurveType:Least Square Method
ZeroThrough:Through
WeightedRegression:None
External Standard



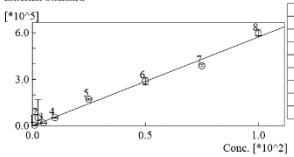
#	Conc. (ppb)	Mean Area
1	1.000	14028.50
2	2.500	160435.75
3	5.000	59577.00
4	10.000	164215.33
5	25.000	532929.67
6	50.000	876337.00
7	75.000	1195181.33
8	100.000	1775288.33

ID#:10 Mass:271.80>236.90 Name:Heptachlor
f(x)=6086.607274*x+0.000000
rr1=0.992902 rr2=0.985855
MeanRF:7130.06 RFSD:5425.48 RFRSD:76.09
CurveType:Least Square Method
ZeroThrough:Through
WeightedRegression:None
External Standard



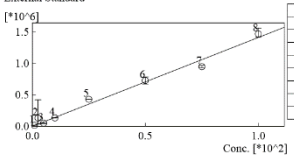
#	Conc. (ppb)	Mean Area
1	1.000	3258.75
2	2.500	50378.25
3	5.000	17670.50
4	10.000	50676.67
5	25.000	175813.67
6	50.000	308832.00
7	75.000	408461.00
8	100.000	637330.00

ID#:11 Mass:291.90>221.90 Name:2,2',5,5'-Tetrachlorobiphenyl (#52)
f(x)=5727.168500*x+0.000000
rr1=0.993130 rr2=0.986308
MeanRF:7198.48 RFSD:5519.62 RFRSD:76.68
CurveType:Least Square Method
ZeroThrough:Through
WeightedRegression:None
External Standard



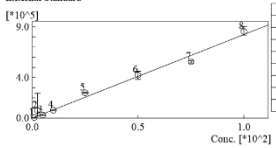
#	Conc. (ppb)	Mean Area
1	1.000	4200.75
2	2.500	51584.00
3	5.000	18442.50
4	10.000	52765.00
5	25.000	172016.00
6	50.000	289027.75
7	75.000	388040.67
8	100.000	595339.67

ID#:12 Mass:262.90>262.90 Name:Aldrin
f(x)=14145.811847*x+0.000000
rr1=0.992345 rr2=0.984749
MeanRF:18883.09 RFSD:13865.27 RFRSD:73.43
CurveType:Least Square Method
ZeroThrough:Through
WeightedRegression:None
External Standard



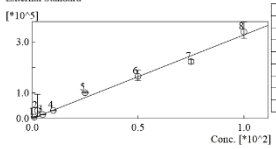
#	Conc. (ppb)	Mean Area
1	1.000	15915.75
2	2.500	131897.00
3	5.000	49224.00
4	10.000	134325.67
5	25.000	430345.33
6	50.000	728499.25
7	75.000	948071.00
8	100.000	1468808.00

ID#:13 Mass:192.90>157.00 Name:Isodrin
f(x)=8238.437466*x+0.000000
rr1=0.992992 rr2=0.986033
MeanRF:10554.05 RFSD:8042.72 RFRSD:76.21
CurveType:Least Square Method
ZeroThrough:Through
WeightedRegression:None
External Standard



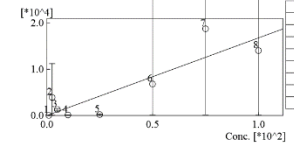
#	Conc. (ppb)	Mean Area
1	1.000	6127.50
2	2.500	75475.00
3	5.000	29631.50
4	10.000	77846.33
5	25.000	249112.00
6	50.000	425053.50
7	75.000	555873.67
8	100.000	852678.00

ID#:14 Mass:354.80>264.90 Name:Heptachlor-exo-epoxide
f(x)=3277.689397*x+0.000000
rr1=0.993864 rr2=0.986771
MeanRF:4713.99 RFSD:3178.97 RFRSD:67.44
CurveType:Least Square Method
ZeroThrough:Through
WeightedRegression:None
External Standard



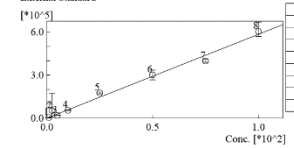
#	Conc. (ppb)	Mean Area
1	1.000	5486.25
2	2.500	30776.25
3	5.000	15320.75
4	10.000	31325.33
5	25.000	101291.00
6	50.000	165316.50
7	75.000	222609.33
8	100.000	339240.67

ID#:15 Mass:185.00>149.00 Name:oxy-Chlordane
f(x)=167.736401*x+0.000000
rr1=0.882768 rr2=0.779279
MeanRF:302.00 RFSD:518.98 RFRSD:171.85
CurveType:Least Square Method
ZeroThrough:Through
WeightedRegression:None
External Standard



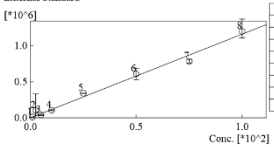
#	Conc. (ppb)	Mean Area
1	1.000	50.67
2	2.500	3910.00
3	5.000	1282.00
4	10.000	109.67
5	25.000	206.33
6	50.000	6803.00
7	75.000	18713.33
8	100.000	14011.00

ID#:16 Mass:352.80>262.90 Name:Heptachlor-endo-epoxide
f(x)=5851.160512*x+0.000000
rr1=0.993717 rr2=0.987474
MeanRF:7407.95 RFSD:5578.86 RFRSD:75.31
CurveType:Least Square Method
ZeroThrough:Through
WeightedRegression:None
External Standard



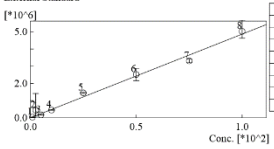
#	Conc. (ppb)	Mean Area
1	1.000	4388.50
2	2.500	52521.50
3	5.000	19879.00
4	10.000	54941.33
5	25.000	175452.67
6	50.000	301627.50
7	75.000	398200.67
8	100.000	603658.33

ID#:17 Mass:372.80>263.90 Name:trans-Chlordane
f(x)=11603.979268*x+0.000000
rr1=0.993372 rr2=0.986787
MeanRF:14790.25 RFSD:10799.56 RFRSD:73.02
CurveType:Least Square Method
ZeroThrough:Through
WeightedRegression:None
External Standard



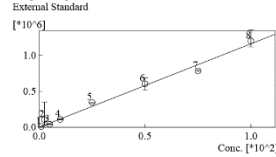
#	Conc. (ppb)	Mean Area
1	1.000	10066.50
2	2.500	103036.75
3	5.000	41494.50
4	10.000	104364.00
5	25.000	342385.67
6	50.000	609083.50
7	75.000	783560.67
8	100.000	1198096.00

ID#:18 Mass:246.00>176.00 Name:o,p'-DDE
f(x)=48658.695614*x+0.000000
rr1=0.993740 rr2=0.987519
MeanRF:61762.15 RFSD:45788.27 RFRSD:74.14
CurveType:Least Square Method
ZeroThrough:Through
WeightedRegression:None
External Standard



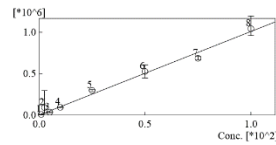
#	Conc. (ppb)	Mean Area
1	1.000	38256.50
2	2.500	434060.00
3	5.000	172260.75
4	10.000	452298.67
5	25.000	1447052.00
6	50.000	2517414.00
7	75.000	3305359.00
8	100.000	5023290.33

ID#:19 Mass:323.90>253.90 Name:2,2',4,5,5'-Pentachlorobiphenyl (#101)
f(x)=11630.134604*x+0.000000
rr1=0.993137 rr2=0.986322
MeanRF:15224.59 RFSD:11369.24 RFRSD:74.68
CurveType:Least Square Method
ZeroThrough:Through
WeightedRegression:None
External Standard



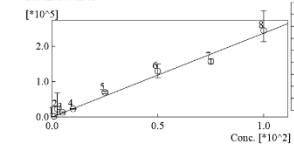
#	Conc. (ppb)	Mean Area
1	1.000	10726.00
2	2.500	107757.25
3	5.000	42757.50
4	10.000	109272.33
5	25.000	347246.67
6	50.000	605834.75
7	75.000	784823.67
8	100.000	1201818.00

ID#:20 Mass:372.80>263.90 Name:cis-Chlordane
f(x)=10098.897724*x+0.000000
rr1=0.993623 rr2=0.987286
MeanRF:12797.97 RFSD:9481.81 RFRSD:74.09
CurveType:Least Square Method
ZeroThrough:Through
WeightedRegression:None
External Standard



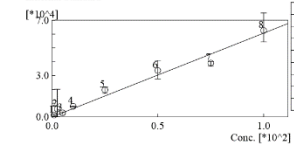
#	Conc. (ppb)	Mean Area
1	1.000	7913.00
2	2.500	89901.75
3	5.000	35555.50
4	10.000	93966.67
5	25.000	298092.33
6	50.000	526643.50
7	75.000	684028.67
8	100.000	1042531.67

ID#:21 Mass:262.90>193.00 Name:Dieldrin
f(x)=2372.877372*x+0.000000
rr1=0.992009 rr2=0.984083
MeanRF:3201.79 RFSD:2266.90 RFRSD:70.80
CurveType:Least Square Method
ZeroThrough:Through
WeightedRegression:None
External Standard



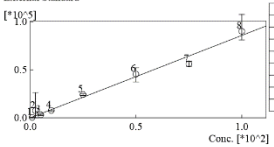
#	Conc. (ppb)	Mean Area
1	1.000	2033.33
2	2.500	21939.00
3	5.000	13037.75
4	10.000	22860.67
5	25.000	69454.33
6	50.000	129698.25
7	75.000	157086.00
8	100.000	244518.67

ID#:22 Mass:235.00>165.00 Name:o,p'-DDD
f(x)=607.602947*x+0.000000
rr1=0.988665 rr2=0.977459
MeanRF:951.41 RFSD:602.79 RFRSD:63.36
CurveType:Least Square Method
ZeroThrough:Through
WeightedRegression:None
External Standard



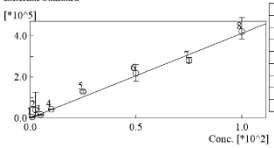
#	Conc. (ppb)	Mean Area
1	1.000	1352.00
2	2.500	5750.00
3	5.000	2916.25
4	10.000	7742.33
5	25.000	19535.67
6	50.000	33662.25
7	75.000	39050.33
8	100.000	62649.00

ID#:23 Mass:194.90>160.00 Name:alpha-Endosulfan (Endosulfan I)
f(x)=857.234166*x+0.000000
rr1=0.990968 rr2=0.982018
MeanRF:1039.97 RFSD:811.17 RFRSD:78.00
CurveType:Least Square Method
ZeroThrough:Through
WeightedRegression:None
External Standard



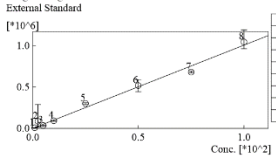
#	Conc. (ppb)	Mean Area
1	1.000	394.00
2	2.500	7489.50
3	5.000	3200.25
4	10.000	7648.67
5	25.000	24231.00
6	50.000	45487.75
7	75.000	55978.33
8	100.000	89963.67

ID#:24 Mass:194.90>160.00 Name:beta-Endosulfan (Endosulfan II)
f(x)=4125.404754*x+0.000000
rr1=0.993347 rr2=0.986739
MeanRF:5892.50 RFSD:4169.04 RFRSD:70.75
CurveType:Least Square Method
ZeroThrough:Through
WeightedRegression:None
External Standard



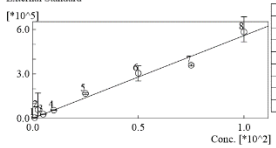
#	Conc. (ppb)	Mean Area
1	1.000	5773.50
2	2.500	40136.50
3	5.000	17673.50
4	10.000	43211.67
5	25.000	128332.67
6	50.000	218810.50
7	75.000	280174.00
8	100.000	421084.33

ID#:25 Mass:406.80>299.90 Name:trans-Nonachlor
f(x)=10084.070598*x+0.000000
rr1=0.993342 rr2=0.986728
MeanRF:12551.83 RFSD:9097.28 RFRSD:72.48
CurveType:Least Square Method
ZeroThrough:Through
WeightedRegression:None
External Standard



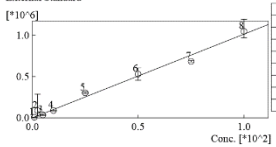
#	Conc. (ppb)	Mean Area
1	1.000	7452.25
2	2.500	86763.75
3	5.000	35363.75
4	10.000	92292.00
5	25.000	301237.33
6	50.000	518625.50
7	75.000	680669.33
8	100.000	1045734.33

ID#:26 Mass:262.90>262.90 Name:Endrin
f(x)=5582.815018*x+0.000000
rr1=0.988288 rr2=0.976714
MeanRF:7885.61 RFSD:6195.06 RFRSD:78.56
CurveType:Least Square Method
ZeroThrough:Through
WeightedRegression:None
External Standard



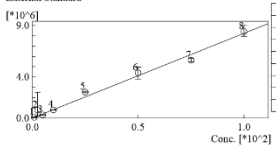
#	Conc. (ppb)	Mean Area
1	1.000	5338.25
2	2.500	57883.75
3	5.000	27996.25
4	10.000	55686.33
5	25.000	168040.67
6	50.000	305250.50
7	75.000	357947.67
8	100.000	582596.00

ID#:27 Mass:406.80>299.90 Name:cis-Nonachlor
f(x)=10152.905961*x+0.000000
rr1=0.993184 rr2=0.986414
MeanRF:12699.50 RFSD:9345.39 RFRSD:73.59
CurveType:Least Square Method
ZeroThrough:Through
WeightedRegression:None
External Standard



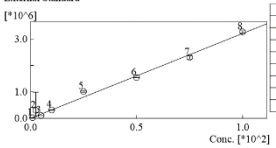
#	Conc. (ppb)	Mean Area
1	1.000	7582.75
2	2.500	88668.00
3	5.000	35658.50
4	10.000	90104.67
5	25.000	303811.00
6	50.000	531710.00
7	75.000	684886.33
8	100.000	1048538.67

ID#:28 Mass:235.00>165.00 Name:p,p'-DDD
f(x)=82403.177071*x+0.000000
rr1=0.993579 rr2=0.987199
MeanRF:110197.64 RFSD:81112.91 RFRSD:73.61
CurveType:Least Square Method
ZeroThrough:Through
WeightedRegression:None
External Standard



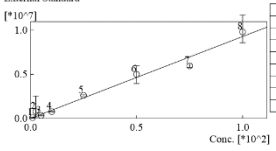
#	Conc. (ppb)	Mean Area
1	1.000	80332.75
2	2.500	773129.50
3	5.000	321139.25
4	10.000	799708.33
5	25.000	2525336.00
6	50.000	4392345.75
7	75.000	5619165.33
8	100.000	8401533.67

ID#:29 Mass:246.00>176.00 Name:p,p'-DDE
f(x)=32083.108331*x+0.000000
rr1=0.994805 rr2=0.989636
MeanRF:41552.53 RFSD:32570.76 RFRSD:78.38
CurveType:Least Square Method
ZeroThrough:Through
WeightedRegression:None
External Standard



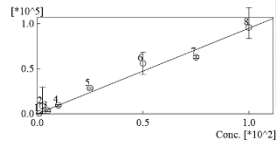
#	Conc. (ppb)	Mean Area
1	1.000	24520.00
2	2.500	302252.25
3	5.000	105218.00
4	10.000	313709.00
5	25.000	1012807.67
6	50.000	1535880.50
7	75.000	2302383.67
8	100.000	3265649.50

ID#:30 Mass:235.00>165.00 Name:o,p'-DDT
f(x)=92738.272392*x+0.000000
rr1=0.989390 rr2=0.978893
MeanRF:115028.15 RFSD:80177.13 RFRSD:69.70
CurveType:Least Square Method
ZeroThrough:Through
WeightedRegression:None
External Standard



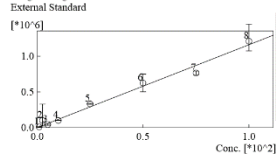
#	Conc. (ppb)	Mean Area
1	1.000	83379.50
2	2.500	777431.25
3	5.000	337321.25
4	10.000	773559.67
5	25.000	2603683.33
6	50.000	4982443.50
7	75.000	5950692.33
8	100.000	9791419.00

ID#:31 Mass:344.90>244.90 Name:Endrin Aldehyde
f(x)=950.013935*x+0.000000
rr1=0.989330 rr2=0.978774
MeanRF:1188.88 RFSD:928.52 RFRSD:78.10
CurveType:Least Square Method
ZeroThrough:Through
WeightedRegression:None
External Standard



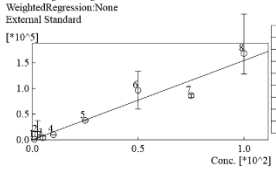
#	Conc. (ppb)	Mean Area
1	1.000	406.25
2	2.500	8526.75
3	5.000	3474.50
4	10.000	9468.00
5	25.000	28416.33
6	50.000	55954.50
7	75.000	62917.67
8	100.000	95776.67

ID#:32 Mass:359.90>289.90 Name:2,2',4,4',5,5'-Hexachlorobiphenyl (#153)
f(x)=11627.278809*x+0.000000
rr1=0.991547 rr2=0.983166
MeanRF:14623.68 RFSD:10514.54 RFRSD:71.90
CurveType:Least Square Method
ZeroThrough:Through
WeightedRegression:None
External Standard



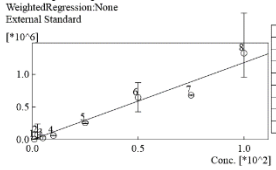
#	Conc. (ppb)	Mean Area
1	1.000	9793.00
2	2.500	100892.75
3	5.000	43360.75
4	10.000	100659.00
5	25.000	332108.33
6	50.000	625152.50
7	75.000	765177.33
8	100.000	1211151.33

ID#:33 Mass:271.80>236.90 Name:Endosulfan Sulphate
f(x)=1545.127128*x+0.000000
rr1=0.969439 rr2=0.939812
MeanRF:1665.72 RFSD:1160.16 RFRSD:69.65
CurveType:Least Square Method
ZeroThrough:Through
WeightedRegression:None
External Standard



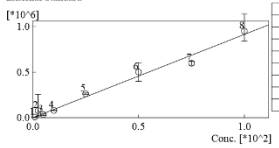
#	Conc. (ppb)	Mean Area
1	1.000	854.00
2	2.500	10912.25
3	5.000	4194.75
4	10.000	10061.00
5	25.000	37661.67
6	50.000	96251.00
7	75.000	86258.67
8	100.000	168022.67

ID#:34 Mass:235.00>165.00 Name:p,p'-DDT
f(x)=11820.882568*x+0.000000
rr1=0.976731 rr2=0.954004
MeanRF:11317.54 RFSD:7498.32 RFRSD:66.25
CurveType:Least Square Method
ZeroThrough:Through
WeightedRegression:None
External Standard



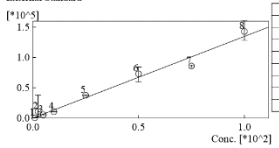
#	Conc. (ppb)	Mean Area
1	1.000	5808.00
2	2.500	70329.25
3	5.000	23968.25
4	10.000	62500.00
5	25.000	257920.67
6	50.000	645580.75
7	75.000	681148.00
8	100.000	1324652.33

ID#:35 Mass:359.90>289.90 Name:2,2',3,4,4',5'-Hexachlorobiphenyl (#138)
f(x)=9146.245206*x+0.000000
rr1=0.990876 rr2=0.981835
MeanRF:11422.16 RFSD:8146.83 RFRSD:71.32
CurveType:Least Square Method
ZeroThrough:Through
WeightedRegression:None
External Standard



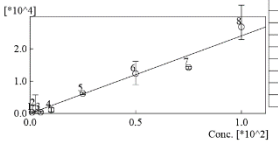
#	Conc. (ppb)	Mean Area
1	1.000	7052.50
2	2.500	78299.25
3	5.000	35126.25
4	10.000	79727.33
5	25.000	262553.33
6	50.000	501317.00
7	75.000	598113.33
8	100.000	950378.00

ID#:36 Mass:180.90>145.00 Name:Endrin Ketone
f(x)=1347.445908*x+0.000000
rr1=0.988620 rr2=0.977369
MeanRF:1572.48 RFSD:1183.83 RFRSD:75.28
CurveType:Least Square Method
ZeroThrough:Through
WeightedRegression:None
External Standard



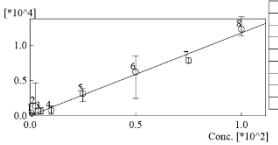
#	Conc. (ppb)	Mean Area
1	1.000	520.50
2	2.500	10988.00
3	5.000	5365.00
4	10.000	10593.67
5	25.000	37608.00
6	50.000	72862.00
7	75.000	85779.00
8	100.000	142653.33

ID#:37 Mass:227.10>169.10 Name:p,p'Methoxychlor olefin
f(x)=240.260525*x+0.000000
rr1=0.979757 rr2=0.959924
MeanRF:268.73 RFSD:178.72 RFRSD:66.50
CurveType:Least Square Method
ZeroThrough:Through
WeightedRegression:None
External Standard



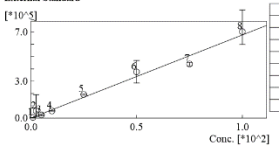
#	Conc. (ppb)	Mean Area
1	1.000	355.75
2	2.500	1632.25
3	5.000	374.75
4	10.000	1147.00
5	25.000	6172.67
6	50.000	12337.25
7	75.000	14253.33
8	100.000	26787.00

ID#:38 Mass:227.10>169.10 Name:o,p' Methoxychlor
f(x)=117.679061*x+0.000000
rr1=0.990389 rr2=0.980870
MeanRF:196.60 RFSD:162.23 RFRSD:82.52
CurveType:Least Square Method
ZeroThrough:Through
WeightedRegression:None
External Standard



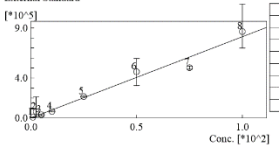
#	Conc. (ppb)	Mean Area
1	1.000	342.75
2	2.500	1358.25
3	5.000	661.75
4	10.000	762.33
5	25.000	3157.33
6	50.000	6218.50
7	75.000	7840.67
8	100.000	12294.67

ID#:39 Mass:393.80>323.90 Name:2,2',3,4,4',5,5'-Heptachlorobiphenyl (#180)
f(x)=6766.327210*x+0.000000
rr1=0.990277 rr2=0.980649
MeanRF:8425.03 RFSD:6025.19 RFRSD:71.52
CurveType:Least Square Method
ZeroThrough:Through
WeightedRegression:None
External Standard



#	Conc. (ppb)	Mean Area
1	1.000	5103.00
2	2.500	57833.75
3	5.000	26081.75
4	10.000	58600.67
5	25.000	191697.67
6	50.000	376065.25
7	75.000	440217.00
8	100.000	702854.00

ID#:40 Mass:273.80>238.80 Name:Mirex
f(x)=8134.530511*x+0.000000
rr1=0.985247 rr2=0.970713
MeanRF:9892.04 RFSD:6414.34 RFRSD:64.84
CurveType:Least Square Method
ZeroThrough:Through
WeightedRegression:None
External Standard

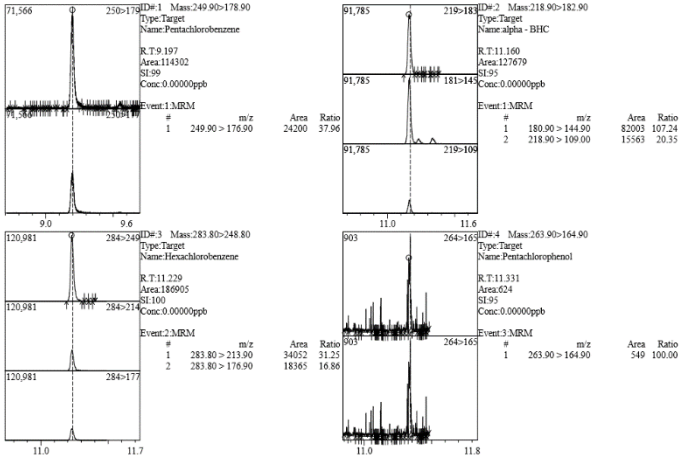


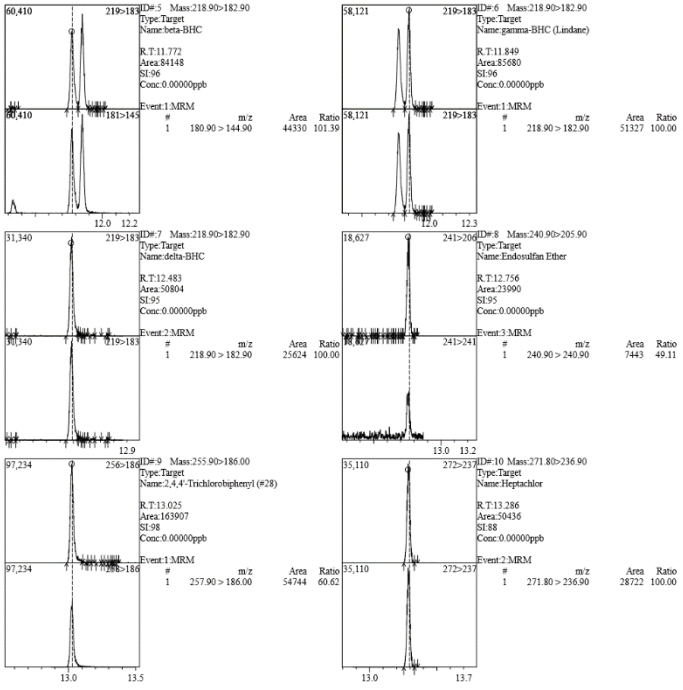
#	Conc. (ppb)	Mean Area
1	1.000	7371.67
2	2.500	63864.00
3	5.000	32730.25
4	10.000	64844.33
5	25.000	214578.00
6	50.000	462935.25
7	75.000	503309.33
8	100.000	863597.00

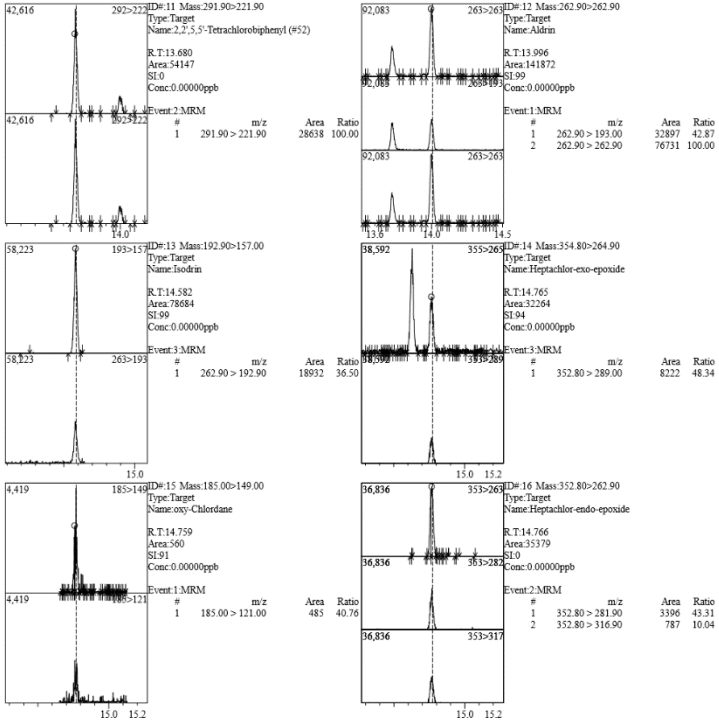
APPENDIX 12: GCMS/MS CHROMATOGRAMS ILLUSTRATING RESULTS OF EXTRACTS OF STANDARDS SPIKES IN BOVINE MILK

==== Shimadzu GCMSsolution Quant. Browser Data Report ====

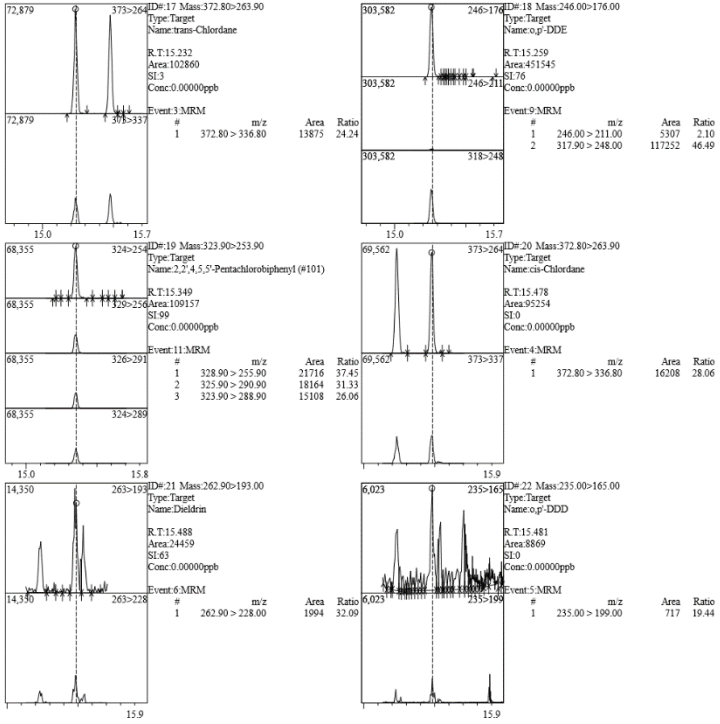
Analyzed by : Admin
Data Acquired : 4/30/2020 2:55:06 PM
Sample Type : Standard
Sample Name : Spike Milk Samples
Sample ID : 388
Sample Amount : 1
Dilution Factor : 1
Vial# : 4
Injection Volume : 1.00
Data File : C:\GCMSsolution\SEP\POPs Analysis\New folder\POPs Std L. 10 - 1.qgd
Method File : C:\GCMSsolution\SEP\POPs Analysis\New folder\POPs MRM 1-1- qgm
Modified by :
Modified : 5/17/2020 12:33:59 PM

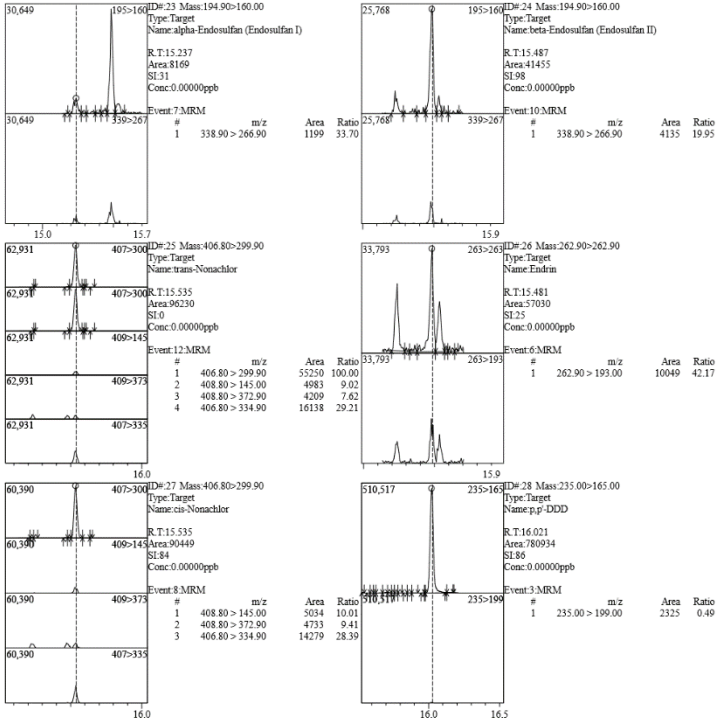




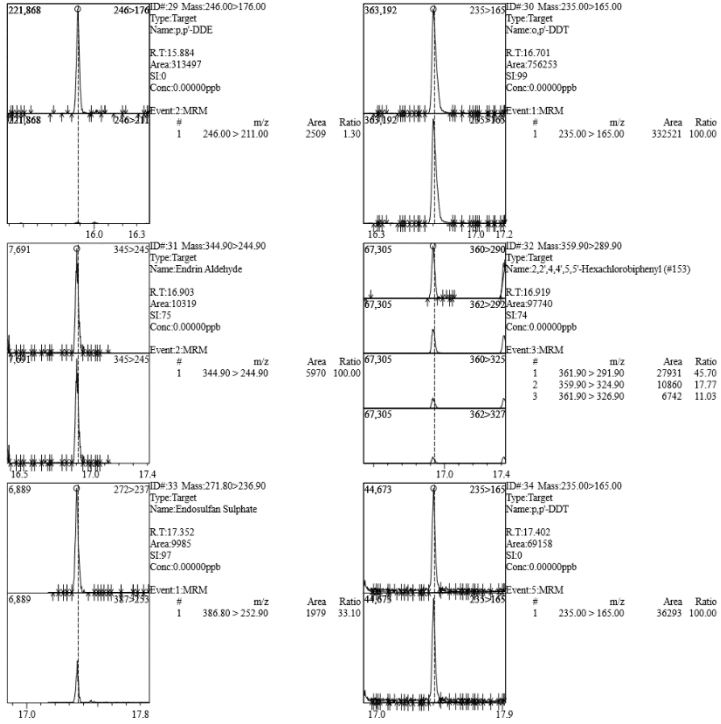


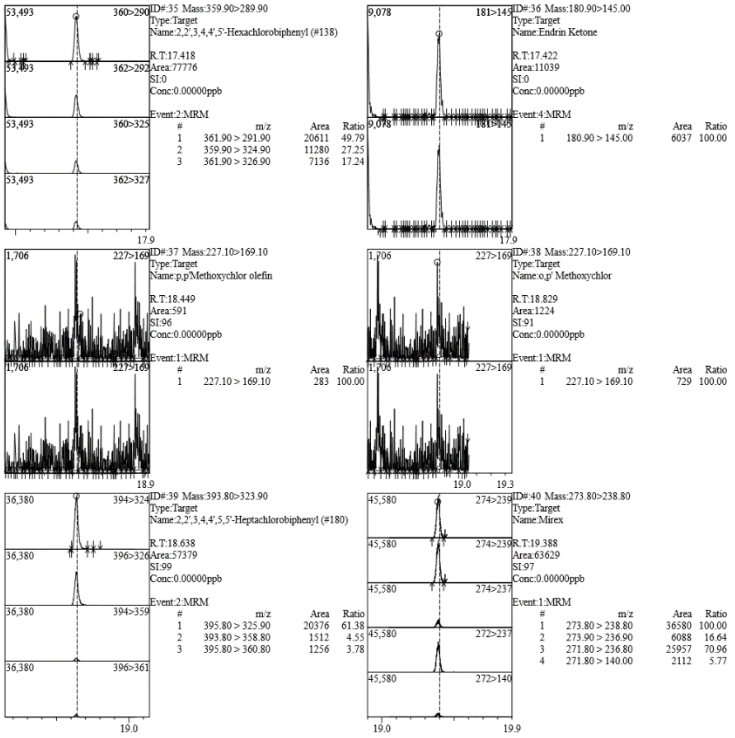
C:¥GCMSsolution¥SEP¥POPs Analysis¥New folder¥POPs Std L. 10 – 1.qgd 5/17/2020 12:48:35





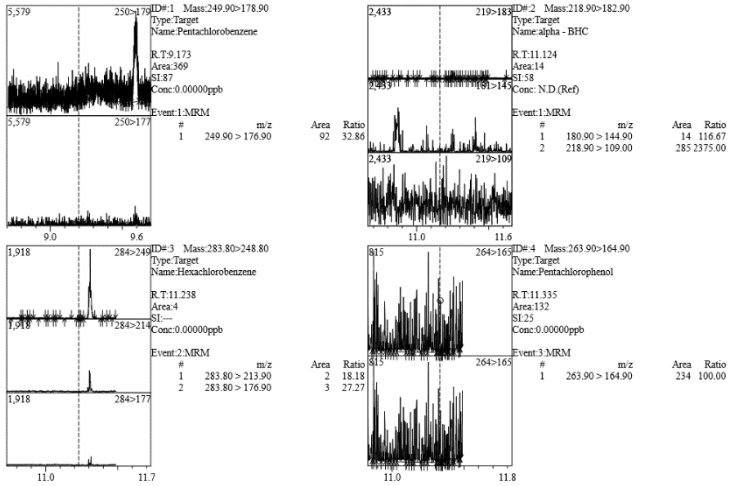
C:¥GCMSsolution¥SEP¥POPs Analysis¥New folder¥POPs Std L. 10 – 1.qgd 5/17/2020 12:48:35

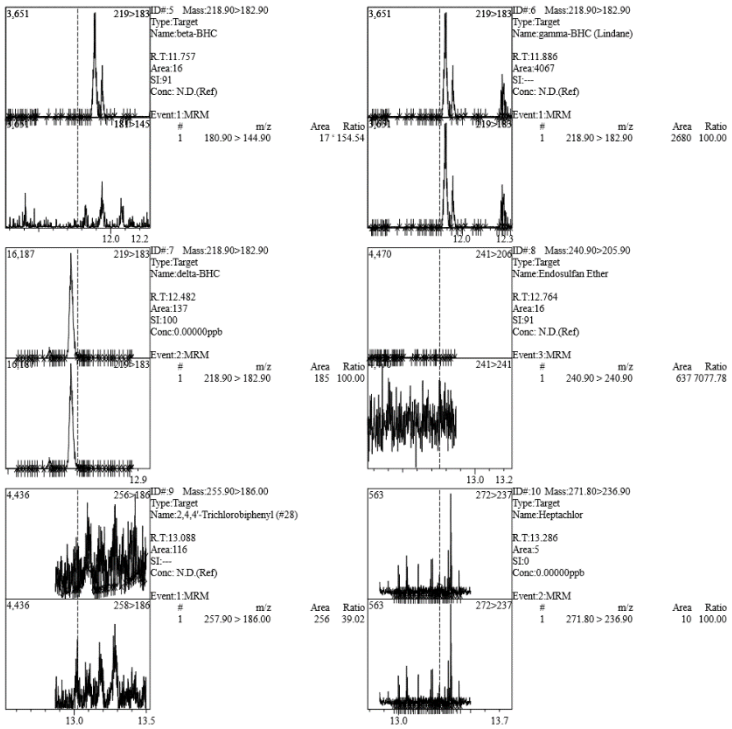




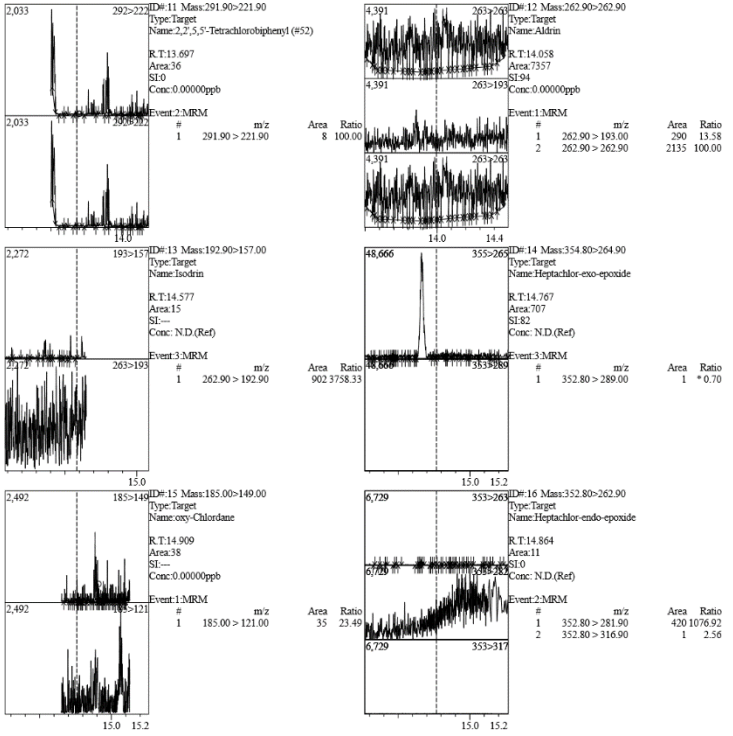
==== Shimadzu GCMSsolution Quant. Browser Data Report ====

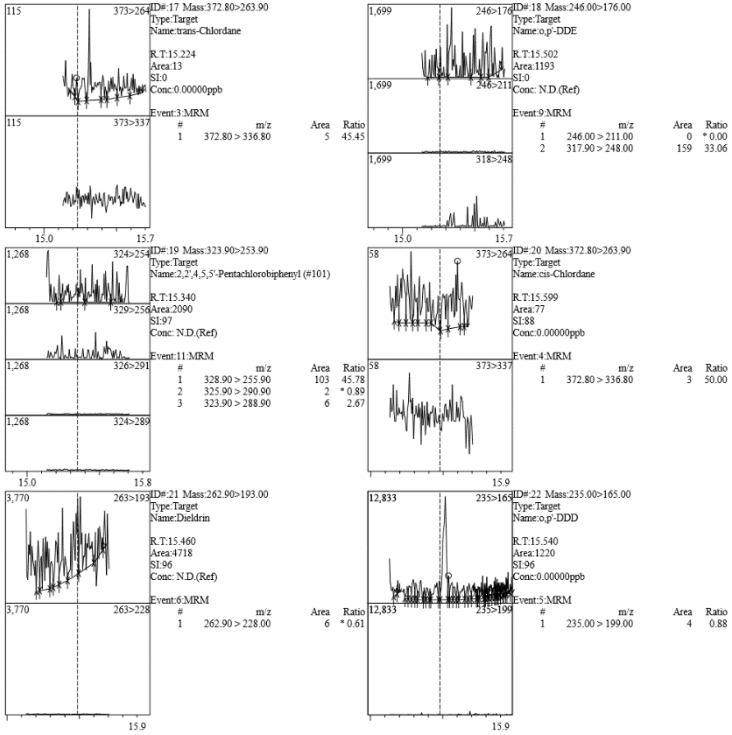
Analyzed by : Admin
Data Acquired : 4/17/2020 10:47:14 PM
Sample Type : Unknown
Sample Name : Human Milk Sample
Sample ID : 103
Sample Amount : 1
Dilution Factor : 1
Vial# : 27
Injection Volume : 1.00
Data File : C:\GCMSsolution\SEP\POPs Analysis\01122020\Human Milk Sample - 103.qgd
Method File : C:\GCMSsolution\SEP\POPs Analysis\New folder\POPs MRM 1-1-.qgm
Modified by : Admin
Modified : 5/13/2020 5:33:12 PM



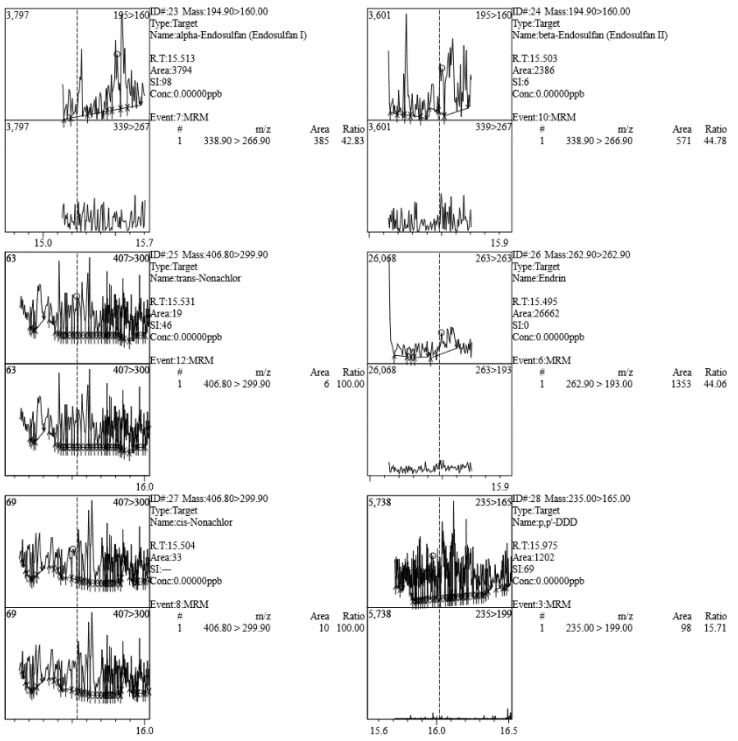


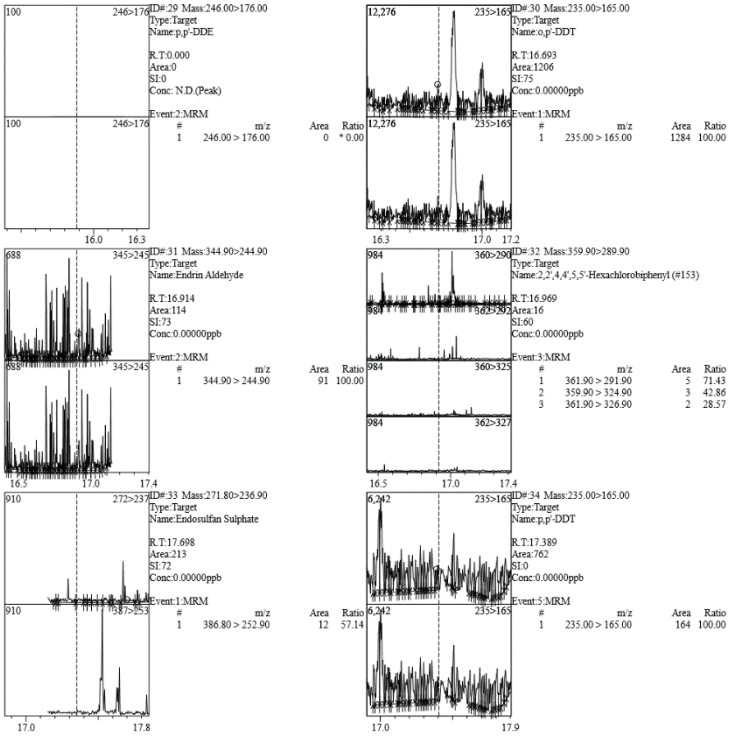
C:¥GCMSsolution¥SEP¥POPs Analysis¥01122020¥Human Milk Sample - 103.qgd 5/15/2020 19:51:35





C:¥GCMSsolution¥SEP¥POPs Analysis¥01122020¥Human Milk Sample - 103.qgd 5/15/2020 19:51:35





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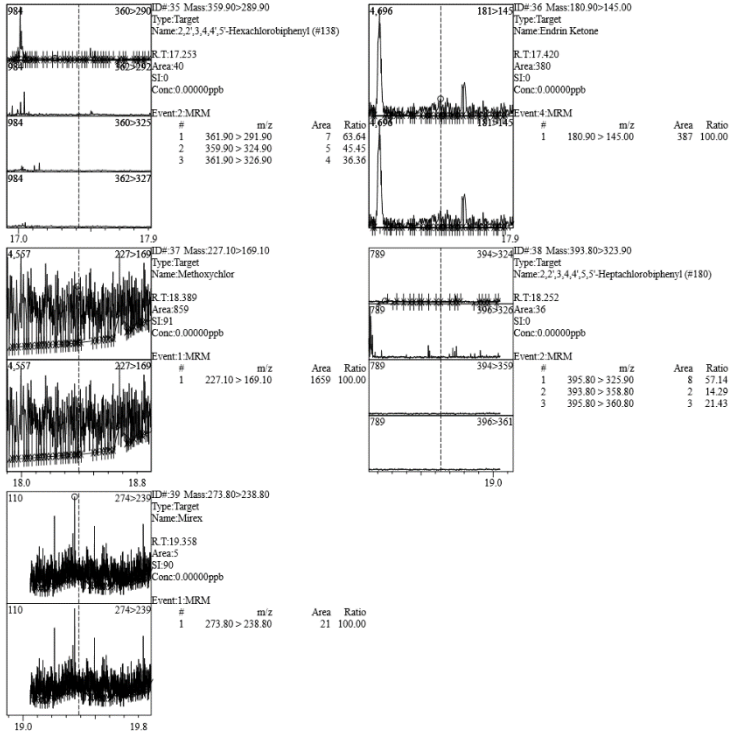

APPENDIX 13: RAW DATA FOR THE LEVELS (ng/g lw) OF STUDIED POPS

Table A13.1. Levels of Persistent Organic Pollutants (ng/g lw) in Human Milk Samples from Southlands Sub Counties, Nairobi County

Compound	KBR 1	KBR 2	KBR 3	KBR 4	KBR 6	KBR 7	KBR 8	KBR 9	KBR 10	KBR 11	KBR 12	KBR 14	KBR 15
∑2Chlordane	-	-	0.639	-	0.133	0.065	-	-	0.452	-	-	-	-
Cis-nonachlor	<0.088	<0.088	0.319	<0.088	<0.088	<0.088	<0.088	<0.088	0.223	<0.088	<0.088	<0.088	<0.088
Trans-nonachlor	<0.065	<0.065	0.320	<0.065	0.133	0.065	<0.065	<0.065	0.229	<0.065	<0.065	<0.065	<0.065
∑4DDT	0.820	0.472	1.024	0.915	2.241	0.855	1.395	0.881	5.266	1.089	6.416	1.080	0.405
p,p'-DDD	0.063	<0.059	0.206	0.078	0.165	0.066	<0.059	0.080	0.264	0.083	0.066	<0.059	0.064
p,p'-DDE	0.543	0.193	0.441	0.152	0.856	0.246	1.138	0.137	2.598	0.228	5.994	0.309	<0.080
o,p'-DDT	<0.065	<0.065	<0.065	<0.065	<0.065	0.153	0.074	<0.065	1.458	0.168	<0.065	0.189	<0.065
p,p'-DDT	0.214	0.279	0.377	0.685	1.220	0.390	0.183	0.664	0.946	0.610	0.356	0.582	0.341
∑4Endosulfan	-	-	-	-	0.138	0.044	0.267	-	0.891	0.194	0.254	0.070	0.150
Endosulfan ether	<0.094	<0.094	<0.094	<0.094	<0.094	<0.094	0.267	<0.094	0.891	<0.094	<0.094	<0.094	<0.094
α-endosulfan	<0.033	<0.033	<0.033	<0.033	<0.033	0.044	<0.033	<0.033	<0.033	0.194	0.106	0.070	0.150
β-endosulfan	<0.041	<0.041	<0.041	<0.041	<0.041	<0.041	<0.041	<0.041	<0.041	<0.041	0.148	<0.041	<0.041
Endosulfan sulphate	<0.038	<0.038	<0.038	<0.038	0.138	<0.038	<0.038	<0.038	<0.038	<0.038	<0.038	<0.038	<0.038
∑3HCH	-	-	-	-	0.470	0.188	-	-	0.105	-	0.456	-	-
α-HCH	<0.055	<0.055	<0.055	<0.055	<0.055	<0.055	<0.055	<0.055	<0.055	<0.055	0.156	<0.055	<0.055
β-HCH	<0.081	<0.081	<0.081	<0.081	0.237	0.103	<0.081	<0.081	0.105	<0.081	0.300	<0.081	<0.081
γ-HCH	<0.084	<0.084	<0.084	<0.084	0.233	0.085	<0.084	<0.084	<0.084	<0.084	<0.084	<0.084	<0.084
HCB	<0.056	<0.056	0.106	<0.056	0.437	0.643	0.182	0.091	0.272	<0.056	0.162	0.101	<0.056
∑2iPCB	-	-	-	-	0.424	-	0.065	-	-	-	0.202	-	0.131
PCB#138	<0.083	<0.083	<0.083	<0.083	0.195	<0.083	<0.083	<0.083	<0.083	<0.083	<0.083	<0.083	<0.083
PCB#180	<0.060	<0.060	<0.060	<0.060	0.229	<0.060	0.065	<0.060	<0.060	<0.060	0.202	<0.060	0.131

Table A13.2. Levels of Persistent Organic Pollutants (ng/g lw) in Human Milk Samples from Eastlands and Westlands Sub Counties, Nairobi County

Compound	NW 2	NE 3	NE 4	NW 4	NE 5	NE 6	NE 7	NE 8	NE 9	NE 10	NE 11	NE 12	NE 13
$\sum_2$Chlordane	1.314	-	-	-	-	1.014	0.575	-	-	0.585	-	-	-
Cis-nonachlor	0.656	<0.088	<0.088	<0.088	<0.088	0.122	0.287	<0.088	<0.088	0.292	<0.088	<0.088	<0.088
Trans-nonachlor	0.658	<0.065	<0.065	<0.065	<0.065	0.892	0.288	<0.065	<0.065	0.293	<0.065	<0.065	<0.065
$\sum_4$DDT	12.608	0.881	0.494	1.369	0.613	128.979	0.666	43.542	8.138	2.996	0.446	0.065	0.890
p,p'-DDD	0.173	<0.059	<0.059	0.095	<0.059	0.597	<0.059	0.751	0.088	0.082	<0.059	0.065	0.117
p,p'-DDE	0.467	0.596	<0.080	0.528	0.093	126.292	0.155	42.368	7.18	0.227	0.206	<0.080	<0.080
o,p'-DDT	0.882	<0.065	<0.065	0.165	<0.065	0.095	0.175	0.117	0.183	0.219	<0.065	<0.065	0.163
p,p'-DDT	11.086	0.285	0.494	0.581	0.520	1.995	0.336	0.306	0.687	2.468	0.240	<0.093	0.610
$\sum_2$Endosulfan	0.062	-	-	-	-	0.075	-	12.405	-	0.175	-	-	-
β-endosulfan	<0.041	<0.041	<0.041	<0.041	<0.041	<0.041	<0.041	<0.041	<0.041	0.175	<0.041	<0.041	<0.041
Endosulfan Sulphate	0.062	<0.038	<0.038	<0.038	<0.038	0.075	<0.038	12.405	<0.038	<0.038	<0.038	<0.038	<0.038
$\sum_2$HCH	0.326	-	-	-	-	0.160	-	0.105	0.176	0.966	-	-	-
α-HCH	0.056	<0.055	<0.055	<0.055	<0.055	<0.055	<0.055	<0.055	<0.055	0.444	<0.055	<0.055	<0.055
β-HCH	0.270	<0.081	<0.081	<0.081	<0.081	0.160	<0.081	0.105	0.176	0.522	<0.081	<0.081	<0.081
HCB	0.182	0.117	0.156	<0.056	0.135	1.307	0.136	0.205	0.159	<0.056	0.188	<0.056	<0.056
$\sum_5$iPCB	0.248	-	-	-	-	4.84	0.642	0.084	0.207	-	-	-	-
PCB#28	0.130	<0.064	<0.064	<0.064	<0.064	2.261	0.642	<0.064	<0.064	0.089	<0.064	<0.064	<0.064
PCB#101	<0.076	<0.076	<0.076	<0.076	<0.076	0.079	<0.076	<0.076	<0.076	<0.076	<0.076	<0.076	<0.076
PCB#138	<0.083	<0.083	<0.083	<0.083	<0.083	0.846	<0.083	<0.083	0.115	0.154	<0.083	<0.083	<0.083
PCB#153	<0.072	<0.072	<0.072	<0.072	<0.072	0.527	<0.072	<0.072	<0.072	<0.072	<0.072	<0.072	<0.072
PCB#180	0.118	<0.060	<0.060	<0.060	<0.060	1.127	<0.060	0.084	0.092	0.656	<0.060	<0.060	<0.060

Table A13.3. Levels of Persistent Organic Pollutants (ng/g lw) in Human Milk Samples from Chesumei Sub Counties, Nandi County

Compound	KCRH 1	KCRH 2	KCRH 3	KCRH 4	KCRH 5	KCRH 6	KCRH 7	KCRH 8	KCRH 9	KCRH 10	KCRH 11	KCRH 12	KCRH 13	KCRH 14	KCRH 15
$\sum_3$Chlordane	-	-	0.616	0.470	-	-	0.076	-	0.398	-	0.065	-	0.203	0.107	-
Cis-chlordane	<0.074	<0.074	0.111	0.100	<0.074	<0.074	<0.074	<0.074	<0.074	<0.074	<0.074	<0.074	<0.074	<0.074	<0.074
Trans-chlordane	<0.058	<0.058	0.168	0.370	<0.058	<0.058	0.076	<0.058	0.077	<0.058	0.065	<0.058	0.203	0.107	<0.058
Trans-nonachlor	<0.065	<0.065	0.337	<0.065	<0.065	<0.065	<0.065	<0.065	0.321	<0.065	<0.065	<0.065	<0.065	<0.065	<0.065
$\sum_3$DDT	-	0.210	-	0.109	0.760	0.496	0.075	0.391	0.764	-	0.124	2.191	1.342	0.114	1.168
p,p'-DDD	<0.059	0.210	<0.059	<0.059	<0.059	0.194	0.075	0.102	<0.059	<0.059	<0.059	0.088	<0.059	<0.059	0.073
o,p'-DDT	<0.065	<0.065	<0.065	0.109	<0.065	<0.065	<0.065	<0.065	0.132	<0.065	0.124	0.080	<0.065	0.114	0.107
p,p'-DDT	<0.093	<0.093	<0.093	<0.093	0.760	0.302	<0.093	0.289	0.632	<0.093	<0.093	2.023	1.342	<0.093	0.988
$\sum_3$Endosulfan	-	-	-	1.809	-	-	-	-	-	-	-	-	7.487	-	-
α-endosulfan	<0.033	<0.033	<0.033	<0.033	<0.033	<0.033	<0.033	<0.033	<0.033	<0.033	<0.033	<0.033	1.775	<0.033	<0.033
β-endosulfan	<0.041	<0.041	<0.041	<0.041	<0.041	<0.041	<0.041	<0.041	<0.041	<0.041	<0.041	<0.041	5.712	<0.041	<0.041
Endosulfan sulphate	<0.038	<0.038	<0.038	1.809	<0.038	<0.038	<0.038	<0.038	<0.038	<0.038	<0.038	<0.038	<0.038	<0.038	<0.038
$\sum_4$HCH	0.372	-	0.159	0.094	0.515	-	0.280	1.552	1.194	0.223	-	-	-	-	0.530
α-HCH	<0.055	<0.055	<0.055	0.094	<0.055	<0.055	<0.055	<0.055	<0.055	<0.055	<0.055	<0.055	<0.055	<0.055	<0.055
β-HCH	<0.081	<0.081	<0.081	<0.081	0.515	<0.081	<0.081	1.552	<0.081	0.223	<0.081	<0.081	<0.081	<0.081	0.530
γ-HCH	0.145	<0.084	<0.084	<0.084	<0.084	<0.084	<0.084	<0.084	0.686	<0.084	<0.084	<0.084	<0.084	<0.084	<0.084
δ-HCH	<0.094	<0.094	0.159	<0.094	<0.094	<0.094	0.280	<0.094	0.508	<0.094	<0.094	<0.094	<0.094	<0.094	<0.094
$\sum_6$iPCB	-	0.368	5.142	0.359	3.325	7.380	0.814	-	1.375	-	0.079	-	-	3.589	-
PCB#28	<0.064	<0.064	0.844	<0.064	<0.064	<0.064	<0.064	<0.064	<0.064	<0.064	<0.064	<0.064	<0.064	<0.064	<0.064
PCB#52	<0.070	<0.070	4.084	<0.070	1.636	<0.070	0.814	<0.070	1.272	<0.070	<0.070	<0.070	<0.070	<0.070	<0.070
PCB#101	<0.076	<0.076	<0.076	<0.076	1.689	7.303	<0.076	<0.076	<0.076	<0.076	<0.076	<0.076	<0.076	3.589	<0.076
PCB#138	<0.083	0.184	0.098	0.121	<0.083	<0.083	<0.083	<0.083	<0.083	<0.083	<0.083	<0.083	<0.083	<0.083	<0.083
PCB#153	<0.072	<0.072	0.116	0.238	<0.072	<0.072	<0.072	<0.072	0.103	<0.072	0.079	<0.072	<0.072	<0.072	<0.072
PCB#180	<0.060	0.184	<0.060	<0.060	<0.060	0.077	<0.060	<0.060	<0.060	<0.060	<0.060	<0.060	<0.060	<0.060	<0.060

Table A13.4. Levels of Persistent Organic Pollutants (ng/g lw) in Human Milk Samples from Chesumei and Nandi Central Sub Counties, Nandi County

Compound	KCRH 16	KCRH 17	KCRH 18	KCRH 19	KCRH 20	NHCH 1	NHCH 2	NHCH 3	NHCH 4	NHCH 5	NHCH 6	NHCH 7	NHCH 8	NHCH 9	NHCH 10
$\sum_3$Chlordane	-	-	-	-	0.44	-	-	-	-	-	-	-	-	-	0.132
Cis-chlordane	<0.074	<0.074	<0.074	<0.074	0.127	<0.074	<0.074	<0.074	<0.074	<0.074	<0.074	<0.074	<0.074	<0.074	0.132
Cis-nonachlor	<0.088	<0.088	<0.088	<0.088	0.156	<0.088	<0.088	<0.088	<0.088	<0.088	<0.088	<0.088	<0.088	<0.088	<0.088
Trans-nonachlor	<0.065	<0.065	<0.065	<0.065	0.157	<0.065	<0.065	<0.065	<0.065	<0.065	<0.065	<0.065	<0.065	<0.065	<0.065
∑ DDT	0.943	1.254	0.962	0.529	1.292	-	-	0.562	-	0.107	0.365	0.102	-	0.300	3.032
p,p'-DDD	0.065	0.102	0.069	0.099	1.292	<0.059	<0.059	<0.059	<0.059	<0.059	<0.059	<0.059	<0.059	<0.059	0.151
o,p'-DDT	<0.065	0.144	0.179	0.176	<0.065	<0.065	<0.065	<0.065	<0.065	<0.065	<0.065	<0.065	<0.065	<0.065	<0.065
p,p'-DDT	0.878	1.008	0.714	0.254	<0.093	<0.093	<0.093	0.562	<0.093	0.107	0.365	0.102	<0.093	0.300	2.881
$\sum_1$Endosulfan	-	-	-	-	-	-	-	-	-	-	0.040	-	0.303	0.919	0.143
Endosulfan Sulphate	<0.038	<0.038	<0.038	<0.038	<0.038	<0.038	<0.038	<0.038	<0.038	<0.038	0.040	<0.038	0.303	0.919	0.143
$\sum_4$HCH	0.622	-	0.303	-	0.324	-	0.448	-	-	4.261	-	-	-	-	0.181
α-HCH	<0.055	<0.055	<0.055	<0.055	0.324	<0.055	<0.055	<0.055	<0.055	<0.055	<0.055	<0.055	<0.055	<0.055	<0.055
β-HCH	<0.081	<0.081	<0.081	<0.081	<0.081	<0.081	0.448	<0.081	<0.081	0.591	<0.081	<0.081	<0.081	<0.081	<0.081
γ-HCH	0.622	<0.084	0.081	<0.084	<0.084	<0.084	<0.084	<0.084	<0.084	3.670	<0.084	<0.084	<0.084	<0.084	<0.084
δ-HCH	<0.094	<0.094	0.222	<0.094	<0.094	<0.094	<0.094	<0.094	<0.094	<0.094	<0.094	<0.094	<0.094	<0.094	0.181
$\sum_6$iPCB	-	-	-	0.109	4.248	0.071	-	0.503	0.204	0.466	0.219	0.137	0.967	-	0.336
PCB#28	<0.064	<0.064	<0.064	<0.064	1.898	<0.064	<0.064	0.207	0.204	0.371	0.103	<0.064	0.342	<0.064	0.122
PCB#52	<0.070	<0.070	<0.070	<0.070	0.698	0.071	<0.070	0.190	<0.070	0.095	0.116	0.137	0.128	<0.070	0.112
PCB#101	<0.076	<0.076	<0.076	<0.076	<0.076	<0.076	<0.076	0.106	<0.076	<0.076	<0.076	<0.076	0.299	<0.076	<0.076
PCB#138	<0.083	<0.083	<0.083	<0.083	0.472	<0.083	<0.083	<0.083	<0.083	<0.083	<0.083	<0.083	<0.083	<0.083	0.102
PCB#153	<0.072	<0.072	<0.072	<0.072	1.180	<0.072	<0.072	<0.072	<0.072	<0.072	<0.072	<0.072	<0.072	<0.072	<0.072
PCB#180	<0.060	<0.060	<0.060	0.109	<0.060	<0.060	<0.060	<0.060	<0.060	<0.060	<0.060	<0.060	0.198	<0.060	<0.060

Table A13.5. Levels of Persistent Organic Pollutants (ng/g lw) in Human Milk Samples from Nandi Central and South Sub Counties, Nandi County

Compound	NHCH 12	NHCH 13	NHCH 14	NHCH 15	NHCH 16	NHCH 17	NHCH 18	NHCH 19	NHCH 20	KSCH - A 1	KSCH - A 2	KSCH - A 3	KSCH - A 4	KSCH - A 5	KSCH - A 6
$\sum_1$Chlordane	-	-	-	-	-	-	-	-	-	0.145	0.398	-	-	0.086	-
Cis-chlordane	<0.074	<0.074	<0.074	<0.074	<0.074	<0.074	<0.074	<0.074	<0.074	0.145	0.398	<0.074	<0.074	0.086	<0.074
$\sum_3$DDT	0.226	-	0.099	0.082	0.978	-	-	0.400	-	0.203	0.599	0.158	0.354	0.248	0.133
p,p'-DDD	<0.059	<0.059	<0.059	0.082	0.068	<0.059	<0.059	<0.059	<0.059	0.203	0.371	0.158	0.064	0.087	<0.059
p,p'-DDE	<0.080	<0.080	<0.080	<0.080	0.198	<0.080	<0.080	<0.080	<0.080	<0.080	<0.080	<0.080	<0.080	<0.080	<0.080
p,p'-DDT	0.226	<0.093	0.099	<0.093	0.712	<0.093	<0.093	0.400	<0.093	<0.093	0.228	<0.093	0.290	0.161	0.133
$\sum_3$Endosulfan	-	-	-	2.163	0.159	3.443	1.515	0.297	0.375	2.347	0.668	0.325	0.093	-	0.386
α-endosulfan	<0.033	<0.033	<0.033	<0.033	<0.033	2.745	1.459	<0.033	<0.033	<0.033	0.668	<0.033	0.093	<0.033	<0.033
β-endosulfan	<0.041	<0.041	<0.041	1.982	<0.041	0.698	<0.041	<0.041	<0.041	<0.041	<0.041	<0.041	<0.041	<0.041	<0.041
Endosulfan Sulphate	<0.038	<0.038	<0.038	0.181	0.159	<0.038	0.056	0.297	0.375	2.347	<0.038	0.325	<0.038	<0.038	0.386
$\sum_2$HCH	-	-	-	0.842	4.814	1.443	-	-	-	0.189	-	1.219	-	-	-
β-HCH	<0.081	<0.081	<0.081	<0.081	<0.081	1.443	<0.081	<0.081	<0.081	0.189	<0.081	0.143	<0.081	<0.081	<0.081
γ-HCH	<0.084	<0.084	<0.084	0.842	4.814	<0.084	<0.084	<0.084	<0.084	<0.084	<0.084	1.076	<0.084	<0.084	<0.084
$\sum_3$iPCB	0.151	-	0.172	0.230	0.137	0.288	0.248	1.137	0.265	0.826	0.611	0.689	-	0.863	0.157
PCB#28	<0.064	<0.064	<0.064	<0.064	<0.064	0.097	0.068	<0.064	<0.064	<0.064	0.151	0.689	<0.064	0.106	0.157
PCB#52	<0.070	<0.070	0.172	0.230	<0.070	0.191	0.180	1.137	<0.070	0.826	0.317	<0.070	<0.070	0.757	<0.070
PCB#101	0.151	<0.076	<0.076	<0.076	0.137	<0.076	<0.076	<0.076	0.265	<0.076	0.143	<0.076	<0.076	<0.076	<0.076

Table A13.6. Levels of Persistent Organic Pollutants (ng/g lw) in Human Milk Samples from Nandi South, East and North Sub Counties, Nandi County

Compound	KSCH - A7	KSCH - A8	KSCH - A9	KSCH - A10	LHC 1	LHC 2	LHC 3	LHC 6	LHC 8	LHC 9	LHC 10	MTT 4	KSCH - M 1	KSCH - M 2	KSCH - M 3	KSCH - M 4
$\sum_3$DDT	0.605	0.075	0.709	1.140	-	-	-	0.240	1.136	0.244	0.085	-	-	0.235	-	0.167
p,p'-DDD	0.268	0.075	0.081	0.078	<0.059	<0.059	<0.059	<0.059	0.088	<0.059	0.085	<0.059	<0.059	<0.059	<0.059	0.167
o,p'-DDT	<0.065	<0.065	0.117	0.108	<0.065	<0.065	<0.065	<0.065	<0.065	<0.065	<0.065	<0.065	<0.065	<0.065	<0.065	<0.065
p,p'-DDT	0.337	<0.093	0.511	0.954	<0.093	<0.093	<0.093	0.240	1.048	0.244	<0.093	<0.093	<0.093	0.235	<0.093	<0.093
$\sum_2$Endosulfan	0.077	-	0.141	-	0.279	0.558	-	-	0.124	0.045	-	-	-	0.595	0.213	-
α-endosulfan	<0.033	<0.033	<0.033	<0.033	<0.033	0.396	<0.033	<0.033	<0.033	<0.033	<0.033	<0.033	<0.033	<0.033	<0.033	<0.033
Endosulfan Sulphate	0.077	<0.038	0.141	<0.038	0.279	0.162	<0.038	<0.038	0.124	0.045	<0.038	<0.038	<0.038	0.595	0.213	<0.038
$\sum_2$HCH	-	-	-	0.302	-	0.360	-	0.224	-	-	-	-	-	-	-	0.306
β-HCH	<0.081	<0.081	<0.081	<0.081	<0.081	0.356	<0.081	<0.081	<0.081	<0.081	<0.081	<0.081	<0.081	<0.081	<0.081	<0.081
γ-HCH	<0.084	<0.084	<0.084	0.302	<0.084	<0.084	<0.084	0.224	<0.084	<0.084	<0.084	<0.084	<0.084	<0.084	<0.084	0.306
$\sum_3$iPCB	0.599	0.377	0.148	0.458	-	0.195	0.145	-	0.188	-	-	0.204	-	-	0.234	0.295
PCB#28	0.090	0.218	0.148	0.305	<0.064	<0.064	0.069	<0.064	<0.064	<0.064	<0.064	0.204	<0.064	<0.064	0.083	0.295
PCB#52	0.509	0.159	<0.070	0.153	<0.070	0.195	0.076	<0.070	<0.070	<0.070	<0.070	<0.070	<0.070	<0.070	0.151	<0.070
PCB#101	<0.076	<0.076	<0.076	<0.076	<0.076	<0.076	<0.076	<0.076	0.188	<0.076	<0.076	<0.076	<0.076	<0.076	<0.076	<0.076

Table A13.7. Levels of Persistent Organic Pollutants (ng/g lw) in Human Milk Samples from Mathira East Sub Counties, Nyeri County

Compound	K 1	K 2	K 3	K 4	K 5	K 6	K 7	K 8	K 9	K 10	K 11	K 12	K 13	K 14	K 15
$\sum_2$Chlordane	0.133	-	-	-	-	-	0.074	0.445	-	-	-	-	-	-	-
Cis-nonachlor	<0.088	<0.088	<0.088	<0.088	<0.088	<0.088	<0.088	0.222	<0.088	<0.088	<0.088	<0.088	<0.088	<0.088	<0.088
Trans-nonachlor	0.133	<0.065	<0.065	<0.065	<0.065	<0.065	0.074	0.223	<0.065	<0.065	<0.065	<0.065	<0.065	<0.065	<0.065
$\sum_4$DDT	0.956	0.338	0.315	0.317	0.210	0.263	0.257	2.733	0.148	0.703	1.152	0.828	0.294	0.447	0.710
p,p'-DDD	<0.059	<0.059	<0.059	<0.059	<0.059	<0.059	<0.059	0.269	<0.059	<0.059	<0.059	0.102	<0.059	<0.059	<0.059
p,p'-DDE	0.082	<0.080	0.315	0.225	<0.080	<0.080	<0.080	0.676	<0.080	0.160	0.381	<0.080	<0.080	<0.080	<0.080
o,p'- DDT	0.190	0.082	<0.065	<0.065	<0.065	<0.065	<0.065	0.537	<0.065	0.077	0.155	0.186	0.117	0.168	0.182
p,p'-DDT	0.684	0.256	<0.030	0.092	0.210	0.263	0.257	1.251	0.148	0.466	0.616	0.540	0.177	0.279	0.528
$\sum_1$Endosulfan	-	-	-	-	-	-	-	-	-	-	0.047	-	-	-	-
Endosulfan sulphate	<0.038	<0.038	<0.038	<0.038	<0.038	<0.038	<0.038	<0.038	<0.038	<0.038	0.047	<0.038	<0.038	<0.038	<0.038
HCB	0.194	0.215	<0.056	<0.056	<0.056	0.054	0.068	<0.056	0.067	<0.056	0.202	<0.056	<0.056	<0.056	<0.056
$\sum_3$iPCB	0.102	-	-	-	-	-	-	-	0.124	-	0.126	1.918	0.064	-	-
PCB#28	0.102	<0.064	<0.064	<0.064	<0.064	<0.064	<0.064	<0.064	<0.064	<0.064	<0.064	<0.064	<0.064	<0.064	<0.064
PCB#138	<0.083	<0.083	<0.083	<0.083	<0.083	<0.083	<0.083	<0.083	<0.083	<0.083	<0.083	0.491	<0.083	<0.083	<0.083
PCB#180	<0.060	<0.060	<0.060	<0.060	<0.060	<0.060	<0.060	<0.060	0.124	<0.060	0.126	1.427	0.064	<0.060	<0.060

Table A13.8. Levels of Persistent Organic Pollutants (ng/g lw) in Human Milk Samples from Kieni East Sub Counties, Nyeri County

Compound	NR 1	NR 2	NR 3	NR 4	NR 5	NR 6	NR 7	NR 9	NR 10	NR 11	NR 12	NR 13	NR 14	NR 15
$\sum_2$Chlordane	-	-	-	-	-	-	-	0.363	-	-	-	-	-	-
Cis-nonachlor	<0.088	<0.088	<0.088	<0.088	<0.088	<0.088	<0.088	0.182	<0.088	<0.088	<0.088	<0.088	<0.088	<0.088
Trans-nonachlor	<0.065	<0.065	<0.065	<0.065	<0.065	<0.065	<0.065	0.181	<0.065	<0.065	<0.065	<0.065	<0.065	<0.065
$\sum_4$DDT	1.053	0.267	0.513	0.462	1.252	0.513	1.487	0.620	1.500	0.292	0.949	0.303	0.926	0.485
p,p'-DDD	<0.059	<0.059	<0.059	0.059	0.061	<0.059	<0.059	<0.059	0.062	<0.059	0.071	<0.059	0.089	<0.059
p,p'-DDE	<0.080	<0.080	<0.080	<0.080	0.179	<0.080	0.109	<0.080	0.814	<0.080	0.637	0.303	0.161	0.156
o,p'-DDT	0.427	0.066	0.065	0.128	0.134	0.138	<0.065	0.120	0.065	<0.065	<0.065	<0.065	0.165	<0.065

Compound	NR 1	NR 2	NR 3	NR 4	NR 5	NR 6	NR 7	NR 9	NR 10	NR 11	NR 12	NR 13	NR 14	NR 15
p,p'-DDT	0.626	0.201	0.513	0.275	0.878	0.375	1.378	0.500	0.559	0.292	0.241	<0.093	0.511	0.329
$\sum_1$HCH	-	-	-	-	0.435	-	-	-	0.094	-	0.176	-	-	-
γ-HCH	<0.084	<0.084	<0.084	<0.084	0.435	<0.084	<0.084	<0.084	0.094	<0.084	0.176	<0.084	<0.084	<0.084
HCB	0.379	0.058	0.065	0.103	0.137	0.120	<0.056	<0.056	0.236	<0.056	<0.056	<0.056	<0.056	<0.056
$\sum_4$iPCB	0.707	-	0.306	0.080	0.641	0.344	-	-	0.084	-	0.061	-	-	-
PCB#28	<0.064	<0.064	<0.064	<0.064	0.123	<0.064	<0.064	<0.064	<0.064	<0.064	0.061	<0.064	<0.064	<0.064
PCB#138	0.319	<0.083	0.081	0.080	0.252	0.344	<0.064	<0.083	<0.064	<0.083	<0.064	<0.083	<0.083	<0.083
PCB#153	<0.072	<0.072	<0.072	<0.072	0.266	<0.072	<0.072	<0.072	0.084	<0.072	<0.072	<0.072	<0.072	<0.072
PCB#180	0.388	<0.060	0.225	<0.060	<0.060	<0.060	<0.060	<0.060	<0.060	<0.060	<0.060	<0.060	<0.060	<0.060

APPENDIX 14: ESTIMATED DAILY INTAKE (ng/kg/day) OF PERSISTENT ORGANIC POLLUTANTS BY BREASTFEEDING INFANT INDICATED AS ABOVE (+) OR BELOW (-) ACCEPTED DAILY INTAKE (ADI)

Table A14.1. Estimated Daily Intake of Persistent Organic Pollutants above (+) or below (-) reference values for Southlands Sub Counties, Nairobi County

Compound	Reference	ADI	KBR 1	KBR 2	KBR 3	KBR 4	KBR 6	KBR 7	KBR 8	KBR 9	KBR 10	KBR 11	KBR 12	KBR 14	KBR 15
$\sum_3$Chlordane	Codex	10000	-	-	-	-	-	-	-	-	-	-	-	-	-
	ATSDR	600	-	-	-	-	-	-	-	-	-	-	-	-	-
	EU	2000	-	-	-	-	-	-	-	-	-	-	-	-	-
$\sum_3$DDT	Codex	20000	-	-	-	-	-	-	-	-	-	-	-	-	-
	ATSDR	100	-	-	-	-	-	-	-	-	-	-	-	-	-
	EU	10000	-	-	-	-	-	-	-	-	-	-	-	-	-
$\sum_3$Endosulfan	Codex	6000	-	-	-	-	-	-	-	-	-	-	-	-	-
	ATSDR	6000	-	-	-	-	-	-	-	-	-	-	-	-	-
	EU	10000	-	-	-	-	-	-	-	-	-	-	-	-	-
$\sum_4$HCH	Codex	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	ATSDR	600	-	-	-	-	-	-	-	-	-	-	-	-	-
	EU	-	-	-	-	-	-	-	-	-	-	-	-	-	-
HCB	Codex	10000	-	-	-	-	-	-	-	-	-	-	-	-	-
	ATSDR	70	-	-	-	-	-	-	-	-	-	-	-	-	-
	EU	10000	-	-	-	-	-	-	-	-	-	-	-	-	-
$\sum_6$iPCB	Codex	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	ATSDR	20	-	-	-	-	-	-	-	-	-	-	-	-	-
	EU	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Notes: Codex = Codex Alimentarius Commission, ATSDR = Agency for Toxic Substances and Disease Registry, EU = European Union.

Table A14.2. Estimated Daily Intake of Persistent Organic Pollutants above (+) or below (-) reference values for Eastlands and Westlands Sub Counties, Nairobi County

Compound	Reference	ADI	NW 2	NE 3	NE 4	NW 4	NE 5	NE 6	NE 7	NE 8	NE 9	NE 10	NE 11	NE 12	NE 13
∑2Chlordane	Codex	10000	-	-	-	-	-	-	-	-	-	-	-	-	-
	ATSDR	600	-	-	-	-	-	-	-	-	-	-	-	-	-
	EU	2000	-	-	-	-	-	-	-	-	-	-	-	-	-
∑4DDT	Codex	20000	-	-	-	-	-	-	-	-	-	-	-	-	-
	ATSDR	100	-	-	-	-	-	+	-	+	-	-	-	-	-
	EU	10000	-	-	-	-	-	-	-	-	-	-	-	-	-
∑2Endosulfan	Codex	6000	-	-	-	-	-	-	-	-	-	-	-	-	-
	ATSDR	6000	-	-	-	-	-	-	-	-	-	-	-	-	-
	EU	10000	-	-	-	-	-	-	-	-	-	-	-	-	-
∑2HCH	Codex	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	ATSDR	600	-	-	-	-	-	-	-	-	-	-	-	-	-
	EU	-	-	-	-	-	-	-	-	-	-	-	-	-	-
HCB	Codex	10000	-	-	-	-	-	-	-	-	-	-	-	-	-
	ATSDR	70	-	-	-	-	-	-	-	-	-	-	-	-	-
	EU	10000	-	-	-	-	-	-	-	-	-	-	-	-	-
∑5iPCB	Codex	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	ATSDR	20	-	-	-	-	-	+	-	-	-	-	-	-	-
	EU	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Notes: Codex = Codex Alimentarius Commission, ATSDR = Agency for Toxic Substances and Disease Registry, EU = European Union.

Table A14.3. Estimated Daily Intake of Persistent Organic Pollutants above (+) or below (-) reference values for Chesumei Sub Counties, Nandi County

Compound	Ref	ADI	KCRH 1	KCRH 2	KCRH 3	KCRH 4	KCRH 5	KCRH 6	KCRH 7	KCRH 8	KCRH 9	KCRH 10	KCRH 11	KCRH 12	KCRH 13	KCRH 14	KCRH 15
∑3Chlordane	Codex	10000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	ATSDR	600	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	EU	2000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Compound	Ref	ADI	KCRH 1	KCRH 2	KCRH 3	KCRH 4	KCRH 5	KCRH 6	KCRH 7	KCRH 8	KCRH 9	KCRH 10	KCRH 11	KCRH 12	KCRH 13	KCRH 14	KCRH 15
∑3DDT	Codex	20000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	ATSDR	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	EU	10000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
∑3Endosulfan	Codex	6000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	ATSDR	6000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	EU	10000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
∑4HCH	Codex	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	ATSDR	600	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	EU	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
∑6iPCB	Codex	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	ATSDR	20	-	-	+	-	-	+	-	-	-	-	-	-	-	+	-
	EU	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Notes: Codex = Codex Alimentarius Commission, ATSDR = Agency for Toxic Substances and Disease Registry, EU = European Union.

Table A14.4. Estimated Daily Intake of Persistent Organic Pollutants above (+) or below (-) reference values for Chesumei and Nandi Central Sub Counties, Nandi County

Compound	Reference	ADI	KCRH 16	KCRH 17	KCRH 18	KCRH 19	KCRH 20	NHCH 1	NHCH 2	NHCH 3	NHCH 4	NHCH 5	NHCH 6	NHCH 7	NHCH 8	NHCH 9	NHCH 10
∑3Chlordane	Codex	10000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	ATSDR	600	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	EU	2000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
∑3DDT	Codex	20000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	ATSDR	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	EU	10000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
∑1Endosulfan	Codex	6000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	ATSDR	6000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	EU	10000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
∑4HCH	Codex	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	ATSDR	600	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Compound	Reference	ADI	KCRH 16	KCRH 17	KCRH 18	KCRH 19	KCRH 20	NHCH 1	NHCH 2	NHCH 3	NHCH 4	NHCH 5	NHCH 6	NHCH 7	NHCH 8	NHCH 9	NHCH 10
	EU	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
$\sum_6$iPCB	Codex	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	ATSDR	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	EU	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Notes: Codex = Codex Alimentarius Commission, ATSDR = Agency for Toxic Substances and Disease Registry, EU = European Union.

Table A14.5. Estimated Daily Intake of Persistent Organic Pollutants above (+) or below (-) reference values for Nandi Central and South Sub Counties, Nandi County

Compound	Reference	ADI	NHCH 12	NHCH 13	NHCH 14	NHCH 15	NHCH 16	NHCH 17	NHCH 18	NHCH 19	NHCH 20	KSCH - A - 1	KSCH - A - 2	KSCH - A - 3	KSCH - A - 4	KSCH - A - 5	KSCH - A - 6
$\sum_1$Chlordane	Codex	10000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	ATSDR	600	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	EU	2000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
$\sum_3$DDT	Codex	20000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	ATSDR	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	EU	10000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
$\sum_3$Endosulfan	Codex	6000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	ATSDR	6000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	EU	10000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
$\sum_2$HCH	Codex	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	ATSDR	600	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	EU	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
$\sum_3$iPCB	Codex	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	ATSDR	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	EU	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Notes: Codex = Codex Alimentarius Commission, ATSDR = Agency for Toxic Substances and Disease Registry, EU = European Union.

Table A14.6. Estimated Daily Intake of Persistent Organic Pollutants above (+) or below (-) reference values for Nandi South, East and Tindiret Sub Counties, Nandi County

Compound	Reference	ADI	KSCH A - 7	KSCH A - 8	KSCH A - 9	KSCH A - 10	LHC - 1	LHC - 2	LHC - 3	LHC - 6	LHC - 8	LHC - 9	LHC - 10	MTT 4	KSCH M - 1	KSCH M - 2	KSCH M - 3	KSCH M - 4
$\sum_4$DDT	Codex	20000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	ATSDR	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	EU	10000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
$\sum_2$Endosulfan	Codex	6000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	ATSDR	6000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	EU	10000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
$\sum_2$HCH	Codex	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	ATSDR	600	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	EU	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
$\sum_3$iPCB	Codex	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	ATSDR	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	EU	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Notes: Codex = Codex Alimentarius Commission, ATSDR = Agency for Toxic Substances and Disease Registry, EU = European Union.

Table A14.7. Estimated Daily Intake of Persistent Organic Pollutants above (+) or below (-) reference values for Mathira East Sub Counties, Nyeri County

Compound	Reference	ADI	K 1	K 2	K 3	K 4	K 5	K 6	K 7	K 8	K 9	K 10	K 11	K 12	K 13	K 14	K 15
$\sum_2$Chlordane	Codex	10000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	ATSDR	600	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	EU	2000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
$\sum_4$DDT	Codex	20000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	ATSDR	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	EU	10000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
$\sum_1$Endosulfan	Codex	6000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	ATSDR	6000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	EU	10000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
HCB	Codex	10000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	ATSDR	70	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	EU	10000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
$\sum_3$iPCB	Codex	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	ATSDR	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	EU	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Notes: Codex = Codex Alimentarius Commission, ATSDR = Agency for Toxic Substances and Disease Registry, EU = European Union.

Table A14.8. Estimated Daily Intake of Persistent Organic Pollutants above (+) or below (-) reference values for Kieni East Sub Counties, Nyeri County

Compound	Reference	ADI	NR 1	NR 2	NR 3	NR 4	NR 5	NR 6	NR 7	NR 9	NR 10	NR 11	NR 12	NR 13	NR 14	NR 15
$\sum_2$Chlordane	Codex	10000	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	ATSDR	600	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	EU	2000	-	-	-	-	-	-	-	-	-	-	-	-	-	-
$\sum_4$DDT	Codex	20000	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	ATSDR	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	EU	10000	-	-	-	-	-	-	-	-	-	-	-	-	-	-
$\sum_1$HCH	Codex	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	ATSDR	600	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	EU	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
HCB	Codex	10000	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	ATSDR	70	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	EU	10000	-	-	-	-	-	-	-	-	-	-	-	-	-	-
$\sum_4$iPCB	Codex	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	ATSDR	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	EU	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Notes: Codex = Codex Alimentarius Commission, ATSDR = Agency for Toxic Substances and Disease Registry, EU = European Union.